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REMEDIATION OF BROMATE CONTAMINATED GROUNDWATER

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REMEDIATION OF BROMATE CONTAMINATED GROUNDWATER

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ABSTRACT

Bromate (BrO_3^-) is a by-product formed at concentrations of 0.4 – 60 μg L^{-1} during potable water ozonation. Following World Health Organisation designation as a 'possible human' carcinogen, a 10 μg L^{-1} drinking water limit was introduced in England and Wales. Discovery of bromate contamination within a UK aquifer highlighted a knowledge gap, addressed by this project, relating to environmental behaviour and groundwater remediation.

Following selection of an anion analysis strategy utilising Ion Chromatography (IC), bromate behaviour in wastewater was investigated as contaminated groundwater ingress to treatment processes was deemed possible. Respiration of wastewater biomass was unaffected by spiking of ≤ 200 mg L^{-1} bromate or bromide, with pilot-scale process dosing trials (≤ 100 mg L^{-1}) using a Membrane Bioreactor (MBR) also exhibiting little negative effect following biomass acclimation.

Bromate reduction to bromide was observed in a continuous-flow suspended growth chemostat bioreactor at retention times of 20 – 80 hours. A biological mechanism was confirmed in this system, with reduction mediated by indigenous groundwater bacteria following glucose addition. Bromate reduction rates were initially low (≤ 27.8 μg L^{-1} hr^{-1}), but acclimation increased rates to > 1000 μg L^{-1} hr^{-1}. An alteration in microbial composition was noted over this period, from a denitrifying 'co-metabolic' culture to predomination of 'high-rate' specific bromate degraders. Operational parameters including pH, temperature, carbon source, influent bromate and glucose, and retention times were investigated, with all parameters apart from pH shown to affect bromate reduction rates. For example increased bromate influent enhanced reduction rate, although potentially toxic effects were noted with an influent > 75 – 80 mg L^{-1}. Batch studies suggested glucose was rapidly fermented (< 48 hours) by the microbial consortium. Nitrate was also rapidly removed (< 4 hours), with sulphate reduction only following removal of bromate. A fixed-film pilot-scale bioreactor system, seeded with biomass from the chemostat culture, reduced > 90% of a 1.1 mg L^{-1} bromate influent within unspiked contaminated groundwater. Plating studies were successful in producing a range of isolates from the mixed chemostat culture. Overall the project demonstrated, for the first time, continuous remediation of bromate groundwater contamination within a bioreactor system.

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1 INTRODUCTION

Over recent years, research into the properties of many chemical and biological components found within water supplies, both naturally-occurring and of anthropogenic origin, has been ongoing. In some cases potential health risks have been identified, subsequently leading to introduction or tightening of legislation relating to their presence within potable water supplies. In England and Wales, one recent major amendment to existing legislation has been the Water Supply (Water Quality) Regulations 2000. A number of contaminants mentioned within these new Regulations were previously listed but are included in the new legislation to tighten up on existing standards following expert advice on health effects. An example of this is lead, for which the maximum allowable concentration in potable water at consumers' taps has been reduced from 50 $\mu g\ L^{-1}$ (or parts per billion) to 25 $\mu g\ L^{-1}$. Others were mentioned for the first time. One of these new parameters is bromate (BrO_3^-) which, following evidence of potential carcinogenicity, has been restricted to a maximum concentration within potable water supplies of 10 $\mu g\ L^{-1}$ from 25 December 2003, where it was previously unregulated.

Bromate is commonly considered as an unwanted product of drinking water treatment processes, formed during disinfection by ozonation. Bromate is formed as a disinfection by-product (DBP) by oxidation of naturally-occurring bromide (Br^-) within the water, where concentrations up to 60 $\mu g\ L^{-1}$ have been observed in treated water for supply (Kirisits and Snoeyink, 1999). Techniques for minimising bromate formation during ozonation have been researched, including pH depression and ammonia addition (Grosvenor, 1999). In addition, several technologies have been evaluated for removal of bromate from ozonated water, including filtration (Marhaba and Medlar, 1993), UV irradiation (Siddiqui and Amy, 1994), chemical reduction (Gordon *et al.*, 2002), and granular activated carbon (Kirisits *et al.*, 2000).

Occurrence of bromate in surface waters or aquifers has not historically been reported (Hutchinson *et al.*, 1997). It is therefore generally thought of only as a low-level man-made contaminant of potable water supplies, for which formation and removal rates can be tightly monitored and controlled. However, following recent advances in

analytical capability, which can now detect bromate at sub-μg L^{-1} concentrations, reports have been made of bromate occurring in the natural water environment (Kruithof and Meijers, 1995).

The current study is based around an aquifer in the South of England where, during routine preliminary sampling for analytical method development, a bromate concentration in excess of the then-proposed Water Supply regulations was detected. Further investigation allowed delineation of a substantial plume of bromate contamination extending from a point source, determined to be a small former chemicals plant involved in production of brominated compounds prior to its' closure. Due to the potentially carcinogenic nature of this pollution, abstraction from major supply boreholes in the area was immediately affected. As a result of the negative and sustained effect of this disruption on water resources in the supply area, development of remediation processes for both abstracted water and the source aquifer was identified as a priority area for research.

1.1 MOTIVATION FOR WORK

At the time of plume delineation, techniques researched for removal of bromate from water supplies were not advanced past laboratory- or preliminary pilot-scale, and all were being developed with the aim of removing low-level ($\leq$ 60 μgL^{-1}) bromate concentrations from treated and thus potable water. No studies had been undertaken to examine application of these methodologies as a potential groundwater remediation strategy. The current project was therefore instigated to investigate an identified knowledge gap. This was to be achieved by reviewing existing techniques available for bromate removal from potable water, and applying this knowledge in development of a laboratory- and pilot-scale remediation system for removal of bromate from groundwater. Advancement of a technology to this stage was aimed at potentially leading to subsequent scale-up to aquifer scale, as part of an integrated aquifer rehabilitation strategy. In addition, with potential for bromate-contaminated groundwater ingress into a sewage treatment system, either accidentally or as part of a pump-to-waste aquifer remediation strategy, bromate behaviour in the presence of sewage sludge was also to be investigated.

Finally, attainment of reliable analytical capability in both groundwater and wastewater matrices to the then-proposed 10 μg L^{-1} drinking water limit was also identified as a priority.

1.2 THESIS STRUCTURE

Following the initial introduction given in this chapter, a review of literature relevant to bromate occurrence, properties, legislation and remediation (both non-biological and biotic) is presented (Chapter 2). Overall thesis aims and objectives are set out (Chapter 3), and an outline of experimental work given in Chapter 4.

With the project dependent on an accurate and reliable anion analysis strategy, Chapter 5 provides a complete account of the technique selection process, including completion of both a literature review to identify available technologies, and subsequent comparative experimental trials of selected potential methodologies.

Chapter 6 summarises all materials and methods utilised during trials investigating both microbial bromate bioremediation within groundwater and also bromate dosing within wastewater systems. Data obtained during these trials are then presented in Chapter 7. Chapter 8 provides a discussion into reasons for and significance of the results in addressing thesis objectives, with the potential for bromate bioremediation as a full-scale aquifer rehabilitation tool also covered. Chapter 9 outlines conclusions reached from the trial in relation to the thesis objectives, with Chapter 10 giving suggestions for future work necessary to develop and enhance the studies completed in this thesis.

2 LITERATURE REVIEW

This chapter outlines the occurrence, manufacture and uses of bromate and other bromine compounds. The subsequent discovery of detrimental effects of bromate to humans and the environment is covered, with current knowledge on toxicology and legislation now in place reviewed. The significance of modern water treatment processes in the formation of bromate within potable water is discussed, with the circumstances leading to bromate discovery within a groundwater supply summarised. Methodologies already investigated to reduce bromate contamination within potable water are outlined, and their efficacy and development potential for use in a groundwater matrix evaluated. The occurrence of and mechanisms operating during reduction of electron acceptors such as nitrate and perchlorate during biotic heterotrophic respiration processes are explained, with this knowledge related to the potential for an analogous biological bromate reduction system. Finally, possibilities of this mechanism for removal of bromate groundwater contamination are described and summarised.

2.1 BACKGROUND TO BROMINE SOURCES AND USE

The element bromine (Br) is a member of the halogen group (Group VII) in the periodic table, and was first discovered in 1825 by the French chemist A.J. Balard (Jolles, 1966), being the third of the halogen family to be documented, after chlorine (1774) and iodine (1811). Due to the unpleasant smell given off by the newly-discovered liquid form, it was given the name bromine, derived from the Greek 'βρώμος' (Bromos), meaning stink. It was not long after the discovery of bromine that an industrial use was found for the newly-catalogued element, with the compound silver bromide developed as the primary constituent of photosensitive photographic 'emulsions' in a fledgling photographic industry in 1840. Gradually other uses were discovered for both bromine and many of its compounds, and an entire industry grew up around the manufacture and distribution of these chemicals. Initially they were only produced in small quantities for the pharmaceutical, dyeing and photographic industries, but development of the automobile industry and inclusion of ethylene dibromide as a part of 'anti-knock' mixture in fuel ensured that by the late 1950's the USA alone was experiencing annual sales of

nearly 90,000 tonnes of bromine (elemental and combined) (Price *et al.*, 1988). This trend of increasing worldwide bromine use continued over the next 25 years, with sales peaking at 403,000 tonnes in 1979. However, changes in the global chemical industry in the 1980's coupled with environmental concerns and the phasing out of leaded petrol subsequently led to a reduction in demand for bromine products and a concurrent over-supply.

Bromine is found in the natural environment, with the Earths' crust estimated to contain between approximately $10^{15} - 10^{16}$ tonnes bromine, mainly within igneous rocks (Jolles, 1966). Bromine is also contained within the hydrosphere, which it reaches following weathering of rocks. However, these stores of bromine are not found in the elemental state, which is highly reactive, but instead in the halide form as bromide (Br^-). The largest bromide source within the hydrosphere is the sea, where the average bromide content has been calculated as around 6.5 mg L^{-1} (Downs and Adams, 1975). Bromide is also found ubiquitously within other water systems, with average concentrations in freshwater of 15–200 μg L^{-1} (Flury and Papritz, 1993), and slightly higher levels in groundwater, especially in regions with saltwater intrusion, dissolution from sedimentary rock, domestic effluents, and road run-off (Hutchinson *et al.*, 1997). The average bromide concentration within natural waters in the United States is thought to be almost 100 μg L^{-1} (Siddiqui *et al.*, 1996d). In addition, bromide can be released as a result of manufacturing processes, including potassium and coal mining, and soda production (von Gunten and Hoigne, 1996).

Whilst the halide ions (chloride, bromide and iodide) are all commonly found within the natural environment, their corresponding oxyanions (chlorate, bromate and iodate) are much rarer. Chlorate and bromate do not occur naturally, and only iodate is known to be present in the oxyanion form (Downs and Adams, 1975). Therefore, bromate has not historically been detected within the terrestrial, freshwater or marine environments, and is not thought of as a component of natural ecosystems.

2.2 MANUFACTURE AND POTENTIAL USES OF BROMATE

Due to the lack of natural sources of bromate, any supplies required for domestic

or industrial usage must be manufactured. The only bromates of industrial importance have historically been the sodium and potassium salts ($NaBrO_3$ and $KBrO_3$). Both are white crystalline solids that dissolve readily in water ($KBrO_3$ solubility at 25°C is 75 g L^{-1}) (Depository Services Program (Canada), 1999), a general characteristic of the Group I, II and III salts with the exception of mercuric and barium bromates (Jolles, 1966). Bromate has traditionally been manufactured by two techniques, either a chemical/electrochemical process or via chemical methodologies. The chemical methods for production of potassium bromate are described by Jolles (1966), with one example being the halogenation of a calcium hydroxide suspension by addition of bromine, to give a solution containing calcium bromide and calcium bromate. Potassium bromate is then precipitated by potassium chloride addition (Jolles, 1966). The electrochemical route involves oxidation of added bromide to bromine at the anode and hydroxide ion production at the cathode. Disproportionation of these two products within the electrolyte solution leads to the synthesis of hypobromite (OBr^-), which is the precursor to bromate formation by either further disproportionation of the OBr^- or its oxidation at the anode. The overall cell process can be represented by Equation 2.1 (Downs and Adams, 1975).

$$Br^- + 3H_2O \rightarrow BrO_3^- + 3H_2$$

Equation 2.1

Bromate has found application in a variety of processes, the majority of which were used in a domestic environment. The basic chemical property used in most of these applications is the ability of bromate to oxidise thiol (-SH) groups, found within the structure of proteins, into disulphide (S-S) groups (Jolles, 1966). The addition of a small amount of bromate to flour, typically 20 – 50 mg L^{-1}, has been widely used to enhance various properties of bread including rheological properties of the dough, tolerance to processing, crumb texture and loaf volume (Dupois, 1997). Bromate has also been used as a neutraliser in solutions for permanent waving of hair. In this case, the active ingredients of the hair waving solution reduce disulphide bonds within cysteine molecules in hair keratin to make hairs more pliable, and the process is then reversed by

bromate addition to 'fix' the hair in the new position (Jolles, 1966; Kutom *et al.,* 1990). Other common applications for bromate included use in the production of fish paste products, cheese, beer, wool and gold (US EPA, 2001; World Health Authority, 1996), the latter application utilising a mixture of sodium bromate and sodium bromide under the name 'mining salts'.

2.3 HEALTH ISSUES AND ECOTOXICOLOGY

The bromate anion is classified by the International Agency for Research on Cancer (IARC, 1999), a part of the World Health Organisation (WHO), as a Group 2B or 'possible human' carcinogen (IARC, 1999). This designation is based on results of a number of separate toxicology studies on laboratory animals investigating the effects of both acute and chronic (short and long-term) exposure, with toxicokinetics, carcinogenicity, genotoxicity and reproductive effects all evaluated (summarised in Depository Services Program (Canada), 1999). Evidence has been noted of tumour induction in rats and mice (Kurokawa *et al.,* 1986), with a dose of 0.38 – 2.1 mg kg^{-1} per day for 100 weeks estimated to result in a 10% increase in cancer risk (US EPA, 2001). Tumours in rats have been noted in a number of areas of the body, including the kidney, thyroid gland and peritoneum (World Health Organisation, 2005). The acute toxicity of bromate to rats is reported as an LD_{50} value (internal dose required to cause 50% test population mortality) between 136 – 495 mg kg^{-1} body weight (World Health Organisation, 1996). Corresponding human toxicity data are limited to acute accidental poisoning cases, where reversible symptoms may include nausea, vomiting, severe gastrointestinal irritation, and depression of the central nervous system. Renal failure and deafness are irreversible effects of bromate poisoning and have been observed following ingestion of 185 – 385 mg L^{-1} bromate (Quick, 1975). Lethal doses are reported as 150 – 385 mg kg^{-1} bodyweight (World Health Organisation, 2005), although doses as low as 5 – 50 mg kg^{-1} body weight following accidental poisoning have also been reported as resulting in death (Depository Services Program (Canada), 1999). Once ingested, bromate may be rapidly absorbed from the gastrointestinal tract and reduced to bromide within body tissues (Fujii *et al.,* 1984) and possibly also to hydrobromic acid within the stomach (Kutom *et al.,* 1990). Other reports however, suggest that bromate is

'surprisingly stable within the body' (Gosselin *et al.*, 1976). Excretion of ingested bromate is mainly in urine as bromate and bromide (Fujii *et al.*, 1984). Bromate can be detected in urine at bromate doses of 5 mg kg^{-1} body weight and above (World Health Organisation, 2000). Following baking, no residues are found in finished bread following addition of up to 50 mg kg^{-1} bromate due to breakdown to bromide during the baking process (World Health Organisation, 2005). However, due to health concerns the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recommended that bromate residues in food following processing should be eliminated, although the US Code of Federal Regulations still allow up to 50 – 75 mg kg^{-1} bromate addition to bread during baking (World Health Organisation, 2005). In the UK, potassium bromate was specifically banned as a flour improver in 1990 (HM Government, 1990). EU regulations do not now permit the use of bromate in any food additive function (Furmage, 2005).

Bromate toxicity has been demonstrated in the natural environment, with one literature review of water-borne organisms (including the crustacean *Daphnia magna*, the flatworm *Polycelis nigra* and juveniles of various fish species) concluding that lethal concentrations (LC_{50}) ranged from 31 mg L^{-1} for newly-hatched striped bass (*Morone saxatilis*) larvae to 2258 mg L^{-1} for *P. nigra*. Therefore, a precautionary ecotoxicity exposure safety value of 3.0 mg L^{-1} bromate in natural water sources was suggested, allowing for a tenfold safety factor in the most sensitive species (Hutchinson *et al.*, 1997). Fish eggs exposed to bromate developed chronic, pathological disorders, especially to the brain and spine (Burton and Richardson, 1981), and a study on marine phytoplankton found 13.6 mg L^{-1} bromate increased cell division in at least two of the four species investigated (Hutchinson *et al.*, 1997). However, there is a paucity of further information on either the ecotoxicology and phytotoxicity of bromate, or chronic effects of bromate exposure on human populations. One limited study has suggested that incidences of cancer in an area with possible chronically elevated potable water bromate concentrations were no higher than for the general population (NHS, 2001), but little further is known about the long-term effects of bromate exposure on human health or the behaviour of bromate within the natural environment.

2.4 LEGISLATION AND RECOMMENDATIONS

2.4.1 WHO Guidelines for Drinking Water Quality

The World Health Organisation Guidelines for Drinking Water Quality, 2nd Edition were published in 1993. Following the designation of bromate by the IARC as a possible carcinogen, WHO Guidelines were updated to include a provisional guideline value of 25 μg L^{-1} bromate in drinking water (World Health Organisation, 1993). This value was based on an excess lifetime exposure cancer risk of 7 x 10^{-5}, which is calculated as the additional risk over that expected in a normal population. Bromate was included in the guidelines due to its formation during water treatment processes, as will be covered in detail in Section 2.5. Many of the subsequent legislative limits enforced were based on this recommendation. The WHO has now updated its recommendation (World Health Organisation, 2005) in the light of revised excess lifetime cancer risks and current analytical capabilities, and a provisional guideline value of 10 μg L^{-1} is now in force.

2.4.2 EC Drinking Water Directive

The major European Union legislation relating to bromate within water matrices is contained within EC Directive 98/83/EC, the Drinking Water Directive. This legislation is concerned with the quality of water intended for human consumption and was adopted in November 1998 (Council of the European Communities, 1998). It replaced the 1980 Directive on Water Quality, and included new or revised standards for a range of parameters. The stated objective of the Directive, as with the previous edition, was to "protect human health from the adverse effects of any contamination of water intended for human consumption by ensuring that it is wholesome and clean". One revision was the introduction of a potable water standard for bromate, set at 10 μg L^{-1}. However, a clause was also included for bromate which set an interim limit of 25 μg L^{-1} for a period of five years until 2008, to allow adequate preparation for the lower limit by all Member States. The Directive was enforced from 25 December 2003, with drinking water quality required to meet the new standards from this date.

2.4.3 Water Supply (Water Quality) Regulations

In England and Wales, publishing of the EC Drinking Water Directive ensured an overhaul of existing Legislation was required. Therefore, the Water Supply (Water Quality) Regulations were published in 2000 and came into force on 25 December 2003 (HM Government, 2000). The standard for bromate, which was not required to be set at 10 μg L^{-1} until 2008, was adopted as the lower concentration from initial implementation of the Regulations.

2.4.4 Worldwide drinking water legislation

Other developed countries have also included bromate as a contaminant of potable water supplies. Bromate legislation for potable water consumption is well-advanced within North America. In the United States, bromate is included in the US Environmental Protection Agency Stage 1 Disinfectants/Disinfection By-products rule, which was published in 1998. As in the EU, the limit for bromate within potable water was set as a Maximum Contaminant Level (MCL) of 10 μg L^{-1} (Kirisits *et al.*, 2002). The Canadian Federal Authorities have also instigated a drinking water limit of 10 μg L^{-1}, which is in force as an Interim Maximum Allowable Concentration (IMAC). The interim nature of the limit was stated to be due to a lack of practical analytical techniques at the time able to detect bromate below 10 μg L^{-1}, and the inability of remediation techniques to remove bromate below these concentrations (Depository Services Program (Canada), 1999).

2.4.5 Future bromate limits

More stringent legislation for bromate within potable water has previously been suggested, with a guideline value of only 0.5 μg L^{-1} proposed by the Dutch research organisation KIWA in 1993 (Hijnen *et al.*, 1999). This limit was based on the estimate at the time by the WHO of a 10^{-6} excess lifetime exposure cancer risk for a concentration of 0.3 μg L^{-1} in drinking water (World Health Organisation, 1993). During recent investigations into establishment of the Stage 2 Disinfectants/Disinfection By-products rule, an update on the Stage 1 legislation, the US EPA evaluated lowering the bromate MCL to 5 μg L^{-1} (US EPA, 2003). However, the eventual proposal was for continuation

of the MCL at 10 μg L^{-1}. These conclusions were reached due to evidence that the requirement for a lower limit could both compromise disinfection efficiency for more recalcitrant microorganisms, and also lead to formation of other undesired compounds in drinking water. These reasons, along with the continuing lack of firm evidence on effects to human health and complexity of routine analysis for concentrations lower than 10 μg L^{-1}, mean it is unlikely in the near future that guideline values for potable water will become more stringent than this level in any country.

Although concentrations of bromate are now strictly regulated within potable water, there is no equivalent standard for bromate in natural water matrices and neither the EC Groundwater Directive (80/68/EEC) or the recent Water Framework Directive (2000/60/EC) include bromate as a contaminant (Council of the European Communities, 1980; Council of the European Communities, 2000), which has subsequently led to the continuing absence of bromate in national legislation within Europe. It is also unlikely that bromate will be included in future groundwater legislation unless further contamination sites are uncovered.

2.4.6 Bromide legislation

Bromide, as a natural constituent of water, is not known to be toxic in concentrations encountered in these matrices. There are therefore no current standards for bromide within potable water..

2.5 FORMATION AND CONTROL DURING DRINKING WATER PROCESSES

In recent years, bromate has become known as a contaminant of potable water supplies due to its formation by oxidation of naturally-occurring bromide during disinfection by ozonation. Bromate can also be introduced into the treatment process by addition of contaminated hypochlorite feedstock. Following the discovery of potential carcinogenic effects of bromate during the mid-1980's (ie. Kurokawa *et al.*, 1986), these effects have become more relevant within the water treatment framework. Therefore from the early 1990's research has focussed on understanding bromate formation and

strategies for minimization, and there is now a larger body of information regarding this area of bromate study than any other single aspect.

2.5.1 Ozonation

Ozone can be produced commercially by exposing oxygen, either pure supplies or that found within the atmosphere, to a high voltage electrical discharge (Grosvenor, 1999). Molecular ozone is a highly effective disinfectant, able to inactivate recalcitrant pathogenic microorganisms such as oocysts of the protozoan *Cryptosporidium parvum,* which is highly resistant to traditional processes utilising free chlorine or monochloramine (Driedger *et al.*, 1991). It is also effective at removing pesticides and other unwanted water constituents such as organics, turbidity, and taste-, odour- and colour-producing compounds (Mills and Meadows, 1995; Grosvenor, 1999). Disinfection by ozonation was first used for drinking water treatment in 1893 in the Netherlands (US EPA, 1999). It has been a widely accepted process in Europe since the early 1980's (Ijpelaar *et al.,* 2002), and is now commonly used in both the United States and Europe to treat potable water (Driedger *et al.*, 1991; Legube *et al.,* 1995). Within the United Kingdom, usage of ozonation increased rapidly in the early 1990's, at least partially due to fears uncovered in the 1970's regarding formation of carcinogenic trihalomethanes (THMs) during disinfection by chlorine (McCann, 1993). However, ozonation does have disadvantages. Ozone can react with natural organic matter (NOM) to produce assimilable organic carbon (AOC) which, due to its readily biodegradable nature can be effective at promoting unwanted biological growth in distribution systems. This can be exacerbated by the short half-life ($t_{½}$) of ozone which is not high enough to supply a sufficient disinfection residue within distribution systems (Song *et al.,* 1996b). These two disadvantages may be overcome by addition of specific treatment techniques such as biological activated carbon (BAC) treatment to remove AOC, and postdisinfection with a chlorine-based disinfectant such as monochloramine respectively. However, another effect of ozonation has been noted which is a more intractable problem. The nature of all disinfection processes means disinfection by-products (DBPs) are not just a consequence of chlorination, and DBP production has been noted from all disinfectants used. Examples given in Teuschler *et al.* (2000) which have been

toxicologically studied include haloacetic acids (HAAs), haloacetonitriles, haloketones, aldehydes, chloral hydrate and chloropicrin, and there are many more which have not been characterised (Teuschler *et al.*, 2000). In the case of ozonation, a variety of both characterised and unknown brominated DBPs can form, of which identified examples include bromoform, bromoacetic acids and bromoacetonitriles (Song *et al.*, 1997; Westerhoff *et al.*, 1998). In addition, it became obvious in the early 1980s that bromate formation was a consequence of ozone disinfection of water containing concentrations of bromide.

Bromate is formed during ozonation via the oxidation of bromide through action of a combination of ozone and hydroxyl radicals (OH·), leading to a complex series of reactions which can be summarised as in Figure 2.1. During the reaction sequences, a range of bromine oxidation states are present either simultaneously or in sequence, which leads to a highly non-linear reaction system (von Gunten, 2003). Oxidation states found within the ozonation system are given in Table 2.1. There are three major oxidation pathways; these can be termed the direct ozonation pathway, the direct-indirect Br^- ozonation combination pathway and the indirect-direct Br^- ozonation combination pathway (Song *et al.*, 1997).

- The direct ozonation pathway occurs when ozone oxidises Br^- to aqueous bromine (OBr^- in equilibrium with $HOBr^-$). The OBr^- produced is then further sequentially oxidised to first BrO_2^- and finally BrO_3^-.

- The direct-indirect combination pathway relies on production of aqueous bromine as with the direct pathway. However, both OBr^- and $HOBr^-$ constituents are oxidised by OH· to form BrO·, which disproportionates to BrO_2^-. Oxidation by ozone then acts to produce bromate.

- The indirect-direct combination pathway does not rely on ozone oxidation in the first step. Instead bromide is oxidised by OH· to form bromine radicals (Br·). Ozone oxidation then acts on Br· to produce BrO· which, as with the direct-indirect pathway, leads to bromate formation by disproportionation and oxidation.

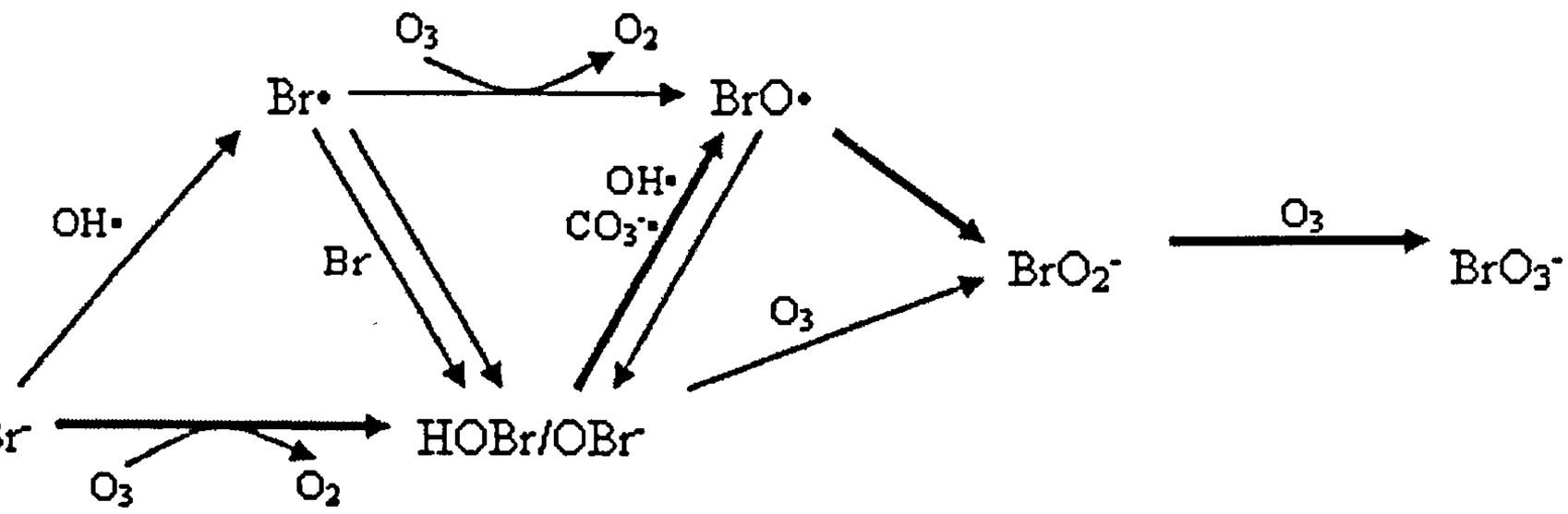


Figure 2.1 - Reaction scheme for bromate formation during ozonation of bromide-containing waters (von Gunten, 2003)

Table 2.1 - Bromine species formed during bromate formation and their oxidation states (von Gunten, 2003)

Species	Chemical formula	Bromine oxidation state
Bromide	Br^-	-I
Bromine radical	$Br\cdot$	0
Hypobromous acid	HOBr	+I
Hypobromite	OBr^-	+I
Bromine oxide radical	$BrO\cdot$	+II
Bromite	BrO_2^-	+III
Bromate	BrO_3^-	+V

The total reaction sequence followed and relative importance of each individual pathway is highly dependent on both water quality and treatment conditions (Song *et al.*, 1997). Factors shown to affect bromate formation during ozonation for potable water production include pH, temperature, ozone dosage, reaction time, alkalinity, bromide content, and NOM content and composition (Song *et al.*, 1996a; Westerhoff *et al.*, 1998). Influent bromide concentration is a major factor, with levels in the range 50 – 100 μg L^{-1} producing quantifiable but manageable bromate concentrations, and concentrations in excess of 100 μg L^{-1} leading to potentially serious bromate problems depending on treatment goals (von Gunten, 2003). Reaction time and ozone dosage are also critical

factors dependent on the aim of the process, an example being inactivation of *C. parvum* oocysts requiring a higher dose than less recalcitrant targets. An effective CT dose (given as a product of disinfection residual (C) and contact time (T)) for 1-log (90%) inactivation of *Giardia*, an example of a less recalcitrant microorganism, was given as 0.5 mg L^{-1} min at 10°C by Amy *et al.* (2000). The authors suggested the equivalent CT value for *Cryptosporidium* inactivation to be in the range 2.5 – 10 mg L^{-1} min. A summary of five studies in Europe and the USA on bromate formation following ozonation showed that, of 156 full-scale plants, around 6% (10 plants) produced water containing greater than 10 μg L^{-1} bromate contamination, with a bromate range between 0.1 – 40 μg L^{-1} (von Gunten, 2003). However, it should be noted that dosages required for *C.parvum* inactivation were not used at the time of the study, and with unfavourable water quality conditions and ineffective bromate formation minimisation techniques, bromate concentrations in finished potable waters have been documented to rise as high as 60 μg L^{-1} (Kirisits and Snoeyink, 1999).

2.5.2 Feedstock contamination

Although formation during ozonation of potable water has provided the main impetus for research into bromate minimisation, another factor which can introduce bromate into a water supply is usage of contaminated feedstock. The major feedstock implicated in this process is sodium hypochlorite, either in commercially available products or on-site electrolytically generated stocks (Drinking Water Inspectorate, 1998). Contamination of feedstock with bromide can occur during the production of hypochlorite solutions from natural salt deposits (US EPA, 2003). Bromide reacts with the hypochlorite solution to form OBr^-, which can subsequently disproportionate during storage to produce bromate (Bolyard *et al.*, 1992). In a study of 40 US water treatment plants, hypochlorite feedstocks were found to contain 141 – 326 mg bromate per gram free chlorine, which contributed up to 3 μg L^{-1} bromate in treated water (Weinberg *et al.*, 2003). A comparable investigation also found bromate within hypochlorite stocks, in the concentration range 82 – 813 mg L^{-1}, at three French water treatment works (Bouland *et al.*, 2001)..

Other feedstocks can also lead to bromate contamination in the finished product. Some used in manufacture of processed food and drink products can include quantities of bromide as an impurity which, if the product is subsequently ozonated may lead to bromate production. Calcium chloride contaminated with bromide was recently responsible for the recall of 500,000 bottles of purified water which, following ozonation led to bromate concentrations of 10 – 22 μg L^{-1} in the finished product (BBC, 2004; Coca-Cola Company, 2004).

2.5.3 Bromate minimisation strategies

There are two broad strategies available for controlling bromate concentrations in treated water supplies. Minimisation strategies aim to provide conditions non-conducive to bromate formation during the ozonation process, whereas remediation strategies are applied after ozonation to remove any bromate formed. Methods for remediation of bromate-contaminated water could feasibly be applied to groundwater treatment and are discussed in Section 2.7. Minimisation strategies are not suitable for groundwater remediation as the bromate contamination is already present prior to treatment. However, they are still an important weapon for bromate minimisation in drinking water. The techniques available for bromate minimisation can be summarised as follows (Adapted from Kruithof and Meijers, 1995):

- Bromide concentration
- Ozone dosage and contact time
- Water temperature
- NOM content
- Hydrogen peroxide concentration
- Hydroxyl radical scavenger concentration
- HOBr/Ammonia concentration
- pH

Bromide concentration in influent water is a function of the situation from which the water was drawn, and as such cannot be controlled. However, as bromide is the root

cause of bromate formation and lower influent bromide leads to lower bromate levels (Song *et al.,* 1996a), it has been suggested bromide control could be utilised prior to ozonation. Kruithof and Meijers (1995) used a surface water source pre-treated by reverse osmosis (RO) and showed that, at a pH of 6.1 – 6.9 no bromate was formed even with a CT dose of 20 mg L^{-1} min, although there was formation observed at pH 8. The authors suggested RO restricted bromate formation 'significantly', although the magnitude of the pH effect compared to that of RO was not quantified. Prados-Ramirez *et al.* (1995) examined microfiltration (0.2 μm) and nanofiltration of river water and concluded that, whilst microfiltration did not remove bromide ions, nanofiltration was successful in reducing bromide concentrations by 63%. However, it is unlikely bromide control could ever become a cost-effective option unless these expensive processes were already part of the treatment stream and, as stated by Mallevialle (1995), there would also then be less requirement for disinfection if they were in place.

Ozone dosage and **contact time** are important factors in determining final bromate concentration. Song *et al.* (1996a) investigated the effect of ozone doses ranging from 1.5 – 6.0 mg L^{-1} and contact times from 1 – 30 minutes in a batch reactor using water with identical quality characteristics including a high bromide content of 400 μg L^{-1}. After a contact time of 5 minutes, an ozone dose of 1.5 mg L^{-1} led to formation of approximately 10 μg L^{-1} bromate, whereas the 6.0 mg L^{-1} dose produced 100 μg L^{-1} bromate. With a contact time of 30 minutes, the difference was even more marked, with little further formation at the 1.5 mg L^{-1} dose but almost 200 μg L^{-1} bromate formation with 6.0 mg L^{-1} ozone. Manassis and Constantinos (2003) also concluded ozone dose was related to bromate production, with a linear relationship elucidated over an ozone dose range of 0.1 – 0.25 mg L^{-1} using bottled water as a matrix. However, applied ozone dose is heavily dependent on process aims such as the level of disinfection required. Therefore it is unlikely that ozone dosage or contact times could be utilised in a specific bromate reduction strategy, and instead should be optimised to give the lowest and most cost-effective dose in the shortest contact time, which in turn will also minimise bromate production.

Water temperature has been shown to affect bromate formation for a given ozone dose, with Kruithof and Meijers (1995) tentatively suggesting bromate formation reduces at a temperature of 5°C, and Song *et al.* (1996a) showing a slight increase in bromate production from 20°C to 30°C. Croué *et al.* (1996) observed bromate concentration increase by a factor of 1.1 when increasing temperature from 20°C to 30°C. However, at higher temperatures inactivation of microorganisms and rate of ozone decomposition both increase (Song *et al.*, 1996a; von Gunten, 2003), which may negate some of these effects under realistic operating conditions. Therefore temperature control is not thought to be a viable formation control strategy.

NOM is a complex mixture of organic matter, mostly derived from plant decay, and is present in all water sources (Nissinen *et al.*, 2001). During ozonation, NOM is able to compete for oxidation by both ozone and HO·, and can also react with aqueous bromine (OBr^-/$HOBr^-$). This latter reaction can either form brominated DBPs, or can result in reduction of the aqueous bromine back to Br^- (Westerhoff *et al.*, 1998) These mechanisms act to reduce available oxidant and aqueous bromine respectively, and therefore bromate production can be expected to reduce in the presence of NOM. Westerhoff *et al.* (1998) achieved a reduction from 3 μM to 0.2 μM bromate by addition of a NOM isolate to water at an ozone dose of 100 μM. Song *et al.* (1996a) noted a decrease in bromate formation from 280 μg L^{-1} to 40 μg L^{-1} with dissolved organic carbon (DOC) concentrations of 6 mg L^{-1} and 1.5 mg L^{-1} respectively at an ozone dose of 6 mg L^{-1}, and Kruithof and Meijers (1995) observed that river water with a DOC content of 2 mg L^{-1} required a lower ozone dose (1.4 mg L^{-1}) for bromate to be detected than a sample with 4 mg L^{-1} DOC (2.8 mg L^{-1} ozone dose). However the nature and composition of NOM, which is dependent on water source, is critical to its effect on bromate formation. This, plus formation of other brominated DBPs in the presence of NOM, means it is unlikely a bromate control methodology can be directly mediated by manipulation of NOM concentration.

Addition of **hydrogen peroxide** (H_2O_2) accelerates ozone decay, reacts with aqueous bromine to regenerate bromide, and increases OH· production. Conflicting results have been obtained, with Kruifhof and Meijers (1995), Lefebvre and Deguin

(1997) and Song *et al.* (1996a) suggesting H_2O_2 addition reduces bromate formation and Croué *et al.* (1996) and von Gunten *et al.* (1995) concluding bromate formation increased with H_2O_2 dose. Song *et al.* (1997) suggested these mixed results can be explained by differential effects on the three pathways, with formation via the direct pathway inhibited due to removal of aqueous bromine, but the other two pathways enhanced by increased OH· levels. In addition, Mallevialle (1995) pointed out that, if the aim was a consistent ozone residual use of H_2O_2 would increase dose required and thus bromate production. Therefore, H_2O_2 addition is not a viable control strategy.

OH· scavengers could be hypothesised to reduce bromate production due to the close involvement of OH· in both indirect formation pathways. Song *et al.* (1997) noted that addition of the OH· scavenger tert-butanol produced a 90% decrease in bromate formation, although actual concentrations were not given. Von Gunten and Hoigne (1994) also found a reduction of 40% in bromate formation with tert-butanol addition, and Krasner *et al.* (1993) observed a 100% reduction with the use of isopropanol. However, despite these results, in a later paper Pinkernell and von Gunten (2001) concluded that requirements for the necessary concentrations of a suitable compound to act as an OH· scavenger would be 'beyond the possibilities of drinking water treatment' and therefore unfeasible.

As can be seen from Figure 2.1, **hypobromous acid** is a key intermediate in both the direct and direct-indirect formation pathways, where Br^- is oxidised to HOBr. This is in equilibrium with OBr^-, with equilibrium on the side of HOBr under typical drinking water treatment conditions (von Gunten, 2003). By removing HOBr, less OBr^- would be available for oxidation via the direct pathway, and the total aqueous bromine would be reduced thus suppressing bromate formation by reactions of the direct-indirect system. Pinkernell and von Gunten (2001) studied possibilities for use of an organic HOBr scavenger (examples included 2-butanone, cyclohexanone, malonic acid and acetic acid) and concluded that, although HOBr is a suitably long-lived intermediate, reaction rates under realistic operating conditions would not be high enough for a feasible process to be developed. However, the use of inorganic **ammonia** to quench HOBr has been trialled, and some encouraging results have been reported. Ammonia, added in the form of

ammonium (NH_4^+), produces an equilibrium reaction with HOBr to form bromamine species (Equation 2.2) (Pinkernell and von Gunten, 2001), which can then be further oxidised by ozone back to Br^- (Equation 2.3). Suppression of bromate formation has been noted, with Song *et al.* (1996a) observing a reduction from 130 μg L^{-1} bromate to 25 μg L^{-1} at a 5 mg L^{-1} ozone dose with addition of 700 μg L^{-1} ammonia (as NH_3-N), and von Gunten (2003) reporting a decrease from 8.5 to 4 μg L^{-1} bromate with 200 μg L^{-1} ammonium addition and a 1.5 mg L^{-1} ozone dose, although higher dosages were found not to further improve reduction of formation. Other reported results range from 50 – 65% reduction in bromate formation with ammonia addition (summary in Song *et al.*, 1997), although another report concludes results range from only 0 – 30% reduction and are not consistent (Gordon *et al.*, 2002). It has also been suggested that the presence of ammonia only delays and does not prevent bromate formation (Mallevialle, 1995), and that results are strongly dependent on NOM source and Br^- concentration (Song *et al.*, 1997). However, despite these shortcomings the weight of evidence suggests ammonia addition can reduce bromate formation and can also lower concentrations of brominated DBPs by removing the HOBr precursor. Ammonia addition may therefore be a useful bromate minimisation technique in waters containing low concentrations of natural ammonia (von Gunten, 2003).

$$HOBr + NH_3 \Leftrightarrow NH_2Br + H_2O$$ (Pinkernell and von Gunten, 2001)

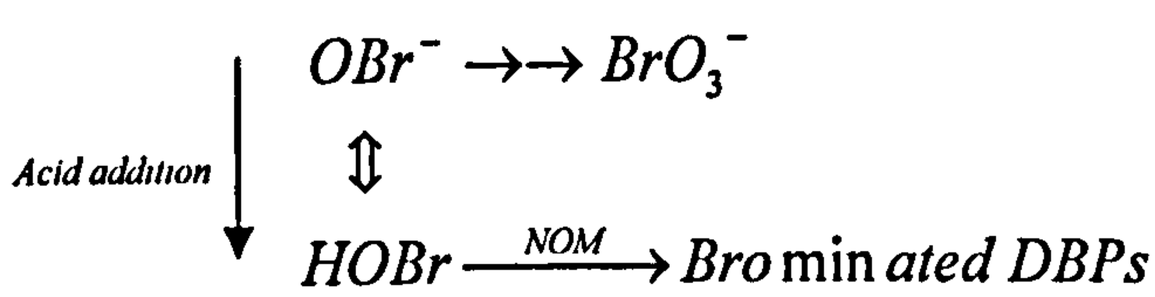
Equation 2.2

$$NH_2Br + 2O_3 \rightarrow 2H^+ + NO_3^- + Br^- + 3O_2$$ (Song *et al.*, 1996a)

Equation 2.3

pH control is another technique which has been widely trialled. pH depression by acid (ie. H_2SO_4) addition leads to two effects on bromate ozonation chemistry. The equilibrium of the two aqueous bromine species is shifted with decreasing pH from the more reactive OBr^- species, instrumental in bromate formation, to HOBr (Grosvenor, 1999). This reduces bromate formation via ozone oxidation of OBr^- (Equation 2.4). However, the direct-indirect formation pathway is independent of OBr^- so, as with

ammonia addition, acid addition will have no effect on this pathway. The more important mechanism by which pH depression reduces bromate formation is due to increased ozone stability, leading to less OH· production and a reduced overall oxidant exposure (ozone and OH·) for a given disinfection target (Kruithof and Meijers, 1995; von Gunten, 2003). Song *et al.* (1996a) reduced bromate formation from 215 μg L^{-1} to 60 μg L^{-1} with a pH reduction from 8.5 to 6.5 at a 6 mg L^{-1} ozone dose. With pH values of 8.0 and 6.0, Pinkernell and von Gunten (2001) produced 10 μg L^{-1} and 4 μg L^{-1} bromate respectively with an ozone exposure of 10 mg L^{-1} min. Song *et al.* (1997) concluded pH depression of one unit would lead to an average 50% reduction in bromate formation. However, Croué *et al.* (1996) reported no 'significant' bromate reduction with a contact time of less than 10 minutes between pH 8.4 and 6.4, and the shift of equilibrium towards HOBr does tend to increase production of brominated DBPs (Song *et al.*, 1996a; Song *et al.*, 1997; Grosvenor, 1999) as can be seen in Equation 2.4 (Adapted from Pinkernell and von Gunten, 2001). However, pH depression is thought to be an effective and cost-effective bromate minimisation strategy for low alkalinity waters (von Gunten, 2003).



Equation 2.4

The most promising options for minimisation of bromate formation were suggested by von Gunten (2003) to be ammonia addition and pH depression. Both were concluded to be feasible for waters containing bromide levels between 50 – 150 μg L^{-1}, where a 50% decrease of bromate formation may be reasonably expected to occur. However, ozonation of water containing bromide in excess of these levels would lead to formation of bromate at concentrations above 10 μg L^{-1} even in the presence of control measures, and blending with uncontaminated supplies or subsequent removal would then be the only effective options available.

2.6 BROMATE GROUNDWATER CONTAMINATION

Bromate at low concentrations (≤ 60 μg L^{-1}) has become researched in recent years due to its' formation during ozonation. Much of this work has concentrated on developing the minimisation techniques outlined in Section 2.5, with investigation also undertaken into removal of low-level contamination from potable water. However, there has been little input into environmental aspects of bromate, so published literature on the subject is sparse. Bromate is not traditionally known as a contaminant of either marine waters (Richardson *et al.*, 1981), surface water sources (Hutchinson *et al.*, 1997) or aquifers (World Health Organisation, 1993; US EPA, 2001). Even so, despite the lack of historical evidence for bromate within natural water sources, more recent investigations have shown increasingly widespread low-level bromate contamination within these matrices. One study showed that bromate can now be found in surface waters such as the river Rhine at levels between 4 – 8 μg L^{-1} (Versteegh *et al.*, 1993; Cited in Hijnen *et al.*, 1999). In addition, bromate contamination has now been detected within groundwater in the UK. Historical spillage from a chemical production plant has led to groundwater contamination in a chalk aquifer, with bromate concentration in areas of the plume exceeding 2 mg L^{-1}.

2.6.1 Background to bromate groundwater contamination incident

The manufacture of bromine compounds, as with most other industrially-important chemicals, is now carried out at a limited number of large plants due to economies of scale and the need for rigorous environmental controls to eliminate spillages and other environmental contamination. However, during the post-WWII years, many chemicals were still produced by small businesses, with environmental concerns rarely considered. One example of this was at the contaminated project site in the South of England which, in 1955 was purchased as a business manufacturing 'fine chemicals known as industrial and pharmaceutical intermediates'. These included alkyl bromides, aromatic bromo-compounds, hydrobromic acid, 4-7-dichloroquinoline, and potassium bromate (Mott Macdonald, 2000a). Site operation continued for a number of years until at least 1978, before being abandoned and later redeveloped.

The incidence of contamination by bromine compounds at and around the site was first discovered during sampling prior to redevelopment, which led to the surface layer (depth of 0.75 – 1.5 m) being removed and replaced (Environment Agency, 2001). At the time bromate was not considered a contaminant, so bromate contamination of either soil or groundwater was not considered.

Following development of more sensitive analytical techniques, coupled with drafting of the new Water Supply (Water Quality) Regulations 2000, groundwater monitoring was undertaken in the area. During method testing in 1999, bromate levels of 15 – 140 μg L^{-1} were identified at local abstraction boreholes. As these concentrations were in excess of the then-proposed Regulations' value of 10 μg L^{-1} bromate for potable supply, abstraction was halted. This situation is still ongoing. Bromide groundwater concentrations are also elevated at the boreholes, with concentrations of 160 – 850 μg L^{-1} recorded during January 2005. Following extensive sampling in the area by the Environment Agency (EA), the source of bromate contamination was traced back to the project site, and a substantial bromate/bromide plume delineated. A schematic of the overall plume shape is given in Figure 2.2.

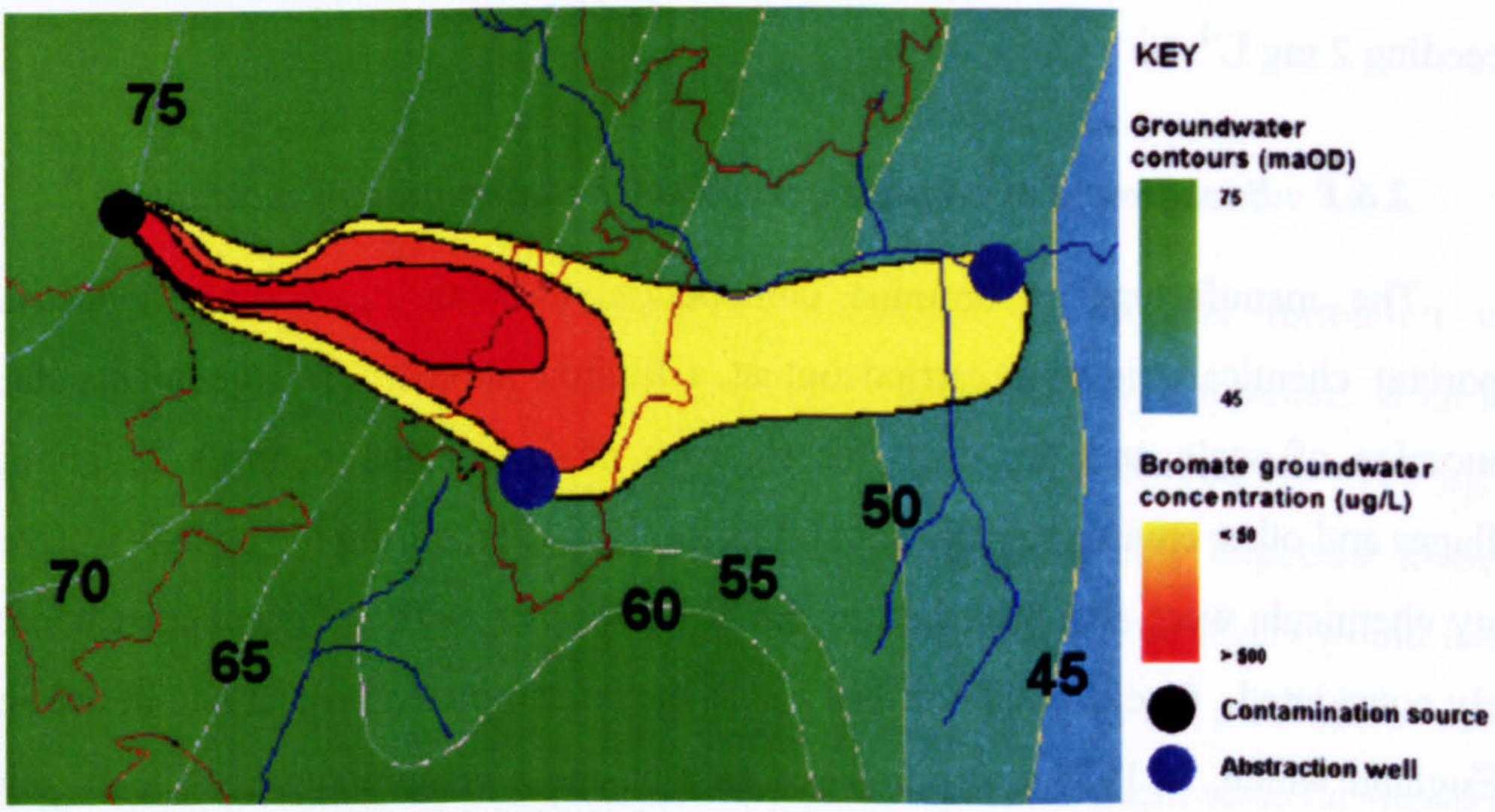


Figure 2.2 - Schematic diagram of overall bromate contamination plume shape, with contamination source and local abstraction boreholes also indicated (data from Veolia Water)

Boreholes sampled varied in depth from under 25 metres to over 100 metres.

They showed that bromate contamination at the source site is uneven, ranging from low groundwater and soil concentrations (< 0.0005 – 3.16 mg L^{-1} and < 0.1 – 0.32 mg kg^{-1} respectively) to significantly higher contamination values (11.7-152 mg L^{-1} and 25.4 – 273 mg kg^{-1} for groundwater and soil respectively) in one area of the site (Environment Agency, 2001). Bromide contamination plots show similar elevated but uneven distribution over the site. Due to the uneven distribution of borehole results, it was not possible to calculate an accurate plume depth within the aquifer.

2.6.2 Contaminated Land legislation in England

Under part IIA of the Environmental Protection Act 1990, which came into force on 1 April 2000, a site can be formally designated as 'Contaminated Land' by the relevant Local Authority if it meets certain legal criteria. The statutory definition of Contaminated Land as set out in the Environmental Protection Act 1990 S78A(2) is stated as:

"Land that appears to the local authority in whose area it is situated to be in such a condition, by reasons of substances in, on or under the land, that – (a) significant harm is being caused or there is a significant possibility of such harm being caused; or (b) pollution of controlled waters is being, or is likely to be, caused"

(Environment Agency, 2002b)

To demonstrate the possibility of harm or pollution, a 'significant pollution linkage' (SPL) must be established, whereby all the following elements must be present:

- A contaminant in the soil or unsaturated zone
- A pathway by which the contaminant can be transmitted from the soil/unsaturated zone to the aquifer or a potable supply borehole, now or in the future
- The presence of the contaminant in the aquifer or potable supply…at a specified concentration which constitutes 'pollution'.

(Environment Agency, 2002c)

In the case of the contaminated site, four SPL's were identified, covering both bromate and bromide contamination, and the site was designated Contaminated Land in June 2002.

Additional legislation, under Regulation 3(a) of the Contaminated Land (England) Regulations 2000 allowed further designation as a 'Special Site', which identifies land affecting waters "which are, or are intended to be, used for the supply of drinking water for human consumption" (Environment Agency, 2002a). This area was only the twelfth site to be given such a designation, and allowed site management to be transferred from the local authority to the Environment Agency.

The designation of land under the Environmental Protection Act 1990 has allowed legal steps to be undertaken to identify 'Appropriate Persons', who are "responsible in law for any ongoing or likely pollution or for any 'significant harm' caused" (Environment Agency, 2002b). A 'Remediation notice' can then be served, which requires those identified to undertake, and pay for 'reasonable' measures to mitigate the pollution threat.

2.6.3 Source reduction of the contaminated aquifer

Following identification of the contamination incident and delineation of the plume, regular monitoring has been undertaken. Identification of 'Appropriate Persons' and serving of 'Remediation Notices' is ongoing. However, the complexity of the situation has led to significant delays in this process. 'Contaminated Land' designations only apply to the actual site and not the contamination plume, and there is currently no clear legislation as to responsibility for plume cleanup. The construction of houses on the contaminated site, whilst not posing a direct threat to residents' health, now prevents a simple site remediation operation and also involves all households in the incident as potential 'Appropriate Persons'. In addition, no cleanup can be attempted until an 'Appropriate Person' has accepted responsibility for the contamination and its' cleanup. Therefore, no source reduction strategy has to date been trialled either at the contaminated site or within the surrounding aquifer.

2.7 WATER TREATMENT AND REMEDIATION

Bromate is thermodynamically a powerful oxidant, which should be able to react readily with organic matter to form the bromide ion (World Health Organisation, 2005). In aqueous solution bromate may be expected to decompose according to either Equation 2.5, or Equation 2.6 at acidic pH in the presence of Br^- (Bourgine *et al.*, 1993).

$$2BrO_3^- \rightarrow 2Br^- + 3O_2$$

Equation 2.5

$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_2O$$

Equation 2.6

Both these reactions can proceed in the laboratory when the concentration of bromate is relatively high. Bromate has also been suggested to degrade abiotically within air-dried soil by up to 64% in 14 days under both aerobic and anaerobic conditions (Rodgers, 1980). However, reaction rates in aqueous solution do not appear significant in a natural context (Lopez-Cueto *et al.*, 2001). Studies have shown that, once in solution bromate is highly stable at room temperature, does not volatilise and is only slightly adsorbed onto soil or sediment (Depository Services Program (Canada), 1999; World Health Organisation, 2005). A recent study on degradation in bromate-contaminated river water also concluded natural removal did not occur at a temperature of 25°C (Fielding, 2002).

Within the contaminated aquifer, although overall plume bromate:bromide ratio increases with distance from the source, it was concluded this was likely to be due to dilution with 'clean' water containing natural bromide (Mott Macdonald, 2000b) and not bromate degradation to bromide. There are thought are thought to be few naturally occurring compounds within the contaminated aquifer available for bromate to oxidise (Mott Macdonald, 2000a), leading to the assumption bromate in the contamination plume is relatively stable. There is anecdotal evidence that pockets of biological bromate degradation exist within the aquifer, and this possibility is further explored during the

project. The only evidence of natural bromate degradation comes from a study on contaminated discharge impacting on a nearby river system (Vivendi Water, 2002). A downstream decline of bromate was observed, not all of which could be attributed to dilution. However, there were acknowledged 'inconsistencies in data' within this preliminary report, which also noted the decline could be due to loss of bromate-loaded water to the underlying aquifer. Therefore, bromate can be assumed to be conservative within aqueous solution, and remediation methodologies are required to remove contamination, whether within treated potable water or untreated groundwater samples.

Studies into remediation of drinking water contaminated with bromate following ozonation have been ongoing for over a decade (e.g. Marhaba and Medlar, 1993; Westerhoff *et al.,* 1994), with a range of strategies identified and trialled. These can be broadly grouped into four categories:

- Physical and electrical techniques
- Chemical techniques
- Bromate bioremediation
- Techniques utilizing activated carbon

All four approaches and their efficacy in removal of predominantly post-ozonation concentrations of bromate are detailed in this section. Potential mechanisms of bromate bioremediation are then outlined in more depth within the following section (Section 2.8). All remediation techniques developed to date are also summarised and compared in Table 2.2.

2.7.1 Physical and Electrical techniques

Filtration techniques can be applied for bromate removal by using membranes to separate higher molecular weight compounds from bulk water. Marhaba and Medlar (1993) tested nanofiltration at 40 and 75 psi, and hyperfiltration at 115 psi. From an initial bromate level of up to 285 μg L^{-1}, 75 – 100% removal was obtained for nanofiltration, and an average of 97% for hyperfiltration was achieved. It was concluded

that nanofiltration was more cost-effective due to lower water pressures, which was later confirmed by Prados-Ramirez *et al.* (1995) who achieved a removal rate of 77% from an initial bromate concentration of 300 μg L^{-1}. Reverse osmosis (Prados-Ramirez *et al.*, 1995) has also been suggested, although little published research has been carried out to date. However, there are major disadvantages related to these physical techniques. The resultant water stream is effectively de-ionised and requires re-ionisation, and a concentrated waste stream is produced, which would need remediation prior to disposal. Unless removed prior to filtration, NOM can lead to membrane fouling and associated efficiency losses (Grosvenor, 1999) Finally the cost for bromate treatment alone is high, and it is unlikely that membrane filtration would be cost-effective without significant process integration (Mallevialle, 1995).

Ion exchange is the process of exchanging certain anions or cations within a water matrix, with ions such as sodium or hydrogen held in a resinous ion exchange material (Vigneswaran and Viswanathan, 1995). It has been a well-known technique for removing the oxyanion nitrate (NO_3^-) from potable water supplies for many years, being used in full-scale plants since the 1980's (Richard, 1989). Nitrate is exchanged for chloride (Cl^-) ions by the resin, which is subsequently either regenerated using a sodium chloride solution (Hall and Croll, 1993), or alternatively disposed to landfill. Bromate removal by ion exchange has not been reported in the published literature, but bromide removal as a bromate minimisation strategy was trialled. Bromide removal by a Magnetic Ion Exchange Resin (MIEX®) was dependent on both alkalinity and bromide influent concentrations in one study, with up to 59% bromide removal at a MIEX® dose of 8 mL L^{-1} and initial 200 μgL^{-1} bromide spike in a raw water source (alkalinity 150 mgL^{-1} as $CaCO_3$). In this case, pre-treatment of water with MIEX® as a bromate minimisation strategy prior to ozonation for potable use was found to reduce bromate formation from 20 μgL^{-1} to 1 μgL^{-1} with a dissolved ozone concentration of 0.35 mgL^{-1} (Johnson and Singer, 2004). It is possible, though as yet unproven, that ion exchange resins such as MIEX® would have a similar effect on bromate contamination.

Ultraviolet (UV) irradiation (wavelength 100 to 400 nm) is widely used for water disinfection (Droste, 1997). The wavelengths 180 – 300 nm also provide enough

energy for bromate decomposition to BrO_2^- and subsequently Br^- (Siddiqui *et al.*, 1996c) . A preliminary study using batch reactors and 50 – 100 μg L^{-1} bromate, concluded irradiation by a low pressure mercury lamp (180 – 254 nm output) led to 3 – 38% removal (Siddiqui *et al.*, 1994b). A 50-times more powerful 200 – 300 nm medium pressure lamp was also investigated (Siddiqui and Amy, 1994) and, in conjunction with continuous flow reactors both these and low pressure lamps (Siddiqui *et al.*, 1996c) were shown to have advantages, in reduced contact time (5 – 20 sec as opposed to 30 sec) and energy-efficiency respectively. However, efficiency is heavily dependent on wavelength and pressure (Grosvenor, 1999), and the high-power low-wavelength lamps required to significantly improve reduction rates above those already achieved may prove not to be cost-effective.

Electrocatalysis uses electrodes modified by treatments such as addition of a molybdenum oxide film (Bertotti and Pletcher, 1996) or a polybasic lanthanide heteropoly tungstate/molybdate complex (Dong *et al.*, 1998). However, no research has to date been undertaken for water remediation using this technique, with both studies focussing on developing sensors for the determination of bromate. The use of **redox catalysis** has been investigated using the catalyst ruthenium-Adams, a form of ruthenium(IV)oxide. Decomposition of bromate to bromide in the presence of the catalyst was prohibitively slow, although increases of 24 – 34 times were noted in the presence of more easily oxidised organic species (Mills and Meadows, 1995).

Photocatalytic decomposition of bromate can be achieved by coupling UV irradiation with a titanium dioxide (TiO_2) catalyst, generating an electron-hole pair (e^-h) capable of reducing bromate to bromide at the semiconductor surface. TiO_2 is widely used in photocatalysis, being biologically and chemically inert, photoactive, stable and inexpensive (Mills *et al.*, 1995). Batch and continuous flow trials with 254 nm UV radiation and platinised TiO_2 used as a dispersion (batch) or coating (continuous flow) concluded bromate reduction was enhanced over UV irradiation alone by 4 – 5 and 4.2 times respectively, with overall disinfection also enhanced (Mills *et al.*, 1995). Long reaction times (15.2 mins and 43 mins respectively) and ionic competition meant the technique was impractical (Mills *et al.*, 1996) and, although recent studies have enhanced

rates by manipulating pH and surface charge (Noguchi *et al.*, 2002), significant further improvement is required prior to commercial use (Noguchi *et al.*, 2003).

Arc discharges are created by discharging a capacitor at 10 – 50 kilovolts, 10 – 100 amps and a pulse rate between 50 – 100 pulses per second across the water flow. This produces an intense, localised high-temperature plasma (10,000 – 50,000 Kelvin), which rapidly expands and contracts, causing shock waves and a burst of UV radiation (Siddiqui *et al.*, 1996a). The UV irradiation reduces bromate, although free radicals and hydrogen atoms produced by the plasma mediate the major reduction processes. **High Energy Electron Beams** (HEEB) produce ionising radiation which induces formation of reducing species including aqueous electrons, hydrogen atoms and hydroxyl radicals (Siddiqui *et al.*, 1996b). Arc discharges and HEEB were found to provide destruction efficiencies of 12 – 45% and 70% respectively, from an initial 100 μg L^{-1} bromate concentration. Destruction efficiency was reduced by electron scavengers (HEEB), water temperature (arc discharge) and DOC (both techniques) (Siddiqui *et al.*, 1996a; Siddiqui *et al.*, 1996b). Arc discharge uses less energy than UV irradiation, but is less efficient (Siddiqui *et al.*, 1996a). HEEB is an effective technique, but high capital costs and presence of contaminants such as nitrate which increase dosage required currently make it economically unfeasible.

2.7.2 Chemical techniques

Coagulants including alum (Al^{3+}) and ferric chloride (Fe^{3+}) were evaluated for adsorption ability, but bromate removal rates were only 5% and 20%, respectively, and it was concluded that coagulating agents were unable to significantly reduce bromate concentrations in natural waters (Hossain *et al.*, 1996; Siddiqui *et al.*, 1994a).

Reducing agents include sulphur compounds such as thiosulphate, sulphite and sulphur dioxide. Sulphite addition induced a bromate removal rate of 16 – 63% at pH 5 – 9.5 and an initial bromate concentration of 7.6 mg L^{-1}. The removal rate increased with pH, with a neutral pH leading to approximately 50% removal (Prados-Ramirez *et al.*, 1995). A subsequent sulphite trial concluded that, although 99% bromate removal could be achieved with initial contamination levels of 0.1 mg L^{-1} at pH 5.5 – 7, reaction time at

pH 7 under drinking water conditions was up to 4 days (Gordon *et al.*, 2002). The authors therefore concluded ferrous iron (Fe^{2+}) was the more promising reducing agent. Fe^{2+} reduces bromate to bromide and oxidises into the ferric form (Fe^{3+}), with any remaining Fe^{2+} oxidised by dissolved ozone or oxygen (Equations 2.7 – 2.9) (Siddiqui *et al.*, 1994a).

$$BrO_3^- + 6Fe^{2+} + 6H^+ \Leftrightarrow Br^- + 6Fe^{3+} + 3H_2O$$

Equation 2.7

$$4Fe^{2+} + O_2 + 4H^+ \Rightarrow 4Fe^{3+} + 2H_2O$$

Equation 2.8

$$Fe^{3+} + 3HCO_3^- \Rightarrow Fe(OH)_{3(s)} + 3CO_2$$

Equation 2.9

Preliminary batch studies using natural water showed the majority of bromate reduction occurred within 10 minutes, and equilibrium was reached after 15 minutes (Siddiqui *et al.*, 1994a; Siddiqui *et al.*, 1994b). Reduction was pH dependent, with higher rates at lower pH (6.5 as opposed to 8.5) and dissolved oxygen (DO) levels. Results indicated bromate reduction was possible within a flocculation basin with a hydraulic residence time of 30 minutes (Siddiqui *et al.*, 1994b). At pilot scale, ferrous chloride ($FeCl_2$) addition (11 and 23 mg L^{-1}) was initially shown to produce unacceptable turbidity, so 14.5 mg L^{-1} ferric chloride ($FeCl_3$) was also added to improve effluent turbidity. Reduction rates at various Fe^{2+} concentrations in 30-minute and 2-hour contact time flocculation basins varied from 4 – 41% and 36 – 70% respectively, with temperature confirmed as a rate-determining factor in the range 14 – 19°C (Krasner *et al.*, 1996). Hossain *et al.* (1996) achieved a 'significant reduction' of bromate with 15 mg L^{-1} ferrous chloride, and a study into use of 'pickling Fe' (a by-product of the steel industry) led to removal of 100 µg L^{-1} bromate by 30 mg L^{-1} iron (as Fe^{2+}) in only 2 minutes at pH 8 (Gordon *et al.*, 2002). Unusually, reduction rate in the artificial matrix used was directly proportional to pH, but 99% removal was achieved with a contact time of 2 – 20

minutes in the pH range 7 – 8. Pickling Fe is produced when concentrated hydrochloric acid is used to clean steel sheets during manufacture, and contains principally Fe^{2+}, with a high chloride content and low Fe^{3+} levels. The residual Fe^{3+} was useful as a coagulant, although no indication was given of other contaminants, which may preclude use in a drinking water situation. A methodology for removal of residual iron was proposed by Krasner *et al.* (1996) utilising pH control to oxidise residual ferrous iron prior to removal in filters. However, despite effectiveness of the system, the issue of cost-effectively removing residual iron from the water stream has not been adequately addressed and, given a UK limit of 200 μg L^{-1} iron in drinking water (DWI, 2000) this problem must be resolved prior to development of a viable full-scale technique.

Zero-valent iron (Fe^0) was first proposed as a remediation methodology for nitrate in the early 1990s. Westerhoff (2003) recently investigated reduction of nitrate, bromate and chlorate using seven sources of Fe^0 at laboratory scale in batch and column tests. Bromate was reduced at a faster rate than either nitrate or chlorate, with approximately 90% of 1.13 g L^{-1} bromate reduced to bromide with an contact time of 20 minutes during column trials, compared with only 10% for nitrate. Fe^0 source was determined as a critical factor in overall reduction rate, but mechanisms leading to these differences were not further investigated. However, use of spiked ultrapure water in the study and lack of other competing groundwater constituents means it is hard to relate these results to a groundwater situation. Other factors including longevity of effectiveness, formation of by-products such as dissolved iron species, and rise in pH due to proton consumption must also be considered prior to trialling this technique at a larger scale (Westerhoff, 2003).

2.7.3 Biological techniques

Bioremediation utilising bacteria is used to degrade many organic compounds including creosote and explosives (Ritter and Scarborough, 1995), and some inorganic species such as manganese (Bernoth *et al.*, 2000), iodate (Rodgers, 1980), nitrate (Smith *et al.*, 2001) and perchlorate (Xu *et al.*, 2003). Bromate bioremediation has not been widely studied to date and there is currently a paucity of literature available on the

subject. However, two preliminary trials confirmed bromate can be degraded to bromide by denitrifying bacteria (Hijnen *et al.*, 1995; Hijnen *et al.*, 1999) supplemented with ethanol as electron donor. Investigations into bromate reduction using Biological Activated Carbon (BAC) have also indicated microbial bromate reduction occurs, even within a mixed bacterial population in the presence of both oxygen and nitrate (Kirisits and Snoeyink, 1999; Kirisits *et al.*, 2001). Microbially-mediated bromate reduction has since been confirmed in recent trials utilising acclimatised activated sludge biomass in an actetate-fed laboratory-scale continuous-flow column (van Ginkel *et al.*, 2005b) and also an autotrophic hydrogen gas-lift bioreactor (van Ginkel *et al.*, 2005a). The acetate-fed continuous-flow column, with an artificial medium influent supply, reduced a bromate influent of 294.4 mg L^{-1} with a retention time (RT) of 48 hours after an acclimatisation period of 14 days (van Ginkel *et al.*, 2005b). Autotrophic laboratory- and pilot-scale gas lift bioreactors were operated with an artificial medium and incinerator effluent respectively (van Ginkel *et al.*, 2005a). Inoculation was with activated sludge (laboratory-scale) and granular sludge from an upflow anaerobic sludge blanket (pilot-scale). Hydrogen was used as electron donor and CO_2 as carbon source. The laboratory-scale reactor removed 340 mg L^{-1} bromate within a 6-hour RT after an acclimatisation phase of around 40 days, and in excess of 90% of 200 mg L^{-1} influent bromate was removed at a similar RT in the pilot-scale reactor. These trials are further discussed in relation to current studies during later sections.

Membrane bioreactors combining membrane technology with biological reactors have been used commercially in wastewater treatment for over 20 years (Stephenson *et al.*, 2001). Membrane bioreactors have also been used in denitrification systems in various configurations (Nerenberg *et al.*, 2002). The development of hollow-fibre membrane biofilm reactors (HFMBfR) for biological nitrate and perchlorate reduction also shows promise for bromate removal. An HFMBfR uses hollow membrane fibres, through which the supplied pressurised hydrogen gas diffuses, encouraging development of an autotrophic biofilm utilising and thus reducing oxidised contaminants. A pilot-scale system currently operating for nitrate and perchlorate reduction is proving cost-effective, and preliminary results suggest 95% bromate removal can also be achieved (Nerenberg and Rittmann, 2004).

2.7.4 Activated Carbon techniques

The **activated carbon** technique is a hybrid process involving physical adsorption of the contaminant followed by chemical (abiotic) reduction. Biological reduction can also be mediated on the carbon surface if a biofilm is allowed to develop, in which case it is termed BAC. Activated carbon in granular (GAC) or powdered (PAC) form is an adsorbant with a highly developed porous structure (Chen *et al.*, 2002), which is utilised in a range of applications including mixture separation, liquid purification and gaseous control emissions (Rio *et al.*, 2004). It has a large internal surface area (600 – 1,600 $m^2\ g^{-1}$ for most GAC) amenable to adsorption, and can be produced from almost any carbonaceous material (i.e., wood, coal, coconut shells) by anoxic carbonisation (Droste, 1997). Activated carbon had previously proved effective in removing inorganic disinfection by-products such as chlorite and chlorate (Siddiqui *et al.*, 1996d), and a preliminary study on bromate removal at concentrations up to 300 $\mu g\ L^{-1}$ achieved almost 100% reduction on GAC for at least 32 hours (Marhaba and Medlar, 1993). Using both batch reactors with PAC, and GAC-filled continuous-flow Rapid Small-Scale Column Tests (Marhaba, 2000) to simulate a pilot plant (Crittenden *et al.*, 1989), PAC was found to be successful but slow with 63 – 99% reduction over 12 – 24 hour time frames (Westerhoff *et al.*, 1994). GAC induced the higher rate of 0.79 mg bromate removed per gram carbon for more than 7 days. However DO, DOC and competing anions including nitrate, sulphate and chloride have all been shown to reduce efficiency in both processes (Bao *et al.*, 1999; Kirisits *et al.*, 2000; Siddiqui *et al.*, 1994a; Siddiqui *et al.*, 1994b). It was concluded GAC was preferable for further studies, either as a full length column or filter cap (Westerhoff *et al.*, 1994).

Prados-Ramirez *et al.* (1995) reported fresh coconut carbon could remove 9 $mg\ L^{-1}$ bromate from distilled water for 122 hours, leading to only 33% breakthrough. However, in the presence of organic contamination, little bromate adsorption was noted. Other investigations concluded GAC-facilitated bromate removal was only possible at impractically low pH levels (Kruithof and Meijers, 1995; Meijers and Kruithof, 1995), and that reduction would not exceed 20% (Schmidt *et al.*, 1995). Subsequent studies have suggested GAC can mediate significant bromate reduction for limited time periods,

with GAC columns removing an average of 30 – 40% and 50% bromate from natural waters for two months and one month respectively (Marhaba, 2000; Siddiqui *et al.*, 1996). At pH 3 almost total bromate removal was achieved, although this decreased to 38% at pH 5.5 (Yang *et al.*, 2000).

Marhaba (2000) suggested that observed decreases in reduction capacity of GAC could be attributed to biological or biofilm growth, although no possible mechanism was outlined and the system was run under ambient oxygen conditions, which may encourage fouling by aerobic microorganisms. In a separate trial, fresh GAC was observed for 2 – 10 months, over which time bromate reduction levels decreased from 1.5 mg bromate per gram carbon to effectively zero due to bacterial colonisation. It was therefore concluded BAC was ineffective at bromate reduction (Asami *et al.*, 1999). However, 86% bromate removal was observed during a BAC filtration trial (Kirisits and Snoeyink, 1999). GAC was used as a matrix for microbial growth and biological reduction under controlled oxygen conditions (2 – 8 mg L^{-1}), and NOM was the source of electron donor with no addition of exogenous carbon. Effects of DO, nitrate concentration and empty bed contact time (EBCT) were evaluated on removal of 10 – 20 µg L^{-1} bromate in groundwater, with both nitrate and DO increases adversely affecting bromate reduction efficiency. An increase in DO concentration of 2 – 8 mg L^{-1} reduced bromate reduction from 56% to 42% at an EBCT of 50 minutes, and nitrate increase (0.3 – 5.0 mg L^{-1}) at a 25 minute EBCT decreased bromate removal from 86% to 76%. EBCT was also shown to be critical, with Figure 2.3 showing percentage removal at EBCTs from 5 – 50 minutes.

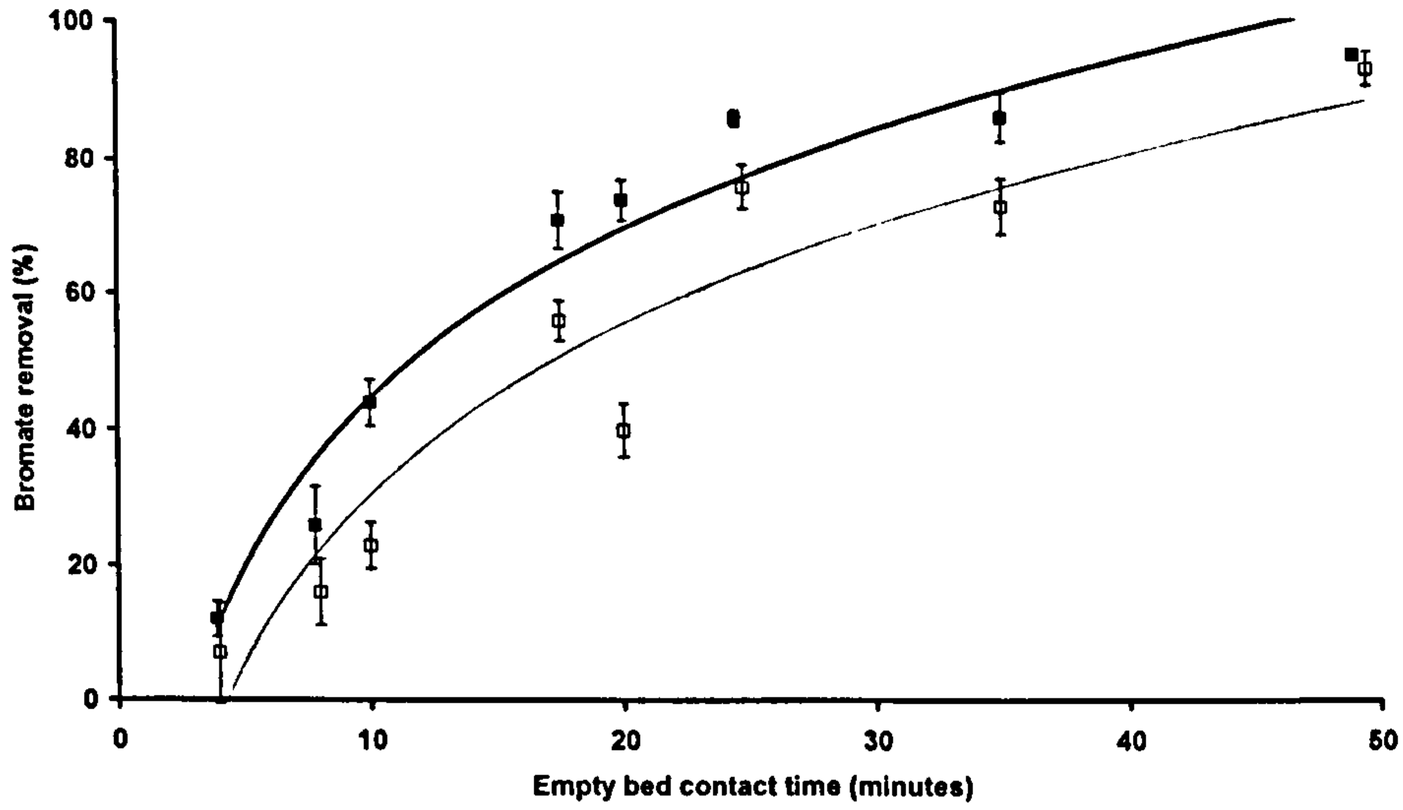


Figure 2.3 – Effect of empty bed contact time on bromate removal in a BAC filter with 0.3 mg L^{-1} (■) and 5.0 mg L^{-1} (□) influent nitrate concentrations (Kirisits and Snoeyink, 1999)

The report suggested effective bromate removal could be achieved by conversion of existing commercial GAC filters. More recent work has shown BAC filters can continuously remove 37 – 41% bromate from an initial bromate concentration of 20 μg L^{-1} for over a year under realistic BAC operating conditions, including influent DO and nitrate concentrations of 2 mg L^{-1} and 0.3 – 5 mg L^{-1} respectively (Kirisits *et al.*, 2002). Abiotic bromate reduction by the GAC was not found to be a significant process after a 45 – 50 day startup period, Therefore, whilst adsorption and abiotic reduction on GAC active sites has the potential to provide almost complete bromate removal in the short term under favourable conditions, GAC type and source water composition are critical in the longer term (Huang and Chen, 2004; Thompson and Megonnell, 2003). It has also been noted that reduction efficiency can decline rapidly using GAC, with one pilot plant receiving up to 163 μg L^{-1} bromate showing rapidly declining removal after 3 months (Huang and Chen, 2004) and Kirisits *et al.* (2000) suggesting an operational lifetime of only 30 days with a 10 μg L^{-1} bromate influent. Therefore, whilst GAC has favourable initial characteristics for low bromate concentrations, BAC can provide continuous biological reduction over a period of months. If it were possible to extrapolate BAC performance to higher bromate concentrations, it could significantly reduce bromate concentrations in highly contaminated groundwater.

2.7.5 Limitations of existing techniques

Bromate contamination in potable water has become a growing problem over the past decade due to a combination of increased use of disinfection by ozonation, improved analytical capabilities and tightening of drinking water limits. Remediation technologies have improved significantly, but few pilot or full-scale trials have currently been completed. In addition, many studies have used distilled or tap water and bromate concentrations in the range 10 – 100 μg L^{-1}. Whilst this is a reasonable approximation of post-ozonation conditions, differences will exist when applied to contaminated groundwater with bromate concentrations up to three orders of magnitude higher. Some trials have investigated bromate influent concentrations above 1 mg L^{-1} (ie. Prados-Ramirez *et al.*, 1995; Westerhoff, 2003) and the recent work by van Ginkel *et al.*, (van Ginkel *et al.*, 2005a; van Ginkel *et al.*, 2005b) utilised bromate concentrations in excess of 200 mg L^{-1} within artificial and industrial effluent matrices. However, no trial available within the published literature has specifically addressed the issue of mg L^{-1} bromate concentrations within a groundwater matrix.

Using the techniques previously investigated for bromate removal and outlined above as a basis, the most developed technologies appeared to be chemical removal by ferrous iron and *ex-situ* treatment by GAC. Both have been undertaken at pilot-scale and have shown some promise under commercially-relevant conditions. In addition, both techniques can utilise modified but pre-existing processes and equipment, with ferrous iron treatment requiring mixing, clarification and filtration tanks and GAC treatment making use of existing columns. However, in both cases further development would be necessary prior to full-scale use.

Another promising, although not proven, technology identified by the above review was biological bromate reduction using a bacterial inoculum as either an *in-situ* or *ex-situ* process. Biological nitrate and perchlorate reduction are both known to occur and have been utilised in groundwater bioremediation systems (Hall, 1997; Polk *et al.*, 2001) demonstrating effectiveness of this mechanism for groundwater bioremediation. Reduction of these related groundwater contaminants is further reviewed below. Even so, despite the availability of data since 1995 demonstrating the occurrence of biotic

bromate reduction within bioreactors (Hijnen *et al.*, 1995) and the success of BAC trials (ie. Kirisits *et al.*, 2002), no work had been undertaken to demonstrate efficacy of this approach for groundwater bromate remediation. Due to the potential of this system, it was therefore proposed a biological bromate reduction system using indigenous groundwater bacteria be investigated for possible development into an aquifer remediation tool.

Table 2.2 – Developmental stage, performance and assessment of potential for full-scale implementation of techniques trialled for bromate removal

	Technique	Operating conditions					Bromate removal			Potential cost of implementation	References	Notes
		Laboratory/ Pilot scale	Batch/ Continuous flow	Throughflow	Bromate range tested	Water type[a]	Efficiency (percentage bromate removed)	Waste stream	Efficiency reduction by interference			
Physical	Nanofiltration/ Hyperfiltration	Pilot	Continuous	n/d	5-285 μgL^{-1}	Real	Averages 89% & 97% for 10 min runs	###	#	###	Marhaba & Medlar, 1993	
	Nanofiltration	Laboratory	Batch	n/a	300 μgL^{-1}	Real	77% (20 litre batch volume)	###	#		Prados-Ramirez *et al.*, 1995	
	UV	Laboratory	Continuous	Approx 4-30 $Lmin^{-1}$	50-100 μgL^{-1}	Real	50-100%	#	###	###	Siddiqui & Amy, 1994	
	UV	Pilot	Continuous	Approx 70-640 $Lmin^{-1}$	15-50 μgL^{-1}	Real	5-46%	#	###		Siddiqui *et al.*, 1996a	
	Arc discharge	Laboratory	Continuous	7.6 $Lmin^{-1}$	100 μgL^{-1}	Real	12-45%	#	n/d	###	Siddiqui *et al.*, 1996a	
	HEEB	Laboratory	Batch	n/a	100-320 μgL^{-1}	Real	up to 100%	#	###	###	Siddiqui *et al.*, 1996b	
	HEEB	Pilot	Continuous	Approx 380 $Lmin^{-1}$	180-700 μgL^{-1}	Real	around 40%	#	###		Siddiqui *et al.*, 1996b	
	Photocatalytic decomposition	Laboratory	Continuous	0.5 $Lmin^{-1}$	50-75 μgL^{-1}	Real	50% with 15-43 min contact time	#	##	###	Mills *et al.*, 1995; Mills *et al.*, 1996	
	Photocatalytic decomposition	Laboratory	Batch	n/a	200 μgL^{-1}	Artificial	up to 100% after 1 hour (pH 5)	#	n/d	###	Noguchi *et al.*, 2002	Long reaction time required
Chemical	Coagulants	Laboratory	Batch	n/a	n/d	Real	5% (Alum) & 20% (Ferric chloride)	##	n/d	n/d	Siddiqui *et al.*, 1994a	Concluded treatment not worth pursuing
	Coagulants	Laboratory	Batch	n/a	n/d	Artificial	4.8% (Alum)	##	n/d		Hossain *et al.*, 1996	
	Reducing agent (Ferrous)	Laboratory	Batch	n/a	25-80 μgL^{-1}	Real	up to 90% in 30 min	##	##	##	Siddiqui *et al.*, 1994b	
	Reducing agent (Sulphite)	Laboratory	Batch	n/a	7.6 mgL^{-1}	Artificial	up to 63% at pH 9.5	##	n/d	##	Prados-Ramirez *et al.*, 1995	High bromate concentration trialled
	Reducing agent (Ferrous)	Laboratory	Batch	n/a	100 μgL^{-1}	Artificial	100% in 2 min (pH 8)	##	##	##	Gordon *et al.*, 2002	
	Reducing agent (Sulphite)	Laboratory	Batch	n/a	100 μgL^{-1}	Artificial	up to 99% in 4 days (pH 7)	##	##	##	Gordon *et al.*, 2002	Reaction time considered unrealistic
	Reducing agent (Ferrous)	Pilot	Continuous	2.75 hour total contact time	15 μgL^{-1}	Real	up to 70% (≤ 41% after 30 min flocculation)	##	##	##	Kramer et al., 1996	
	Zero-valent iron (Fe^0)	Laboratory	Continuous	5-20 min EBCT[b] for 2-4 bed volumes	1.2 $g\,L^{-1}$	Artificial	up to 90% with EBCT of 20 min	##	n/d	n/d	Westerhoff, 2003	
Biological	Denitrifiers	Laboratory	Batch	n/a	1 mgL^{-1}	Artificial	up to 99% in 23 days	#	n/d	#	Hijnen *et al.*, 1995	High bromate concentration trialled
	Denitrifiers	Pilot	Continuous	250 $Lmin^{-1}$; 18-36 min contact time	15+35 μgL^{-1}	Real	up to 71%	#	##	#	Hijnen *et al.*, 1999	
	HFMBfR	Pilot	Continuous	1.5 $Lmin^{-1}$	n/d	Real	up to 95%	#	##	##	Nerenberg *et al.*, 2002; Nerenberg & Rittmann, 2004	Not trialled for bromate reduction (perchlorate/nitrate only)
	BAC	Laboratory	Continuous	25-50 min EBCT[b]	10-11 μgL^{-1}	Real	up to 86% for 30 days	#	# / ##	# / ##	Kirisits & Snoeyink, 1999	Cost low where GAC already installed
	BAC	Laboratory	Continuous	20 min EBCT[b]	20 μgL^{-1}	Real	40% for 10 days during sustained run	#	# / ##	# / ##	Kirisits *et al.*, 2001	
	BAC	Laboratory	Continuous	20 min EBCT[b]	10-50 μgL^{-1}	Real	37-41% continuous, up to 74% peak	#	# / ##	# / ##	Kirisits *et al.*, 2002	Sustained run (>11 months)
Activated carbon	PAC	Laboratory	Batch	n/a	25-100 μgL^{-1}	Real	5-99% over 24 hrs	##	##	n/d	Siddiqui *et al.*, 1994a; Westerhoff *et al.*, 1994	GAC considered more effective and worth pursuing
	GAC	Pilot	Continuous	Approx 1.3 $Lmin^{-1}$	3-290 μgL^{-1}	Real	up to 100% for 32 hrs	#	n/d	# / ##	Marhaba & Medlar, 1993	
	GAC	Laboratory	Continuous	10-15 EBCT[b] equivalent	50 μgL^{-1}	Real	up to 100% for at least 1000 bed volumes	#	###	# / ##	Siddiqui *et al.*, 1994a; Siddiqui *et al.*, 1994b	
	GAC	Laboratory	Continuous	Approx 11-38 $Lmin^{-1}$ equivalent; 4-10 min EBCT[b] equivalent	50 μgL^{-1}	Real	up to 100% for around 1000 bed volumes	#	n/d	# / ##	Westerhoff *et al.*, 1994	50-80% breakthrough after 4000 bed volumes
	GAC	Laboratory	Continuous	0.0125 $Lmin^{-1}$; 10 min EBCT[b] equivalent	9 mgL^{-1}	Artificial	67% after 122 hours	#	n/d	# / ##	Prados-Ramirez *et al.*, 1995	High bromate concentration trialled, with short run times
	GAC	Laboratory	Continuous	20 min EBCT[b]	n/d	Artificial	No removal observed	n/a	n/a	# / ##	Meijers & Kruithof, 1995; Kruithof & Meijers, 1995	
	GAC	Laboratory	Continuous	0.17 $Lmin^{-1}$ equivalent; 5-10 min EBCT[b] equivalent	25-100 μgL^{-1}	Real	30-40% for 60 days equivalent	#	###	# / ##	Siddiqui *et al.*, 1996d	
	GAC	Pilot	Continuous	0.32 $Lmin^{-1}$	50 μgL^{-1}	Real	>60% for 30 days	#	##	# / ##	Azmi *et al.*, 1999	Efficiency reduction noted as GAC coloured to BAC
	GAC	Pilot	Continuous	0.51 $Lmin^{-1}$; 20 min EBCT[b]	up to 320 μgL^{-2}	Real	Average 75% for 3 months	#	###	# / ##	Bae *et al.*, 1999	Suggested BAC may be more suitable in long-term & GAC type critical
	GAC	Pilot	Continuous	3.7 $Lmin^{-1}$; 10 min EBCT[b]	50 μgL^{-1}	Real	Average 50% over 1 month	#	n/d	# / ##	Marhaba, 2000	Suggested GAC may be cost-effective
	GAC	Laboratory	Continuous	3 min EBCT[b]	9-10 μgL^{-1}	Real	Above 50% for 1000-41000 bed vols (dependent on water source)	#	###	# / ##	Kirisits *et al.*, 2000	Suggested BAC may be more suitable in long-term
	GAC	Pilot	Continuous	0.63 $Lmin^{-1}$; 15 min EBCT[b]	17-47 μgL^{-1} (1 month), then 94-163 μgL^{-1}	Real	100% continuously for one month, then declining to ~5% after 11 months	#	###	# / ##	Huang & Chen, 2004	

Key

Waste stream
- # - Little residual other than bromide (and low-level microbial biomass) produced by treatment
- ## - Treated water includes chemical or carbon residues
- ### - Concentrated ionic waste stream produced which requires treatment prior to disposal

Interference (Examples include pH, organic matter, oxygen, nitrate, competing anions)
- # - Little efficiency reduction noted under real conditions
- ## - Moderate efficiency reduction noted under real conditions
- ### - High efficiency reduction noted under real conditions

Potential cost of implementation (based on comments within publications)
- # - Low potential capital and/or operating costs
- ## - Medium potential capital and/or operating costs
- ### - High potential capital and/or operating costs

[a] *'Real' describes a natural water matrix, 'Artificial' describes a distilled water sample spiked with bromate*

[b] *Empty bed contact time*

[c] *Tried tested on RSSCT (Rapid Small-Scale Column Test) configuration; data given for theoretical scale-up to pilot scale*

n/a - Not applicable

n/d - No da

2.8 BROMATE BIODEGRADATION

2.8.1 Redox reactions in the subterranean environment

Bacterial activity within the subterranean environment and a possible link to contaminant degradation has been studied since the 1980s, when evidence began to accumulate that anthropogenic pollution, particularly hydrocarbons, could be reduced by the activities of subsurface microorganisms. Microbes are now known to have the ability to remediate a wide range of organic and inorganic pollutants during their normal metabolic processes. This includes traditionally recalcitrant organic compounds such as trinitrotoluene (TNT) and methyl-*tert* butyl ether (MTBE), and also common inorganic pollutants including nitrate and iron (Chapelle, 2001).

Bacterial metabolism proceeds via the two basic mechanisms of fermentation and respiration. Fermentation operates in the absence of an external electron acceptor such as oxygen but does not allow complete oxidation of a complex food source such as glucose. Conversely, cellular respiration enables a cell to harvest more of the energy stored within a food source, but requires an acceptor for electrons generated. During this process, reactions occur which lead to oxidation and catabolism of the food source and simultaneous reduction of a suitable electron acceptor (Campbell, 1993).

Oxygen is the preferred bacterial respiratory electron acceptor for many bacterial species due to a high energy yield, and is preferentially utilised under aerobic conditions. However, under anoxic conditions or at low oxygen concentrations many microbes, including facultative anaerobes, are able to use alternative respiratory electron acceptors such as nitrate (during denitrification). If nitrate becomes limiting then other, lower energy yielding electron acceptors including iron, manganese and sulphate may be utilised by species able to metabolise them. To give an understanding of the anticipated order of utilisation, the major redox reactions occurring in the subterranean environment can be categorised in order of Gibbs Free Energy changes (kilojoules per mole organic matter) of a standard reaction ($\Delta G_0(W)$) with the model organic compound (CH_2O) at pH=7 (Equations 2.10 – 2.15) (Christensen *et al.*, 2001)

Aerobic respiration: $CH_2O + O_2 \rightarrow H_2O + CO_2$ $\Delta G_o(W) = -501.6$ kJ mol^{-1}

Equation 2.10

Denitrification: $5CH_2O + 4NO_3^- + 4H^+ \rightarrow 7H_2O + 5CO_2 + 2N_2$ $\Delta G_o(W) = -476.5$ kJ mol^{-1}

Equation 2.11

Bromate reduction: $6CH_2O + 4BrO_3^- \rightarrow 6H_2O + 6CO_2 + 4Br^-$ $\Delta G_o(W) = -453$ kJ mol^{-1}

Equation 2.12

Iron reduction: $CH_2O + 4Fe(OH)_3 + 8H^+ \rightarrow 11H_2O + CO_2 + 4Fe^{2+}$

$\Delta G_o(W) = -117.0$ kJ mol^{-1}

Equation 2.13

Sulphate reduction: $2CH_2O + SO_4^{2-} + H^+ \rightarrow 2H_2O + 2CO_2 + HS^-$ $\Delta G_o(W) = -104.5$ kJ mol^{-1}

Equation 2.14

Methanogenesis: $2CH_2O \rightarrow CH_3COOH \rightarrow CH_4 + CO_2$ $\Delta G_o(W) = -92.0$ kJ mol^{-1}

Equation 2.15

Other electron acceptors, including chlorate (Logan, 1998), perchlorate (Herman and Frankenberger, 1998; Urbansky, 2002), selenate (Losi and Frankenberger, 1997), chromate (Losi *et al.,* 1994), iodate (Waite and Truesdale, 2003) and bromate (Hijnen *et al.,* 1995) have also been studied. Biological bromate reduction proceeds according to Equation 2.12 and, based on thermodynamics, would be expected to occur in preference to all but denitrification and aerobic respiration. This was shown in practice by Hijnen *et al.* (1995; 1999) who attempted to induce bromate reduction in denitrifying bacterial species within a batch suspended growth system. It was noted that, when supplemented with ethanol bromate reduction to bromide did occur. However, the reduction rate of 0.6 – 0.8 μg L^{-1} min^{-1} from an initial concentration of 25 – 35 μg L^{-1} at 12°C was over 100 times lower than the equivalent rate for denitrification (Hijnen *et al.,* 1999), and both oxygen and nitrate were found to be inhibitory (Hijnen *et al.,* 1995). This inhibition

provided evidence for the preferential utilisation of oxygen and nitrate over bromate within a denitrifying system.

The range of microbial life within an aquifer is extremely diverse, with a wide range of strains co-existing within the ecosystem. Under aerobic conditions, aerobic and facultatively anaerobic heterotrophic bacteria predominate. For example, the majority of known denitrifiers are heterotrophic (Korom, 1992), with the range of denitrifiers including typically aerobic heterotrophs such as *Pseudomonas* species, which can utilise both nitrate and oxygen and mainly denitrify only under oxygen-limiting conditions (Cartmell, 1997). Under anoxic conditions, other species including fermenters, iron and sulphate reducers and methanogens instead start to predominate.

Interactions between these different species are very complex and poorly understood in many cases. However, one interaction thought to impact on remediation capacity within an aquifer is that of synergism between fermentative and respirative bacteria (Chapelle, 2001). The wide range of organic carbon compounds present within an aquifer means it is not possible for a single bacterium to have enzymes for degradation of all carbon sources it is likely to encounter. For example, the ubiquitous *Pseudomonad* species are always respirative and do not possess the capability for fermentation. Fermentation capability is also absent from many anaerobic species such as sulphate reducers and methanogens (Chapelle, 2001). However, these bacteria are capable of oxidising fermentation products such as acetate, which fermenting strains are unable to degrade. By this mechanism both populations benefit, and complex carbon sources can be fully utilised. Therefore, although it is reported a wide variety of organic compounds including methanol, ethanol, glucose, acetate, formic acid, molasses and whey reported can be utilised during respiratory processes such as denitrification (Mateju *et al.*, 1992), in the subsurface environment synergistic relationships may also be important in the remediation of anthropogenic groundwater contamination.

2.8.2 Analogous compounds

Biological bromate reduction had been demonstrated as a viable, although slow method of removing bromate from water under laboratory conditions. However, the

complexity of an aquifer system makes it extremely difficult to evaluate whether natural bromate bioremediation would occur within groundwater. The environmental behaviour and mechanism of bromate bioremediation are still sparsely studied and therefore poorly understood, but they are likely to be at least partially analogous to other inorganic oxyanions. Two widely reported and topical examples are nitrate (NO_3^-) and perchlorate (ClO_4^-).

2.8.2.1 Nitrate

Nitrate is the most common groundwater contaminant (Korom, 1992) and is found as a diffuse pollutant in many agricultural areas following application within nitrogenous fertilisers. It is highly soluble and mobile within aqueous solution, and any excess applied to fields readily leaches down below the rooting zone and into nearby surface and groundwater sources (Cartmell, 1997). An estimate from the early 1990s suggests that around 25% of nitrogen applied to arable land in Eastern England is lost by leaching (Parker and Chilton, 1991). Once lost from the agricultural system, nitrate can contribute to eutrophication of natural water bodies, leading to accelerated growth rates of certain algal and higher plant species and subsequent disturbance of natural ecosystems. In addition, nitrate contamination of drinking water has been implicated in both stomach cancers and the disorder methaemoglobinaemia ('blue-baby syndrome'), in which the bacterial conversion of nitrate to nitrite in a baby's stomach leads to formation of methaemoglobin instead of haemoglobin within blood. This reduces blood oxygen supply to vital organs such as the brain and may lead to brain damage or death by oxygen starvation. Methaemoglobinaemia is not a major cause of death, with around 2000 cases and a 10% mortality rate reported worldwide from 1945 until the 1960s (Cartmell, 1997). The carcinogenicity of nitrate is also controversial, with nitrate readily absorbed and excreted in urine. However, WHO guidelines recommend that total nitrogen within drinking water should not exceed 50 mg L^{-1}, with EU legislation setting a 50 mg L^{-1} potable water limit for nitrate alone (World Health Organisation, 1993; Council of the European Communities, 1998).

Following years of unconstrained nitrate application which led to around 20% of groundwater beneath European agricultural land exceeding the 50 mg L^{-1} limit in the late

1980s (European Environment Agency, 1995), nitrate within groundwater in the EU is now strictly regulated. The Nitrate Directive (91/676/EEC) imposes measures designed to protect water from nitrate pollution (Council of the European Communities, 1991), and has been implemented in English law with the introduction of Nitrate Vulnerable Zones (NVZs) within which farmers have to adhere to rules designed to limit nitrate leaching (HM Government, 1996; HM Government, 2002). However, nitrate is thought to move very slowly within groundwater (Cartmell, 1997). It is not uncommon for the nitrate residence time within various aquifer types to exceed 50 years, with one study suggesting a maximum residence time of 50 – 70 years in a glacial outwash aquifer in Minnesota, USA (Puckett and Cowdery, 2002). Therefore, as with bromate, nitrate can be thought of as largely conservative within a groundwater system.

Natural bacterial denitrification does occur given the requirements of suitable bacterial strains, an electron donor and anaerobic conditions or restricted oxygen availability (Korom, 1992), and is an integral part of the natural environmental cycling of nitrogen. Most subsurface habitats are thought to have the potential to denitrify, with one study concluding bacterial strains able to undertake heterotrophic denitrification can form up to 20% of the total aquifer bacterial population (Madsen, 1993; Bengtsson and Bergwall, 1995). Even so, despite the widespread occurrence of denitrifying organisms, the role of denitrification in aquifers is extremely difficult to evaluate or generalise. The major factors controlling denitrification are pH, temperature, DO concentration and the presence of organic carbon (OC) as an electron donor (Cartmell, 1997). **pH** is positively correlated with denitrification rate, with the optimum range being pH 7 – 8 (Hiscock *et al.,* 1991). Denitrification is measurable at a **temperature** of 0 – 5°C but optimum rates are found at 60 – 70°C (Knowles, 1982), which is much higher than would ever be encountered within a UK aquifer. Differences in the concentration of **dissolved oxygen** and other electron acceptors within an aquifer can be caused by preferential utilisation of the more efficient electron acceptors such as oxygen and then nitrate, leading to stratification of an aquifer into different redox zones. This stratification is a fundamental property of redox processes in groundwater systems (Chapelle, 2001). Although the zones (oxic, denitrifying, sulphate reducing etc.) have been shown to overlap, degradation patterns are extremely site-specific (Korom, 1992). An example of spatial

variation in aquifer denitrification is caused by preferential oxygen utilisation as the electron acceptor, typically leading to higher nitrate concentrations near the surface (Puckett and Cowdery, 2002). Even in a largely aerobic aquifer denitrification may occur at anaerobic microsites (Gale *et al.*, 1993; Gale *et al.*, 1994), and differing oxygen sensitivity of bacterial species means that species composition of the population can be an important factor in determining aquifer denitrification rates (Cartmell, 1997). Availability of **organic carbon** (as electron donor) is the final key rate-determining factor. Aquifers are generally carbon-limited with the majority of groundwaters suggested to have a DOC concentration below 2 mg L^{-1} (Thurman, 1985). This has been shown in practice, with Smith and Duff (1988) and Vogel *et al.* (1981) both concluding carbon availability was the major factor limiting denitrification within aquifers in the United States and Kalahari desert respectively. Interaction with surface water or vegetation can resupply an aquifer with OC (Korom, 1992), but this will only occur in specific areas and will accentuate any spatial variations. Due to the complexity of estimating whether nitrate reduction occurs within aquifers, there is no complete consensus in the literature as to its significance. Some authors state it is not significant and dilution effects can account for observed concentration reductions (Howard, 1985), whilst others suggest that although degradation rates are small (in the region of 0.005 – 0.047 mmol NO_3 yr^{-1}), they can be significant over time (Puckett and Cowdery, 2002).

Denitrification has been investigated as a biological remediation system in a range of pilot- and full-scale remediation trials, encompassing both *in-situ* and *ex-situ* processes. Recent examples of *in-situ* denitrification applications include pilot trials of a system injecting formate as an electron donor which removed 80 – 100% nitrogen up to 15 metres from the injection point (Smith *et al.*, 2001), and a comparable process injecting glucose (Godbold, 2002). *Ex-situ* biological drinking water denitrification has been developed using many different unit processes, including fluidised-bed reactors, packed-bed reactors, biofilters and even denitrifying bacteria immobilised in alginate beads or a polymer matrix (Mateju *et al.*, 1992).

2.8.2.2 Perchlorate

Perchlorate salts are widely used in industry, primarily as ammonium perchlorate in solid rocket fuel, but also in the manufacture of car air bags, explosives and pyrotechnics. Therefore, perchlorate contamination is typically a localised problem from point sources, with many affected sites found near military-related facilities (Gullick *et al.*, 2001). Despite the strength of perchlorate as an oxidising agent, chemical perchlorate reduction does not readily occur due to kinetic barriers, and in practice it is very slow to react. Perchlorate is extremely soluble (Urbansky, 2002) and does not appear to adsorb onto or react with the bulk aquifer matrix (Kim and Logan, 2001). It was also not known as an environmental contaminant until as recently as 1996 (Coates and Achenbach, 2004), when a combination of toxicology research and analytical advances led to discovery of widespread perchlorate contamination in the USA. One example is found in the State of California, where at least 38 drinking water wells were found to exceed an 18 $\mu g\ L^{-1}$ State advisory limit, a limit which has now been further reduced to 4 $\mu g\ L^{-1}$ (Giblin *et al.*, 2000a; Logan and LaPoint, 2002). Perchlorate analytical technologies can now detect concentrations of around 4 $\mu g\ L^{-1}$, US advisory limits between 4 – 32 $\mu g\ L^{-1}$ have been introduced in some States, and a recent Environmental Protection Agency draft reference dose of 0.03 $\mu g\ kg^{-1}$ body weight may lead to implementation of a Federal drinking water limit of 1 $\mu g\ L^{-1}$ (Yang *et al.*, 2000; Logan and LaPoint, 2002; Nerenberg *et al.*, 2002).

Information on perchlorate and the related oxyanion chlorate (a compound used in weed control, as a paper bleaching agent and in water disinfection (Logan, 1998)) in groundwater systems is limited, but relevant literature is now growing. It is thought perchlorate and chlorate (together known as (per)chlorate), are stable and mobile in aqueous systems and can migrate substantial distances within aquifers (Gullick *et al.*, 2001). Ion exchange systems are among technologies being trialled for (per)chlorate treatment along with reverse osmosis, nanofiltration, activated carbon, and electrochemical reduction (Urbansky, 1998; Gullick *et al.*, 2001; Brown *et al.*, 2002; Theis *et al.*, 2002; Urbansky, 2002; Amy *et al.*, 2003; Gisch, 2003). Biological *ex-situ* perchlorate reduction has also been investigated at laboratory and pilot-scale in recent

years (Xu *et al.*, 2003; Nerenberg *et al.*, 2002), with construction of full-scale bioreactors now also reported.(Polk *et al.*, 2001; Hatzinger *et al.*, 2000).

It can be seen that there are some strong similarities between the environmental characteristics of bromate and those of both nitrate and (per)chlorate, and these could be used in the elucidation of bromate behaviour and development of treatment technologies.

2.8.3 Biotic heterotrophic reduction mechanisms

The mechanism of biological bromate reduction is poorly understood, and there has been little research to date into this area. No trial to date has investigated mechanisms and pathways operating to mediate the bromate bioremediation observed during published studies. However, the biological denitrification process has been widely studied since 1882, when the term 'denitrification' was coined by U. Gayon and G. Dupetit (Korom, 1992). Many of the mechanisms occurring during bacterial denitrification have been elucidated, and reviews have been published focussing on this subject (ie. Hiscock *et al.*, 1991; Mateju *et al.*, 1992). In addition, the recent issues surrounding groundwater (per)chlorate contamination have led to studies into bacterial reduction of these compounds which, combined with the extensive denitrification literature, may provide insights into bromate reduction pathways.

The range of bacteria known to possess an ability to denitrify encompasses at least 27 genera, which were collated and listed by Mateju *et al* (1992). Denitrification can be carried out by heterotrophic bacteria which require OC as an electron donor, and also autotrophs which use inorganic electron donors such as Mn^{2+}, Fe^{2+} and HS^- (Korom, 1992). The denitrification pathway utilised is typically shown as a series of reducing steps (Figure 2.4), commencing with nitrate (oxidation state +5) and completed with the evolution of dinitrogen gas (oxidation state 0).

NO_3^-	$\rightarrow$	NO_2^-	$\rightarrow$	NO	$\rightarrow$	N_2O	$\rightarrow$	N_2
nitrate		nitrite		nitric oxide		nitrous oxide		dinitrogen

Figure 2.4 - Proposed denitrification pathway (Payne, 1981)

The reduction of nitrate is catalysed by a discrete enzyme system at each individual step, with some denitrifiers able to complete the entire pathway but many only capable of carrying out one or two steps (Hiscock *et al.*, 1991). The initial step of nitrate reduction to nitrite is mediated by membrane-bound nitrate reductases, with nitrite reductases catalysing reduction of nitrite to either nitric oxide or nitrous oxide. The less-well characterised nitric oxide and nitrous oxide reductases then complete the pathway to dinitrogen (Mateju *et al.*, 1992), which resists further chemical change due to its stable triple N bond (Korom, 1992). Only true denitrifying batceria can fully mediate nitrate reduction to nitrogen gas, with other nitrate reducing strains ('incomplete denitrifiers') only able to complete the initial step of nitrate reduction to nitrite (Drysdale *et al.*, 1999). Thus the overall denitrifying microflora may be thought of as a group of complementary bacterial strains, only together able to totally convert nitrate to nitrogen gas.

It has been known since the 1920s that some of these nitrate-reducing strains can also reduce chlorate to chlorite (Quastel, 1925). The mechanism was thought to be a competitive interaction between nitrate and chlorate as a terminal electron acceptor for nitrate reductase, with bacterial growth and energy conservation not a consequence of chlorate utilisation (Coates *et al.*, 1999; Mateju *et al.*, 1992). However, it is now known not all denitrifying bacteria are capable of chlorate utilisation (Herman and Frankenberger, 1998), and specific chlorate reductases have since been isolated from denitrifying species (Logan, 1998). In addition, recent studies have shown certain species have the ability to use (per)chlorate as a terminal electron acceptor for anaerobic respiration and conserve energy for growth from this reduction pathway. Reduction of (per)chlorate to chlorite is mediated by (per)chlorate reductases, and a second reaction disproportionates chlorite utilising a highly conserved chlorite dismutase enzyme (Wolterink *et al.*, 2003) in a reaction suggested to be a detoxification mechanism to remove toxic chlorite (Herman and Frankenberger, 1998). The end-product is therefore chloride in a pathway first elucidated in 1996 (Figure 2.5).

$$ClO_4^- \rightarrow ClO_3^- \rightarrow ClO_2^- \rightarrow Cl^-$$

perchlorate chlorate chlorite chloride

Figure 2.5 - Proposed perchlorate reduction pathway (Rikken *et al.*, 1996)

Until recently only six bacterial species capable of anaerobic respiration linked to (per)chlorate reduction had been identified, but it is now thought to be a much more ubiquitous process (Coates *et al.,* 1999), with the (per)chlorate degradation mechanism in certain species shown to be mediated by specific perchlorate and chlorate reduction pathways (Coates *et al.,* 1999; Wolterink *et al.,* 2003). One recent paper by Xu *et al.* (2004), discussing the relationship between chlorate, perchlorate and nitrate reduction, concluded that generalisations between (per)chlorate reducing bacterial strains were often inappropriate. It had previously been shown in strains Perclace and *Dechlorosoma suillum* that a single enzyme was responsible for both perchlorate and nitrate reduction (Chaudhuri *et al.,* 2002; Giblin and Frankenberger, 2001). However, it was found *Dechlorosomas sp.* KJ contained separate perchlorate and nitrate pathways, and *Pseudomonas sp.* PDA was able to mediate the complete chlorate degradation pathway but unable to reduce either perchlorate or nitrate (Xu *et al.,* 2004). Another recently identified chlorate reducing strain, *Pseudomonas chloritidismutans* strain AW-1, has also been shown as incapable of perchlorate or nitrate reduction (Wolterink *et al.,* 2002; Wolterink *et al.,* 2003). Xu *et al.* (2003) suggested that, whilst all perchlorate reducing bacteria are also able to reduce chlorate, not all chlorate reducers can degrade perchlorate. Thus previously observed variable effects of nitrate concentration on (per)chlorate reduction (Logan, 1998) may be a consequence of different reduction pathways, some utilising the nitrate reduction system and some completely independent.

Early bromate bioremediation studies provided no evidence for a specific reduction pathway mediated by bacteria exhibiting energy conservation during anaerobic respiration of bromate, and capable of growth using this substrate. It had been concluded by most authors that biological bromate reduction was a side reaction of the nitrate reduction pathway (Hijnen *et al.,* 1995; Kirisits and Snoeyink, 1999), and that bromate reduction occurred by a process known as co-metabolism. Co-metabolism in this context

refers to a reaction which is incidentally catalysed by an enzyme that has another biochemical purpose, and which does not necessarily generate energy for the cell (Nerenberg *et al.*, 2002). It is commonly applied to electron donors (Saez and Rittmann, 1991), but has also been used to describe reduction of other anions by the nitrate reductase enzyme. For example, it was shown that *Escherichia coli* nitrate reductase catalyses the reduction of bromate (Morpeth and Boxer, 1985). However, the assumption of bromate reduction by co-metabolism is made in the absence of experimental evidence, and the recent (per)chlorate studies suggest oxyanion reduction pathways are more complex and interlinked than previously known. Lack of naturally-occurring bromate (and therefore evolutionary selection pressures) was cited as a reason for lack of a specific bromate pathway (Hijnen *et al.*, 1995), but perchlorate contamination is also of recent anthropogenic origin (Coates *et al.*, 1999). In addition, it has recently been demonstrated by Wolterink *et al.* (2002; 2003) that, whilst unable to reduce nitrate, *P.chloritidismutans* strain AW-1 is capable of bromate reduction, although this reaction could not be utilised for growth. Recent observations by van Ginkel *et al.* (2005b) have now shown, for the first time, that a continuously-fed enrichment culture inoculated with activated sludge can reduce bromate in the absence of other electron acceptors. Isolated biomass from this culture was also unable to reduce (per)chlorate or nitrate in batch flasks. This study concluded that bromate was reduced within the continuous flow system by bacteria utilising only bromate for growth, thus implying that energy conservation had also occurred. However, a great deal of further work is required to elucidate the complex mechanisms of biological bromate reduction, via putative anaerobic respiration and co-metabolic pathways, and also possibly via other as yet uninvestigated pathways.

3 THESIS AIMS AND OBJECTIVES

This project was based around the recent delineation of a plume of bromate groundwater contamination within an aquifer in the South of England. As a novel groundwater pollutant with no previous precedent, little data were available on either environmental effects of, or remediation strategies for bromate. The primary aim of this study was therefore to investigate the potential for microbial bromate reduction as a mechanism to assist aquifer decontamination, by development and initial optimisation of a bioremediation system. A further aim, given a possibility of bromate contamination impacting on sewage treatment works, was to provide baseline data on any potential disruption to biological wastewater treatment processes. For successful achievement of these overall aims, the project objectives were to:

- develop accurate and reproducible analysis of bromate and other anions within groundwater and wastewater matrices
- provide information relating to bromate behaviour within a wastewater environment, including potential effects of bromate contamination on sewage sludge biomass, at both laboratory- and pilot-scale
- assess occurrence and feasibility of bromate reduction by groundwater micro-organisms, at laboratory-scale, using batch and continuous-flow trials
- study parameters affecting efficacy and reduction rate of a biological bromate-reducing culture, and elucidate potential mechanisms for microbial reduction
- attempt isolation of bromate-reducing bacterial strains present within an enriched culture and demonstrate reduction ability of any isolated strains
- develop and investigate use of a bromate reducing culture for remediation of bromate-contaminated groundwater within a pilot-scale bioreactor
- provide a preliminary assessment on potential effectiveness of a biological bromate remediation system at aquifer scale

4 OUTLINE OF EXPERIMENTAL WORK

This chapter gives a brief overview of practical studies undertaken under the three generalised headings of analytical development, wastewater dosing trials, and investigations into groundwater bromate bioremediation, and summarises reasons why use of the methods selected was justified in the context of the current project.

4.1 DEVELOPMENT OF ANION ANALYSIS

The requirement for accurate and reproducible analytical capability of anions was identified as a prerequisite for successful project completion and thus a priority of investigation. A literature review identified known techniques, most of which had been developed primarily for analysis of treated potable water supplies. A comparative trial of the six most promising methodologies was then undertaken, using both standard samples and also spiked groundwater and wastewater matrices. The technique most suitable for use in the current situation was identified, and this was subsequently utilised for analysis of bromate and also other oxyanions including bromide, nitrate, nitrite and sulphate throughout subsequent trials.

4.2 BROMATE CONTAMINATION IN WASTEWATER

With abstraction from the bromate-contaminated aquifer occurring via both large supply boreholes and by small-scale private pumping, ingress of bromate-loaded groundwater into sewage treatment processes was deemed likely, either accidental or as part of a pump-and-treat remediation strategy. Infiltration of toxic components into these biological treatment systems can give rise to operational problems such as alterations of sludge settling characteristics, caused by changes within the biomass including filamentous bulking and deflocculation (Spencer-Davies and Murdoch, 2001). Little information was available as to whether bromate contamination would pose a threat to sludge biomass and therefore overall operational stability. Therefore, a practical investigation of bromate within a wastewater matrix encompassing three areas of study was undertaken to ascertain and quantify any effects of passing bromate-loaded water through sewage treatment processes.

- Wastewater sludge biomass respiration inhibition - Respirometry measures and logs oxygen uptake of a sample, and is commonly used in wastewater processes for routine monitoring of treatment plant performance and assessment of wastewater characteristics (Young, 1999). Respiration rate is a measure of bacterial energy metabolism and, as metabolism is an essential biological process, oxygen uptake is a good estimator of biomass growth and therefore overall 'health' of the wastewater biomass (Spencer Davies and Murdoch, 2001). Inhibition of respiration can also be measured by respirometric techniques, whereby differences in oxygen uptake rate against control values are logged following exposure of sludge bacteria to potentially toxic components. This methodology was applied to give a basic understanding of overall sludge biomass health during periods of bromate loaded water ingress.

- Wastewater sludge biomass sorption – Bromate is thought to adsorb only slightly to soil or sediment (Depository Services Program (Canada), 1999), but there are no data available on bromate sorption to organic compounds such as wastewater sludge biomass. Therefore, a study was undertaken to investigate sorption and overall behaviour of bromate when introduced to a sludge biomass matrix.

- Wastewater process dosing – Whilst laboratory-scale trials provide data on specific aspects of a process when viewed in isolation, pilot and full-scale trials give results based on all parameters operating within the overall system, which may not be apparent at a smaller scale. A pilot-scale Membrane Bioreactor (MBR) was utilised to investigate effects on sludge biomass of dosing with bromate-contaminated water. The two-chambered submerged MBR system, which was in operation prior to dosing trials commencing, contained an existing biomass. This allowed the trial to concentrate only on changes elicited by bromate spiking of influent supplies, which would suitably emulate sudden and sustained ingress of bromate-contaminated water to a sewage treatment process.

4.3 GROUNDWATER BROMATE BIOREMEDIATION

The primary project aim was to investigate and evaluate remediation of bromate-contaminated groundwater by utilisation of a biological system incorporating indigenous groundwater bacteria. Within the aquifer, conditions potentially favouring development of bacteria possessing the capacity for biological bromate reduction have been in place for an unknown but possibly extended period of time. Anecdotal evidence of natural small-scale microbial bromate reduction within the aquifer had been suggested, but no firm data were available. This facet of the project was therefore aimed at enriching indigenous aquifer bacteria, with the objective of developing a viable bromate bioremediation process for aquifer decontamination.

Initial trials were carried out utilising a laboratory-scale two-vessel anoxic suspended growth chemostat. A continuous-flow configuration allows appropriate selection pressures to be maintained for extended periods of time, and also facilitates precise manipulation of parameters of interest under continuously anoxic conditions. Following initiation, the chemostat system was kept under continuous-flow conditions for the entire project timescale, with three experimental phases carried out during this period.

- Phase I (Proof of concept) – In the first phase of operation, confirmation of an ability for the chemostat system to establish a viable bromate reducing culture was the priority. Bromate concentrations contained within two different contaminated groundwater matrices were investigated, in addition to an artificially-spiked high-bromate influent source. A range of glucose concentrations were trialled at each bromate concentration, to study bromate reduction potential of this new culture under both carbon limiting and carbon excess conditions. Groundwater sources with relatively high nitrate concentrations (30 – 40 mg L^{-1} as nitrate) were utilised, due to a hypothesis previously advanced that bromate reducing activity only occurs as a side reaction of the nitrate reduction pathway (Hijnen *et al.,* 1995; Kirisits and Snoeyink, 1999). Use of high nitrate concentrations would allow proliferation of nitrate reducing strains, thus possibly providing a sufficient microbial population to reduce bromate via this proposed 'co-metabolic' reduction mechanism.

- Phase II (Two-stage reduction and enrichment) – Bromate reduction observed during Phase I provided evidence that bioremediation was occurring within the chemostat system. Phase II was aimed at enrichment of the culture by provision of conditions giving a selective advantage to bacteria capable of bromate reduction. The two bioreactors were placed in series to allow a two-stage enrichment process. Retention times and reactor temperature were increased to accommodate any slow-growing strains, with conditions shown to be carbon-excess during Phase I utilised throughout. Influent water supply was altered to a borehole source close to the contaminated site. Using nitrate concentrations comparable to Phase I a range of spiked bromate concentrations was initially trialled (Phase IIa), with the remainder of the phase (Phase IIb) using continuous conditions over an extended period to provide a stable environment for possible selection of putative higher rate specific bromate-reducing microbes. Nitrate spiking was discontinued for phase IIb to increase the possibility of enrichment for a bacterial strain capable of bromate reduction independent of denitrification.

- Phase III (Parameter assessment) – Encouraging results from Phase II allowed assessment of the resultant culture by manipulation of operational parameters. Using constant conditions on a test and control basis, individual parameters were altered. Parameters investigated were carbon concentration and operational temperature (Phase IIIa), and culture effectiveness in the absence of the possibly-competing electron acceptors nitrate and sulphate (Phase IIIb).

To more fully understand bromate reduction within the bioreactors, an investigation of the chemostat enrichments at a microbial level was undertaken. By gaining knowledge of bacterial identity and diversity within cultures produced by the applied selection pressures, a more targeted approach can be taken to suggest manipulations of the various operational parameters. The approach taken in this section of study was utilisation of agar plates to attempt further isolation of strains within the chemostat culture, with a storage technique also briefly investigated to allow preservation of the mixed culture and any isolated strains without the requirement for continued maintenance of a bioreactor system.

Bench-scale bioremediation batch flask trials were carried out to investigate individual parameters, and to test efficacy of isolated cultures. The batch flask technique was utilised as it is simple and also allows rapid, simultaneous investigation of a wider set of conditions than continuous-flow culture methodologies. Parameters investigated using batch trials were bromate concentration, carbon source, temperature and carbon/anion usage profiles. Trials were also completed to assess plate culture viability once reintroduced into a liquid medium.

Successful enrichment of the chemostat culture provided a necessary step for development of a successful bromate bioremediation system. However, conditions within the chemostat provided a strong selective advantage for microbes capable of reducing bromate. To investigate robustness of the culture when introduced into non-ideal but industrially-relevant conditions, the final study area involved construction and commissioning of a two-vessel *ex-situ* pilot-scale bioreactor system. The reactors were operated as fixed-film systems utilising an unspiked groundwater matrix sourced from close to the source of contamination. Two experimental phases were undertaken:

- Phase A (Inoculation and startup) – Conditions during reactor startup were optimised to emulate those encountered by the chemostat culture, with inoculum from this system added to provide a source of enriched microbes. Bromate reduction within the system, operated in batch suspended growth configuration, was closely monitored.

- Phase B (Reactor operation) – Following initiation of continuous flow conditions, reactor acclimation and performance were monitored (Phase Bi). Using the two reactors on a test and control basis, effects of retention time were investigated (Phase Bii), with operational stability at low winter temperatures over an extended experimental period also evaluated (Phase Biii).

5 DEVELOPMENT OF ANION ANALYSIS

This chapter outlines the importance to the current project of investigating, sourcing and developing a reliable strategy for anion analysis in groundwater and wastewater matrices. Analytical requirements are described, with available technologies summarised. A targeted selection of strategies are identified as warranting further investigation, and the process of evaluating these strategies for use in groundwater bromate analysis is outlined. Results of the comparative trials are given and the basis for selection of the anion analytical strategy used in the project justified.

5.1 REQUIREMENTS FOR ANION ANALYSIS

One of the primary project requirements was identified as adoption of a suitable anion analysis strategy. Routine detection of anions, notably bromate but also including bromide, nitrate, nitrite and sulphate, was defined as a priority for investigation. The major attributes required by a suitable strategy were determined as:

- Analysis of bromate at low concentrations, with a necessary minimum requirement of 10 μg L^{-1}, the current (from 25 December 2003) UK potable water limit

- An ability to detect bromate over a wider concentration range than conventionally required in potable water analysis. Within the contaminated site a maximum bromate concentration of 152 mg L^{-1} has been reported (Environment Agency, 2002a)

- Detection of bromide, nitrate, nitrite and sulphate, either simultaneously with bromate or as a separate methodology

- Cost-effective, quick and routine analytical runs, due to a requirement for frequent sampling

- Successful application for use on both groundwater and wastewater samples

Investigation of bromate analytical techniques within the published literature confirmed development of a range of methodologies over the preceding two decades, superseding relatively crude titrimetric methods (Watson, 1994). An extensive range of literature was available (ie. Schminke and Seubert, 2000; Thompson *et al.*, 2000; Koscielna, 2004), with technologies providing detection limits of sub-μg L^{-1} concentrations available, and detection to ng L^{-1} concentrations fast becoming routine. However, research had been directed towards detecting bromate as a DBP following ozonation of potable water, with only sparse literature available for bromate analysis specifically on untreated groundwater or surface water sources. No published literature dealing with bromate analysis within wastewater matrices was available. These matrices would provide a more demanding test for the techniques due to the presence of potentially interfering compounds, both organic and inorganic, held in solution. Inorganic compounds found within groundwater matrices include nitrate, sulphate, chloride and bicarbonate, with a wide range of organic compounds such as sugars, amino acids, carboxylic acids and proteins also present (Ingrand *et al.*, 2002). Therefore, prior to adoption of an anion analysis strategy it was necessary to trial a range of available technologies for use under the unique conditions required.

5.2 AVAILABLE TECHNOLOGIES

5.2.1 Ion chromatography (IC) techniques

Most techniques currently available for bromate analysis use IC as the underlying separation mechanism. There are three main IC technologies used for bromate detection. 'Traditional' IC is coupled with conductivity detection (IC-CD) and provides the basis of formally-approved bromate analytical methodologies in areas such as the United States and UK (US EPA, 1997; Environment Agency, 1997; British Standards Institute, 2001). Two other variants with lower Method Reporting Levels (MRLs) have subsequently been developed for bromate analysis – use of a post-column reaction (IC-PCR) whereby a bromate-reactive compound is injected post-column into the eluent stream and the spectrally active product analysed spectrophotometrically, and incorporation of an on-line Inductively Coupled Plasma – Mass Spectrometer (IC-ICPMS) into the eluent stream.

5.2.1.1 Ion Chromatography with Conductivity Detection

The use of IC-CD for bromate analysis was officially recognised in 1989 with publication of US Environmental Protection Agency (US EPA) method 300.0, later amended in 1993 as Revision 2.1 (Hautman *et al.*, 2001). Subsequent refinements to the technique have generally been related to sample volume, column capacity and sample pre-treatment. Pre-treatment can involve sample pre-concentration (Thompson *et al.*, 2000) and/or removal of interfering anions, of which the most important is chloride due to close elution with bromate. One pre-treatment method is by use of commercially-available silver (for halide removal), barium (for sulphate removal) and hydrogen (for metal and carbonate removal) cartridges (Environment Agency, 1997; Sacher *et al.*, 1995; van der Jagt *et al.*, 1995). High capacity anion exchange columns are now utilised along with large sample volumes, generally 200 – 1000 µL, which can more efficiently resolve trace bromate signals whilst reducing or eliminating the need for pre-treatment and pre-concentration. US EPA method 300.1 is based on such a system, using the high-capacity Dionex AS9-HC column to give a Method Detection Limit (MDL) of 1.4 µg L^{-1} and MRL of 5 µg L^{-1} (Hautman *et al.*, 2001).

5.2.1.2 Ion Chromatography with Post-Column Reaction

To overcome the limitations of IC-CD a post-column step can be added. This extra step is specific to bromate and results in analysis being less prone to interference, thus reducing the requirement for sample pre-treatment. Hydrobromic acid reacts with bromate to form tribromide (Br_3^-), which can be detected at 268 nm within a range of 0.5 – 10,000 µg L^{-1} (Inoue *et al.*, 1997) or to a lower limit of 0.05 µg L^{-1} (Delcomyn *et al.*, 2001) with no sample pre-treatment. Chlorpromazine is a phenothiazine derivative, oxidised by bromate under acidic conditions, forming a spectrally-active species. Detection within a 0.5 – 100 µg L^{-1} range was noted without sample pre-treatment (Walters *et al.*, 1997). Sulfonaphtholazoresorcinol reacts with bromate to give a decrease in fluorescence at 585 nm, with a calibration range of 0.28 – 15 µg L^{-1} bromate suggested by Gahr *et al.* (1998). Fuschin can react with bromate in acidic medium, forming a strongly-absorbing product at 530 nm. A range of 0.1 – 100 µg L^{-1} was noted by Achilli and Romele (1999; 2000) using a standard carbonate/bicarbonate eluent, with Valsecchi

et al. (1999) trialling a tetraborate eluent and sample size up to 1.5 mL with no pre-treatment to improve resolution. Iodide in acidic solution (KI-$(HN_4)_6Mo_7O_{24}$) reacts with bromate to form I_3^-, which can be detected at 352 nm. Salhi and von Gunten (1999) used iodide with a carbonate eluent and 500 µL sample loop, giving a 0.1 µg L^{-1} detection limit with little interference from matrix anions. US EPA method 326.0 is based on the iodide reaction, and utilises a carbonate eluent and 225 µL sample volume to give bromate detection limits of 0.17 µg L^{-1} (US EPA, 2002). σ-dianisidine reacts with bromate to form a product that can be detected at 450 nm. A methodology incorporating this technique has also been published by the US EPA, designated Method 317.0. With a carbonate eluent and 225 µL sample volume, Method 317.0 can provide bromate detection limits of 0.12 µg L^{-1} (Hautman *et al.*, 2001) although pre-treatment may be necessary for some water types to remove chlorite (Wagner *et al.*, 2000).

5.2.1.3 Ion Chromatography with Inductively-Coupled Plasma Mass Spectrometry

The IC-ICPMS technique has been developed for detection of very low (sub-µg L^{-1}) bromate concentrations without pre-treatment. Nowak and Seubert utilised a high-capacity column with large sample volume (> 500 µL) and an ammonium nitrate eluent to give a detection limit of 50 – 65 ng L^{-1} (Seubert and Nowak, 1998; Nowak and Seubert, 1998). An ammonium nitrate eluent was used by Creed and Brockhoff (1999) and Schminke and Seubert (2000) with US EPA Method 321.8 also using this eluent along with large sample volumes (500 – 580 µL) and no pre-treatment to give an MDL of 0.3 µg L^{-1} (Hautman *et al.*, 2001). An ammonium carbonate eluent was used by Divjak *et al.* (1999) with a small sample size (50 µL), giving low detection limits (0.67 µg L^{-1}) and short analysis times (4 minutes). Carbonate/bicarbonate eluents were not used initially with IC-ICPMS due to column clogging by sodium and potassium salts (Fernandez *et al.*, 2001) but, with suppression, these eluents can be utilised to give detection limits on 'real' water samples of 1 ng L^{-1} using 500 µL (Fernandez *et al.*, 2001) or 100 µL (Dudoit and Pergantis, 2001) samples with no pre-treatment.

5.2.1.4 Advanced Ion Chromatography-based techniques

Other related technologies are also being developed which claim advantages in detection ability or freedom from interference. Electrospray ion chromatography-tandem mass spectrometry (IC-MS/MS) uses IC with a methanol/ammonium sulphate eluent to give a detection range of 0.05 – 10 μg L^{-1} bromate (Charles and Pepin, 1998), although extensive sample preparation was required. Ion chromatography with Atmospheric Pressure Ionisation Mass Spectrometry (IC-APIMS) is a development of IC-ICPMS, and utilises a sodium-based eluent for suppressed IC, or an ammonium citrate eluent in non-suppressed mode. Sample pre-treatment was necessary for 'real' water samples, with detection limits of 0.5 μg L^{-1} and 40 μg L^{-1} respectively (Buchberger and Ahrer, 1999).

5.2.2 Other techniques

Non IC-based methodologies have also been investigated and developed. These mainly fall into two categories: techniques using mass spectrometry (MS) detection; and relatively cheap and simple spectrophotometric methodologies. Methods based on electrochemical techniques have also been investigated recently for bromate analysis.

5.2.2.1 Mass Spectrometry-based techniques

Gas Chromatography-Mass Spectrometry (GC-MS) is an established analytical methodology, and has been utilised for bottled water bromate analysis. Nyman *et al.* (1996) converted bromate into a chlorobromostyrene derivative, which was then identified and quantified by GC-MS. Concentrations of 1 μg L^{-1} were detected in bottled water, although potential interferences were noted which may preclude use on a more demanding matrix. Gas Chromatography-Mass Spectrometry with Negative Chemical Ionisation (GC-NCIMS) reduces bromate to bromine, followed by reaction with organic material to form volatile, detectable species. The technique is sensitive and rapid, but exhibited strong interference in chlorinated waters (Magnuson, 1998). Negative Thermal Ionisation-Isotope Dilution Mass Spectrometry (NTI-IDMS) was found by Diemer and Heumann (1997) to be a very accurate method, but time-consuming sample preparation may preclude its use for routine analytical purposes. Electrospray Ionisation-high Field

Asymmetric waveform Ion Mobility Spectrometry-Mass Spectrometry (ESI-FAIMS-MS) is capable of analysing bromate at ng L^{-1} concentrations with minimal interference (Barnett *et al.*, 1999), and has also been shown to detect the related contaminant perchlorate in wastewater and river water samples at sub µg L^{-1} concentrations (Ells *et al.*, 2000). However, no further work has been published on bromate analysis using this technique. ICPMS coupled with flow injection (FI-ICPMS) has also shown some promise, with low detection limits (0.13 µg L^{-1}) and short analysis times (10 minutes per sample) in 'real' samples (Elwaer *et al.*, 2000).

5.2.2.2 Spectrophotometry techniques

These methodologies utilise similar reactions to IC-PCR, but with direct spectrophotometric detection. Phenothiazones produce a coloured species following reaction with bromate under acid conditions. Chlorpromazine is the most widely trialled phenothiazone. Gordon and co-workers (Gordon *et al.*, 1994; Gordon and Bubnis, 1995) concluded chlorpromazine was accurate over a 1 – 40 µg L^{-1} range, although there was interference from some metal ions. Triflouperazine, another phenothiazone, can be detected at 504 nm, with a detection limit of 0.67 µg L^{-1} and range of 1 – 700 µg L^{-1} (Farrell *et al.*, 1995). However, it has been noted spectrophotometric methods using phenothiazones are susceptible to interference by humic substances found in natural water samples (Mitrakas *et al.*, 2000). Reduced fuschin can be reacted with bromine, produced from bromate by reaction with metabisulphate. The red product is detected at 530 nm, within a range of 1 – 40 µg L^{-1}. Pre-treatment is required to remove heavy metals, but the method is then straightforward (Romele and Achilli, 1998). Methylene blue can also be reacted with bromate under acidic conditions, with analysis at 745 nm. The method produced a detection range of 4 – 50 µg L^{-1} and was largely free of interference, although hypochlorite had to be removed by hydroxylamine hydrochlorite addition. Although not sensitive enough to analyse low bromate levels (<10 µg L^{-1}), the authors concluded this technique showed promise as a simple field method (Ingrand *et al.*, 2002).

5.2.2.3 Electrochemical techniques

Electrochemical techniques utilizing potentiometry have been applied for the detection of bromide, such as the use of ion selective electrodes (ISEs) (Farange and Janjic, 1982; Jwo and Cheng, 1989; Adamcikova *et al.*, 2003). These electrodes are capable of selectively measuring the concentration of a particular ionic species. The performance of an ISE is based on the selective passage of charged species from one phase to another leading to the creation of a potential difference. Operation involves dipping the electrodes in a test solution and reading the signal from a voltmeter. ISEs are reasonably sensitive, with analytical capability of commercially-available electrodes for bromide in the range 40 μg L^{-1} – 1 g L^{-1} (Ahmad *et al.*, 2001). ISEs perform better in clean solutions without interferences. The main disadvantages are that the limit of detection can be high and selectivity poor in some environmental samples.

Very limited reports have been found in the literature for bromate detection using electrochemical methods. The use of a glassy carbon electrode modified with a sol-gel thin containing heteropolyanion was found to exhibit high electrocatalytic response for bromate reduction (Wang *et al.*, 2001). A linear relationship was found between the catalytic current taken at –0.4 Volts and bromate concentration in 0.5 Molar H_2SO_4. However, this electrode failed to actively catalyse bromate at pH 4.6 since the reduction process needs proton participation (Cheng and Dong, 2000). A composite film containing heteropolyanion fabricated on a modified gold electrode was also investigated (Wang and Du, 2003). This electrode showed good catalytic activity for the reduction of bromate in acidic solutions. More recently a tungsten oxide film was used as an amperometric sensor for the analytical determination of bromate, chlorite and nitrite ions. Detection limits evaluated using cyclic voltammetry were high, being approximately 8, 27 and 32 g L^{-1} respectively (Casella and Contursi, 2005). Other amperometric sensors based on a titania sol-gel matrix have also been investigated for determination of bromate, iodate and hydrogen peroxide, giving a linear range from 1.6 – 350 mg L^{-1} and detection limit of 0.4 mg L^{-1} for bromate in acidic aqueous solutions (Li *et al.*, 2005). However, most sensors reported for bromate analysis are still in the development stage.

5.3 COMPARISON OF METHODOLOGIES

5.3.1 Selection of techniques for trial

Due to the range of possible methodologies available for analysis of bromate, a comparative study was initiated to elucidate the most suitable technique for application in this project. Two systems were available for use and comparison; a Shimadzu vp-series High Performance Liquid Chromatography system (Shimadzu, Milton Keynes, UK) incorporating an Ultra-violet/Visible light detector; and a Dionex ICS-2500 Ion Chromatography system (Dionex (UK) Ltd, Leeds, UK) with an electrochemical (including conductivity) detector. These two systems were trialled using four analytical columns, three in conjunction with the Shimazdu HPLC system and the latter column incorporated with the Dionex IC system:

- Hamilton PRP-X100 (4.1 x 150 mm) anion exchange column (Hamilton Company, Reno, USA)
- Hamilton PRP-X110 (4.1 x 150 mm) anion exchange column (Hamilton Company, Reno, USA)
- Metrohm MetroSupp-5 anion exchange column (Metrohm UK, Buckingham, UK)
- Dionex AS9-HC 4 mm i.d. anion exchange column (Dionex (UK) Ltd, Leeds, UK)

In addition, a spectrophotometric technique was compared for use as a simple laboratory or field based method, based on the reaction between bromate and methylene blue, with detection at 745 nm (Haigh, 2002). Finally, an external laboratory (Veolia Water Laboratories, Staines, Middlesex, England) provided data for IC-PCR analysis on groundwater samples.

5.3.2 Materials and Methods

The six techniques trialled were examined for bromate analysis in both groundwater and wastewater samples. Equipment, methodologies and operating conditions investigated are given in Table 5.1. Where possible, manufacturers' or suppliers' recommended conditions were utilised, with any deviations from these conditions also outlined in Table 5.1.

Two clean water matrices were investigated; standard solutions containing anions of interest (AnalaR grade potassium bromate and a proprietary IC anion standard solution (Fisher Scientific, Loughborough, UK) in Milli-Q (18.2 MΩ) water); and a bromate-contaminated groundwater source collected from two areas within the contaminated aquifer. Further information on the two groundwater sources utilised ('Hatfield' and 'House Lane') is given in Section 6.1, with selected properties of the water supplies outlined in Table 6.1. For the purposes of this trial the 'Hatfield' source was named groundwater GW-A and the 'House Lane' source groundwater GW-B. Fresh wastewater samples were collected from a pilot-scale MBR, using the sludge biomass as a matrix for spiking with AnalaR-grade potassium bromate and potassium bromide solutions. Wastewater samples were filtered using a 0.22 μm syringe-tip filter (Millex PVDF 25 mm filter; Millipore, Carrigtwohill, Co.Cork, Ireland) prior to analysis. No further pre-preparation, concentration or preservation was carried out on any groundwater or wastewater samples, which were stored in a cold room at 6°C prior to analysis. No dilutions were made with the exceptions of the groundwater samples for Methods 1 and 6, in both cases to bring results within the calibration range. Standard solutions and eluent were made up shortly before analysis from stock solutions, and eluent was degassed by sonication immediately prior to use.

Anion concentrations trialled with each system were based on the recommended ranges of the technique, with a lower bromate detection limit ascertained for each method. The bromate detection limit was assumed for the purposes of this trial to be the lowest concentration trialled where a discernable analytical peak, or change in absorbance for spectrophotometry, was reproducibly obtained. The range of bromate

concentrations studied in each case is given in Table 5.2. In each case a calibration line was obtained for the bromate standard concentrations studied, with an indication of linearity given by the R-squared value (Table 5.2). Bromide detection was also studied, with a calibration to give R-squared values carried out where possible (Methods 2, 3 and 5 only).

Table 5.1 - Operating conditions of techniques studied

		High Performance Liquid Chromatography			Ion Chromatography	
	Spectrophotometry	Direct UV detection	Direct UV detection	Post-column reaction	IC-CD	IC-PCR
Method number	1	2	3	4	5	6
Equipment	Jenway 6505 spectrophotometer	Shimadzu vp-series system	Shimadzu vp-series system	Shimadzu vp-series system with Metrohm post-column reactor unit	Dionex ICS-2500 system	Dionex DX-600 system
Column	n/a	Hamilton PRP-X100 (4.1 x 150 mm) + guard column	Hamilton PRP-X110S (4.1 x 150 mm) + guard column	Metrohm MetroSupp-5	Dionex AS9-HC 4 mm i.d. (Dionex AG9-HC 4 mm i.d. guard column)	Dionex AS9-HC 2 mm i.d. (Dionex AG9-HC 2 mm i.d. guard column)
Based on methodology	Haigh, 2002	Application note #318 (Developed by Hamilton Company, Reno, USA)	Application note #356 (Developed by Hamilton Company, Reno, USA)	Application work AW CH6-0777-032003 (Developed by Metrohm UK, Buckingham, England)	Application Note 81 (Developed by Dionex Corp., Sunnyvale, USA)	Application note 149 (Developed by Dionex Corp., Sunnyvale, USA)
Eluent	n/a	1.7 mM sodium bicarbonate + 1.9 mM sodium carbonate + 0.1 mM sodium thiocyanate	1.7 mM sodium bicarbonate + 1.9 mM sodium carbonate + 0.1 mM sodium thiocyanate	100 mM sulphuric acid + 45 µM ammonium molybdate tetrahydrate	9 mM sodium carbonate	6.75 mM sodium carbonate
Eluent flow rate	n/a	2 mL min^{-1}	2 mL min^{-1}	0.7 mL min^{-1}	1 mL min^{-1}	0.38 mL min^{-1}
Injection/sample volume	41.2 mL	50 µL (1.5 mL vials)	50 µL (1.5 mL vials)	200 µL (1.5 mL vials)	250 µL (5 mL filtercap vials)	200 µL
Post-column reagent	n/a	n/a	n/a	0.26 M potassium iodide	n/a	0.26 M potassium iodide (US EPA, 2002) at 0.4 mL min^{-1}
Other reagents	250 µL methylene blue (200 mg L^{-1}) + 8.6 mL hydrochloric acid (37%)	n/a	n/a	n/a	n/a	n/a
Method notes (Method 1) / Alterations to published methodology (Methods 2-6)	Mix reagents and 40 mL sample in 50 mL volumetric flask, complete to 50 mL with sample; wait 10 min and measure using 4 cm cuvettes	Absorbance at 220 nm instead of conductivity detection	Absorbance at 220 nm instead of conductivity detection	Metrohm MetroSupp-5 instead of Phenomenex Star Ion A300 HC		Autosuppression external water mode at 38 mA; AMMS III suppressor; PCR heater temperature 40°C
Anions studied	Bromate	Bromate; Bromide	Bromate; Bromide	Bromate	Bromate; Bromide	Bromate
Detection	Absorbance at 745 nm	Absorbance at 220 nm (Shimadzu SPD-10A vp)	Absorbance at 220 nm (Shimadzu SPD-10A vp)	Absorbance at 352 nm (Shimadzu SPD-10A vp)	Conductivity (Dionex ED50)	Absorbance at 352 nm (Dionex AD25)

5.3.3 Analytical trial results

All the techniques trialled were able to detect bromate within both standard solutions and an undiluted groundwater matrix in the mg L^{-1} range, with only HPLC using the Hamilton PRP-X100 column unable to detect bromate at lower (µg L^{-1}) concentrations. Results obtained for each technique are given in Table 5.2. Example chromatograms for standard samples are given in Figure 5.1 and chromatograms for groundwater samples in Figure 5.2. Wastewater samples provided a much more demanding matrix, with extensive interference observed using spectrophotometry and direct UV detection. Example chromatograms for wastewater samples are given in Figure 5.3. In all figures, an enlarged section of the trace containing bromate and bromide peaks is also given in boxed inserts where necessary.

Spectrophotometry (Method 1) was trialled using bromate concentrations in the 10 – 50 µg L^{-1} range, but was prone to sampling error upon duplication, even using standard samples. An R-squared value of only 0.6809 was obtained for this method. Groundwater samples were also investigated and, for GW-B (diluted 10 times to come within the calibration range), an average result of 201.7 µg L^{-1} was obtained (Standard deviation = 58.3 µg L^{-1}, n=3). Analysis of uncontaminated groundwater samples indicated the technique was not affected by interfering compounds. However, wastewater samples were prone to extensive interference and therefore no calibration was possible. A previous study on bromate analysis in groundwater using the methylene blue technique (Haigh, 2002) obtained a more acceptable R-squared calibration value of 0.8978, but suggested that use of a more accurate method in tandem was also necessary for quality control purposes. The study concluded a linear relationship with absorbance within the range 10 – 150 µg L^{-1} would be obtainable, but that higher concentrations would require dilution.

HPLC techniques using direct UV detection (Methods 2 and 3) were able to simultaneously detect bromate and bromide, and peaks were well defined in both standard and groundwater samples with elution times under 10 minutes. However, the limitations of the PRP-X100 column were apparent, with a bromate detection limit of

only 5 mg L^{-1} (Figures 5.1a and 5.2a). The PRP-X110S column was able to detect bromate up to two orders of magnitude lower, with a detection limit of 50 µg L^{-1} (Figures 5.1b and 5.2b). Other peaks were apparent in groundwater samples, most notably chloride, but did not interfere with either the bromate or bromide peaks. R-squared values showed good linear correlation (> 0.97 in all cases) within the calibration ranges used, for both bromate and bromide (Table 5.2). PRP-X100 calibration was carried out in the range 5 – 100 mg L^{-1}, and two ranges were trialled for the PRP-X110S, with a higher (500 – 20,000 µg L^{-1}) and lower range (50 – 1,000 µg L^{-1}) both giving good linear correlation. Wastewater samples showed greater interference with both methodologies. Bromate and bromide were detected with the PRP-X100 column at a concentration of 50 mg L^{-1}, although bromate peak resolution was poor (Figure 5.3a). No bromate or bromide was detected in a wastewater matrix with a 20 mg L^{-1} spike of both anions. Analysis of spiked wastewater samples with concentrations up to 20 mg L^{-1} using the PRP-X110S column resulted in no bromate or bromide detection. Extensive interference was observed around the elution time of both anions (Figure 5.3b).

IC-CD (Method 5) was trialled using a range of anions in the standard solutions, which all eluted within a 25 minute run time. Peaks were well separated in both standard and groundwater samples, although the relatively long run time was required to ensure adequate separation of bromate from the much larger chloride peak found in groundwater samples (Figures 5.1d and 5.2d). Sample preparation to eliminate closely-eluting anions would have reduced the chloride peak but, as with the other techniques trialled, this was not found to be necessary for bromate and bromide peaks to be well resolved. Good linear correlation was achieved using all anions, with bromate calibrated within the range 5 – 2,000 µg L^{-1} (Table 5.2). Less baseline noise was observed than with any of the HPLC methodologies. Analysis of bromate and bromide-spiked wastewater samples led to anomalously high bromate readings within some samples in the range 2 – 1000 µg L^{-1}, suggesting the bromate peak was being partially masked by interference. Bromide concentrations were also anomalously high at low concentrations with some samples (20 µg L^{-1}), although this effect was not noted at higher concentrations (≥ 500 µg L^{-1}). The chromatogram obtained with a 20 µg L^{-1} bromate and bromide spike is given in Figure

5.3d. However, trials with previously freeze-dried MBR sludge samples showed some wastewater samples could be successfully analysed with IC-CD without sample pre-treatment. Even so, careful investigation of peaks should be undertaken prior to data collection to ensure interference is not occurring.

Post-column reaction methods were trialled using both HPLC (Method 4) and IC (Method 6) systems. Due to the specificity of the applications only bromate detection was possible. With the HPLC methodology, well-resolved peaks were obtained using standard and groundwater samples, with a detection limit of 10 μg L^{-1}. Interference was minimal even in samples with high ionic loadings, although some baseline noise was evident (Figures 5.1c and 5.2c). Good bromate peak resolution was also obtained with wastewater samples, specificity of the post-column reaction eliminating much of the interference obtained with other methods (Figure 5.3c). IC-PCR trials on standard and groundwater samples were carried out by the external laboratory. Although a range of anions can be analysed on the system, which incorporates conductivity and UV detection capabilities, only PCR detection of bromate was investigated for this trial. As with the HPLC-PCR system, good detection capabilities of standard and groundwater samples (Figures 5.1e and 5.2e) with minimal interference and a detection limit of 0.5 μg L^{-1} was obtained. An R-squared value of 0.9996 within the range 10 – 50 μg L^{-1} was achieved. It was not possible to analyse wastewater samples by IC-PCR for this trial.

Table 5.2 - Bromate concentration range studied and bromate/bromide detection in standard and groundwater samples

		Spectrophotometry	High Performance Liquid Chromatography			Ion Chromatography	
		Method 1	Method 2	Method 3	Method 4	Method 5	Method 6
Bromate range studied ($\mu g\ L^{-1}$)		10 – 50	1,000 – 100,000	1 – 20,000	1 – 100,000	1 – 2,000	1-50
Bromate detection limit ($\mu g\ L^{-1}$)		10	5,000	50	10	5	0.5
Simultaneous bromide detection?		No	Yes	Yes	No	Yes	No[a]
Sample run time (min)		n/a	12	15	15	25	25
Bromate elution time (min)		n/a	5.7	3.3	10.6	6.5	5.7
Bromide elution time (min)		n/a	7.7	3.8	n/a	12.8	n/a
R-squared calibration values	Bromate	0.6809	0.9985	0.9985[b]; 0.9984[c]	0.9995	0.9993	0.9996
	Bromide	n/a	0.9933	0.9923[b]; 0.9752[c]	n/a	0.9963	n/a

[a]Simultaneous detection possible with addition of conductivity detector; [b] Higher calibration range; [c]Lower calibration range; n/a – not applicable

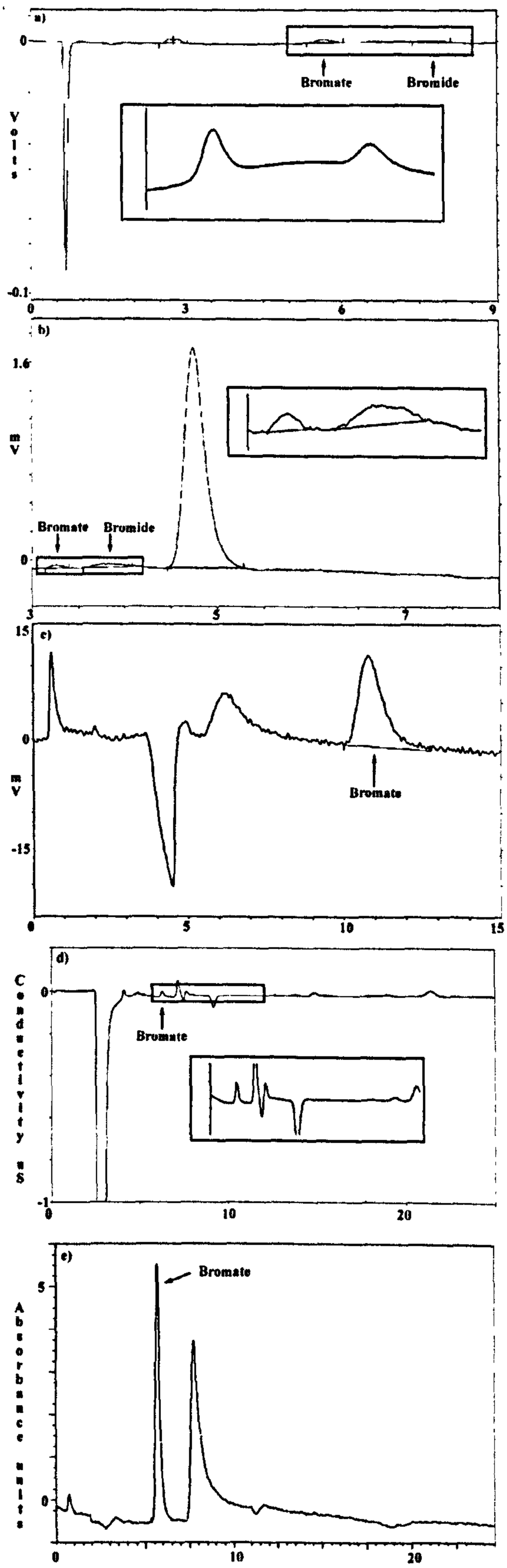


Figure 5.1 - Peak traces for standard samples containing bromate and bromide spikes; a) Method 2, 5 mg L^{-1}; b) Method 3, 50 µg L^{-1}; c) Method 4, 10 µg L^{-1} BrO_3^-; d) Method 5, 10 µg L^{-1} BrO_3^-; e) Method 6, 10 µg L^{-1} BrO_3^-. Time (minutes) given on horizontal axis for all graphs.

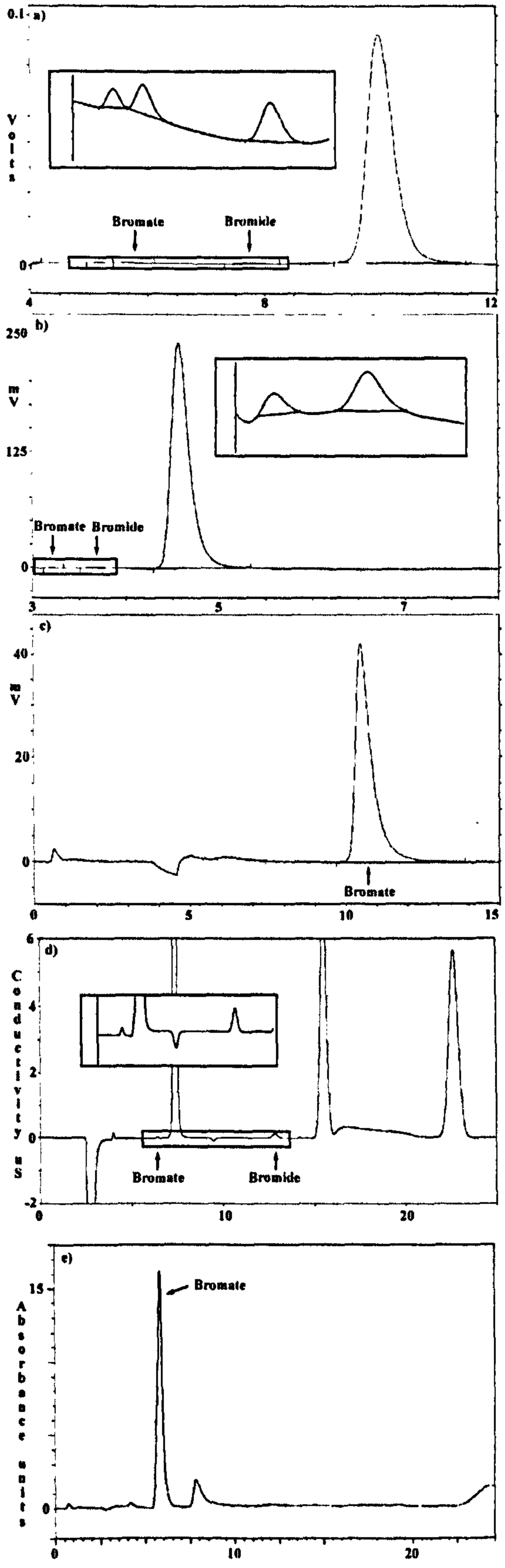


Figure 5.2 - Peak traces for groundwater samples; a) Method 2, GW-A; b) Method 3, GW-A; c) Method 4, GW-B; d) Method 5, GW-B; e) Method 6, GW-B (10 x dilution). Time (minutes) given on horizontal axis for all graphs.

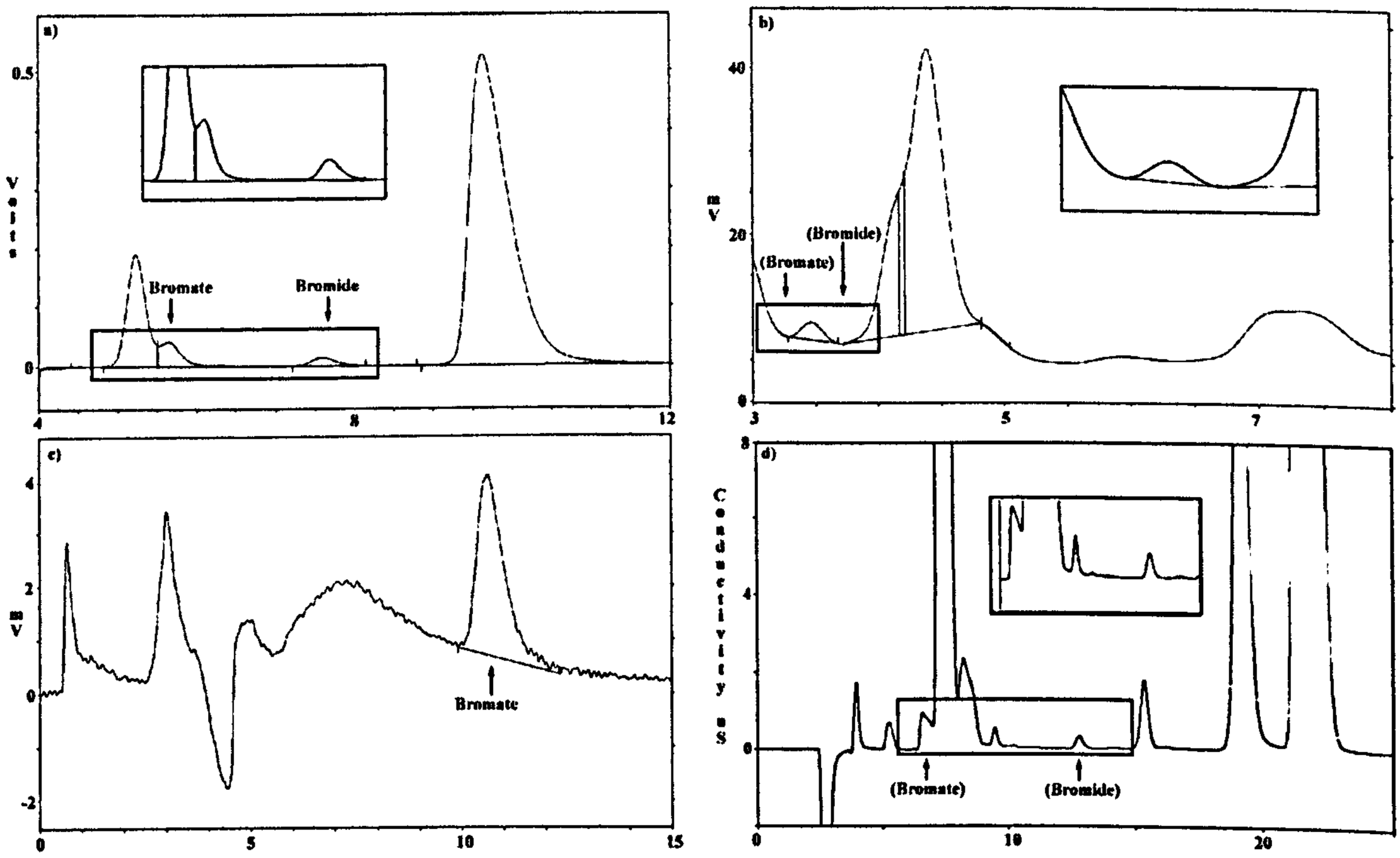


Figure 5.3 - Peak traces for wastewater samples containing bromate and bromide spikes; a) Method 2, 50 mg L^{-1}; b) Method 3, 20 mg L^{-1} (no peaks visible) ; c) Method 4, 20 µg L^{-1} BrO_3^-; d) Method 5, 20 µg L^{-1} (peak interference). Time (minutes) given on horizontal axis for all graphs.

5.4 SELECTION OF AN ANION ANALYTICAL METHODOLOGY

Following completion of the trial, a number of conclusions could be reached regarding the systems tested. Although all were capable of detecting bromate within groundwater, some were not able to fulfil the attributes outlined as necessary for successful application within the context of this project.

- Spectrophotometry using the methylene blue technique is a cheap method able to give a rough indication of bromate concentrations in groundwater matrices, for example during an initial field study, but inherent inconsistencies preclude it for use where accurate and reproducible data is required. This method would also not be of use for wastewater studies, and was not further pursued within the context of the project.

- HPLC with direct UV detection is capable of accurate bromate and bromide detection in groundwater, but column choice is critical. Although producing similar peak traces, the PRP-X100 column was unable to detect bromate at concentrations below 5 mg L^{-1}. The PRP-X110S column could detect bromate at considerably lower concentrations, but the detection limit of 50 µg L^{-1} was still too high for use at the lower concentrations required for this project. Neither system was able to satisfactorily analyse wastewater samples.

- IC-CD using the Dionex AS9-HC column produced comparable results to the HPLC systems, but use of a high-capacity column and large injection loop (250 µL) led to increased resolution at lower concentrations, and allowed analysis of samples at the 10 µg L^{-1} bromate regulatory limit. The method, comparable to US EPA Standard Method 300.1, is the technique of choice for groundwater analysis if simultaneous detection of a range of anions is required using a standard conductivity-based IC as, apart from the column, no additional equipment is required.

- HPLC and IC using PCR are both specific to bromate and, as such are less prone to interference from other compounds. If bromate is the only compound to be analysed, then these methodologies provide detection in groundwater to concentrations below regulatory limits. PCR-based techniques are also the method of choice for samples with high ionic loadings, such as wastewater samples. HPLC-PCR was able to detect bromate at ≤ 20 µg L^{-1} in wastewater and, although not tested in this trial it is likely IC-PCR would have similar or greater capabilities. HPLC-PCR is the cheaper option, only requiring a post-column reactor and pump in addition to a standard HPLC system. However, the eluent used in this application precludes simultaneous detection of other anions, which can be achieved using an IC system with post-column capability and the additional equipment required to carry out conductivity as well as absorbance detection.

Selection of an anion analysis methodology for this project was based on the trials outlined above. Equipment availability and cost also constrained method choice, but the most suitable strategy was determined to be Method 5 (IC-CD) for both groundwater and wastewater analysis. With a detection limit of 5 µg L^{-1}, IC-CD was able to analyse bromate in groundwater to the required lower limit and, with sample dilution was also suitable for analysis of high concentrations. Limited wastewater analysis was undertaken during the project, with only sorption trials (Section 6.3.2) requiring investigation of bromate and bromide concentrations. These samples were successfully analysed using IC-CD with no peak interference noted, and this method was therefore again utilised to provide comparable results to groundwater analysis.

Use of the IC-CD method incorporating conductivity detection led to simultaneous analysis of a range of anions, including those required for the project. A sample run-time of 25 minutes allowed duplicate analysis of approximately one sample per hour, and autosampler capability of 66 samples facilitated high volume throughput where necessary. Analysis of standard samples showed all analytes to have linear characteristics within the required concentration range, and they were calibrated within

the limits given in Table 5.3. No sample pre-treatment was required, and interference with the peaks of interest did not occur even with chloride eluting close to bromate. Analysis of higher concentrations was achieved by sample dilution where necessary (5x, 25x and 50x dilutions). In practice a minimum 5x dilution was generally utilised, leading to a limit of detection (LOD) of five times the undiluted LOD value. Any concentration lower than this was recorded as being one half of the LOD (Table 5.3). This method of recording concentrations below the LOD has been used previously (Kirisits *et al.*, 2000) and is considered as good or better than more complicated mathematical methods (Clarke, 1998).

Table 5.3 - Calibration range and practical LOD for anions of interest

Anion	Undiluted calibration range (mg L^{-1})		Recorded LOD (mgL^{-1}; using 5x dilution)
	Minimum	Maximum	
Bromate	0.005	2.0	0.01
Bromide	0.010	2.0	0.03
Nitrate	0.020	2.4	0.10
Nitrite	0.025	2.0	0.06
Sulphate	0.012	2.4	0.03

6 MATERIALS AND METHODS

This chapter commences by detailing influent supplies and analytical procedures utilised in the overall project. Materials and methods employed for the three trials investigating bromate contamination within wastewater are then outlined, comprising respiration inhibition, biomass sorption and wastewater process dosing trials. Finally, procedures undertaken to study groundwater bromate bioremediation are given, consisting of trials using a laboratory-scale chemostat, biomass isolation and characterisation, batch flask trials and use of a pilot-scale bioreactor system.

6.1 INFLUENT SUPPLIES

6.1.1 Wastewater biomass and influent supplies

MBR biomass was used during wastewater trials as a matrix representative of biomass contained within biological sewage treatment systems. Biomass was maintained for all wastewater trials within the two-vessel pilot-scale MBR utilised during process dosing trials (Section 6.3.3). Feed supplied to these bioreactors was macerated partially settled municipal sewage from the Cranfield University sewage treatment works.

6.1.2 Groundwater

Influent supplies for all groundwater trials were collected from boreholes within the contaminated aquifer. Four separate boreholes were utilised, each producing water with a different concentration ratio of bromate and bromide contamination. Selected characteristics of the four abstracted water supplies are given in Table 6.1.

Within each borehole, seasonal variations and plume movement led to abstraction at different times over the project timescale producing water with slightly varying characteristics, including levels of bromate and bromide contamination. This is illustrated in Figure 6.1, a plot of bromate and bromide concentrations from April 2000 – January 2005. It can be seen the abstraction borehole exhibited a generally increasing trend of bromate and bromide contamination within abstracted samples over the five-year sampling period. Anion values averaged over the entire project period were therefore not

used for data analysis, with values obtained during each individual trial instead given in the relevant results section.

Table 6.1 - Major parameters of influent water supplies

Parameter	Water supply			
	Hatfield**	Nashes Farm**	Orchard Garage***	House Lane***
Distance from source site (km)*	5.7	1.3	0.5	< 0.01
Borehole depth (metres below datum)*	2	28	10	12
Borehole usage	9 ML abstraction borehole, now only used for sampling	Occasional washing of vehicles	Regular washing of vehicles	Occasional sampling
Pre-sampling procedure	Run for 15 – 20 minutes	Run for 5 – 10 minutes	Run for 5 – 10 minutes	Run for 5 – 10 minutes
pH	7.04	6.99	7.04	7.03
Total Organic Carbon (mg L^{-1})	1.5	1.9	n/d	n/d
Bromate as BrO_3 (mg L^{-1})	0.23	1.38	1.02	< 0.01
Bromide as Br (mg L^{-1})	0.64	3.97	2.72	11.13
Nitrate as NO_3 (mg L^{-1})	30	41	32.16	8.49
Nitrite as NO_2 (mg L^{-1})	0.011	0.010	< 0.06	< 0.06
Sulphate as SO_4 (mg L^{-1})	24	36	23.21	54.78
Chloride as Cl (mg L^{-1})	26	32	20.27	> 60

* Approximate values

** Sampled 22 January 2003; Analysis completed by Veolia Water Laboratories, Staines, UK

*** Sampled 23 May 2005

n/d – no data

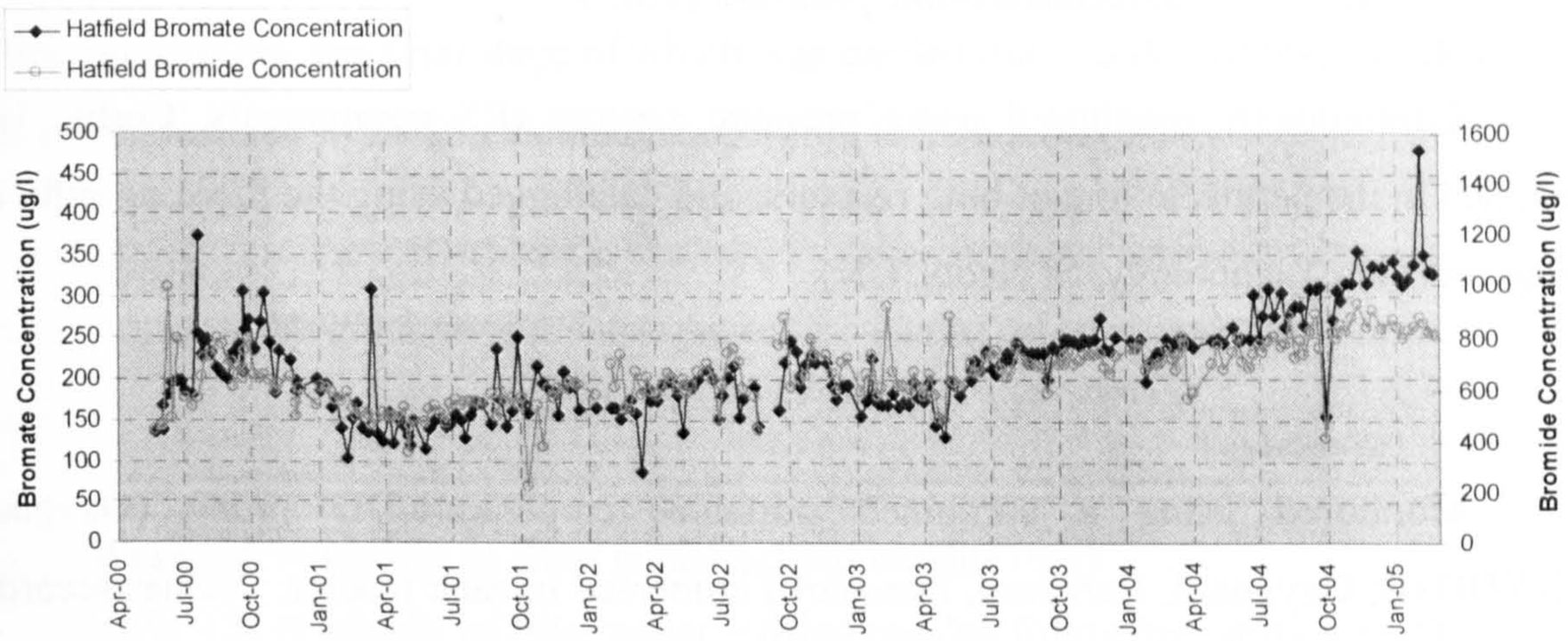


Figure 6.1 - Bromate and bromide concentrations in abstracted samples from Hatfield between April 2000 and January 2005 (data from Veolia Water)

6.2 GENERAL ANALYTICAL PROCEDURES

General analytical procedures are given for both wastewater and groundwater trials. Wastewater procedures relate to analysis carried out for wastewater process dosing trials only unless otherwise indicated.

6.2.1 Wastewater analysis

6.2.1.1 Flux

Flow rate measured manually by permeate collection within a measuring cylinder over a specified time. Flux (L m^{-2} hr^{-1}, or LMH) calculated using Equation 6.1.

$$Flux\,(LMH) = \frac{Permeate\ flow\ (L\,hr^{-1})}{Membrane\ surface\ area\,(m^2)}$$

Equation 6.1

6.2.1.2 Transmembrane pressure (TMP)

Continuously monitored using pressure sensors (RS components, Corby, UK) installed in the permeate line of both reactors, and datalogged using the PicoLog software program (Pico Technology, St Neots, UK).

6.2.1.3 Temperature/dissolved oxygen (DO)

Monitored using a combined portable temperature/DO probe (Oxyguard OXYHO1B; Oxyguard, Berkerød, Denmark) immersed in each reactor. Values recorded upon stabilisation of each reading.

6.2.1.4 Mixed liquor suspended solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS)

Tests were carried out using Standard Methods 2540D and 2540E respectively (APHA, 1998) on process dosing and respirometry samples. MLSS gives a measure of the sample solids content retained on a filter following drying, and MLVSS the proportion lost upon ignition (APHA, 1998). For MLSS determination, a 10 mL sample of well-mixed sludge was filtered under vacuum (Speedivac 2 rotary vacuum pump, D. Benway Ltd, Boreham Wood), using a Buchner flask and filter system fitted with a dried, pre-weighed glass-fibre filter paper (Whatman GC/F 70 mm diameter, Fisher Scientific Ltd, Loughborough, UK). Following filtration, the filter paper was removed and placed in an oven (Gallenkamp Hotbox oven, OVB 350, Walton-on-Thames) for a minimum of 3 hours, before being cooled in a dessicator, reweighed, and MLSS mass calculated according to Equation 6.2.

$$MLSS = \frac{(A-B)\times 1000}{Sample\,volume\,(mL)}$$

Equation 6.2

Where A = Weight of filter paper + dried residue (mg)

B = Weight of filter paper (mg)

MLVSS content was determined by ignition of the MLSS residue in a furnace

(Carbolite, Sheffield, UK) set at 500 ± 50°C for 4 hours (process dosing trials only). Following cooling, the final stage of which was carried out in a dessicator, the filter paper was reweighed and MLVSS calculated according to Equation 6.3.

$$MLSS = \frac{(A-B)\times 1000}{Sample\,volume\,(mL)}$$

Equation 6.3

Where A = Weight of filter paper + dried residue (mg)

B = Weight of filter paper + dried residue following ignition (mg)

6.2.1.5 Extracellular Polymeric Substance (EPS) and Soluble Microbial Product (SMP)

EPS and SMP are both bacterial-derived organic materials which can influence and enhance membrane fouling in an MBR process (LeClech, 2002). In this context EPS refers to the organic material bound to biomass recovered by a process of heating and solvent extraction following the procedure of Zhang *et al.* (Zhang *et al.*, 1999). SMP refers to the soluble/colloidal fraction with supernatant collected after centrifuging the biomass sample for 5 minutes at 5000 rpm. Protein and carbohydrate concentrations were determined by absorbance at 595 and 480 nm (Jenway 6505 spectrophotometer, Patterson Scientific Ltd, Luton, UK) with reference to bovine serum albumin and glucose standards respectively.

6.2.1.6 Carbonaceous Oxygen Demand (COD)

Investigated in EPS and SMP fractions plus influent and permeate samples during process dosing trials. Measured by Merck 'Spectroquant' test kit no. 14895 (VWR International, Lutterworth, UK). A 2 mL sample was pipetted into the supplied reaction cell, the cap replaced and contents vigorously mixed. The cell was heated for 120 minutes at 148°C in a Hach COD heater (Hach Instruments, Loveland, USA) and cooled. The completed test was measured spectrophotometrically at 447 nm using a Merck Nova 60 spectrophotometer (VWR International, Lutterworth, UK). Calibration range of test 15 – 300 mg L^{-1} COD.

6.2.1.7 Ammonia (NH_4^+)

Measured by Merck 'Spectroquant' test kit no. 14559 (VWR International, Lutterworth, UK). A 0.1 mL sample was pipetted into the supplied reaction cell, the cap replaced and contents mixed. One dose of supplied reagent NH_4-1K was added to the reaction cell, the cap replaced and contents mixed. The cell was left to stand for 15 minutes and the completed test measured spectrophotometrically at 712 nm using a Merck Nova 60 spectrophotometer (VWR International, Lutterworth, UK). Calibration range of test 4.0 – 80.0 mg L^{-1} NH_4-N (5.2 – 103.0 mg L^{-1} NH_4^+).

6.2.1.8 Particle Size Distribution

Gives a measure of the size of biomass flocs formed within the MBR reactor. Assessed within process dosing samples using the Mastersizer 2000 (Malvern Instruments, Malvern, UK), which outputs results as standard 'percentile' size readings, represented as d(0.1), d(0.5) and d(0.9). For example d(0.5) represents the size at which 50% of the sample is larger and 50% smaller.

6.2.1.9 Dynamic viscosity (μ)

Quantifies a fluids' resistance to flow, assessed using a Brookfield DV-E viscometer (Brookfield viscometers Ltd, Harlow) which measures the viscous drag of a rotating spindle at a constant shear rate within a 16 mL sludge sample. Rotation speeds of 20 – 100 rpm were trialled, with results outputted in units of mPa s^{-1}.

6.2.1.10 Capillary Suction Time (CST)

Gives a measure of the relative dewaterability of a 6.4 mL sludge sample through a filter paper in a quick and easy test, although lack of reproducibility means it is not an absolute parameter (LeClech, 2002). Measured using a Triton 200 CST filterability tester and standard filter papers (both Triton Electronics Ltd, Essex, UK), which outputs the length of time required for liquid within the sample to move a set distance along the filter paper by capillary action.

6.2.1.11 Cake filtration resistance (α)

Gives a measure of the fouling potential of a sludge biomass. Analysis was performed using the methodology outlined in Germain (2004), using KST 47 Pressure Filtration apparatus (M-Tech Diagnostics Ltd, Warrington, UK). Sludge samples were placed in the filter cell reservoir and filtered across a 0.2 µm membrane (M-Tech Diagnostics Ltd, Warrington, UK) at a pressure of 1 bar. A beaker placed on a balance collected the filtrate, and the filtrate mass was recorded at one-second intervals by a data-logging computer connected to the balance. Specific cake resistance (m kg^{-1}) was then calculated using the method given in Germain (2004).

6.2.1.12 High Performance Size Exclusion Chromatography (HPSEC)

HPSEC allows characterisation of the molecular size distribution of a solution, with larger molecules eluting faster from the analytical column than smaller components. HPSEC was carried out using a Shimadzu vp-series HPLC system (Shimadzu, Milton Keynes, UK), fitted with a BioSep SEC-3000 analytical column (supplied by Phenomenex, Macclesfield, UK). Eluent was 0.01 Molar sodium acetate at a flow rate of 1 mL min^{-1}, with detection by absorbance at 254 nm (Shimadzu SPD-10A vp uv/vis detector; Shimadzu, Milton Keynes, UK).

6.2.2 Groundwater analysis

6.2.2.1 Flow rate

Monitored using manual measurement techniques. Chemostat groundwater and glucose influents, and pilot-scale bioremediation reactor nutrient influents were measured using a pipette containing a known volume of influent, with the time taken to empty the pipette measured giving an estimate of flow rate. Pilot-scale bioremediation system groundwater flow rate was measured by timing the interval required for emptying a beaker containing a known volume of influent groundwater.

6.2.2.2 Temperature

Chemostat supernatant temperature monitored via standard laboratory mercury

thermometers (Fisherbrand; Fisher Scientific, Loughborough, UK) permanently mounted within each reactor via an airtight seal. Pilot-scale bioremediation system supernatant temperature was monitored using digital thermometers (Maplin Electronics, Barnsley, UK) with probes permanently mounted within each reactor via an airtight seal. Influent temperatures was measured using a portable temperature probe (Hanna HI 8424; Hanna Instruments, Leighton Buzzard, UK).

6.2.2.3 pH

Measured during all groundwater trials using a portable pH meter (Hanna HI 8424, Hanna Instruments, Leighton Buzzard, UK) placed within the sample vessel. Meter was calibrated using two standard solutions prior to each use

6.2.2.4 Dissolved oxygen (DO)

Measured in influent and supernatant samples for continuous flow trials (chemostat and pilot-scale) using a portable DO probe (Jenway 9071 meter, Patterson Scientific Ltd, Luton, UK), which was calibrated prior to each use. Influent readings were taken from within the relevant influent storage vessel, and supernatant readings immediately following sample collection. It was not possible to have DO probes permanently mounted within the experimental systems. Although supernatant samples were always analysed for DO as soon as possible following collection, it is possible small amounts of oxygen may have become entrained during the collection procedure. Therefore, supernatant readings taken may be slightly higher than those found actually within the reactors and should be interpreted as maximum readings. No DO analysis was undertaken on effluent samples due to the length of time required for sample collection.

6.2.2.5 Biomass

Monitored in samples taken from the supernatant sampling ports in both continuous-flow trials. Biomass analysis was also undertaken on all steady-state samples, and during selected batch trials. Biomass was measured by optical density using a spectrophotometer reading absorbance at 600 nm (Jenway 6505 spectrophotometer, Patterson Scientific Ltd, Luton, UK) against an RO water (15.2 MΩ)

blank. All readings were taken in triplicate and the mean value used. For most bacteria absorbance at 600 nm approximates to microbial biomass concentration expressed as grams dry weight per litre (Butler *et al.*, 2003), and this relationship was assumed for all readings.

6.2.2.6 Total Organic Carbon (TOC)

Soluble carbon in continuous-flow trial samples was measured using a Shimadzu TOC-5000A analyser (Shimadzu, Milton Keynes, UK). Samples were filtered prior to analysis using a 0.45 µm syringe-tip filter (Nalgene 25 mm SFCA filter; Nalgene, Rochester, NY, USA), to remove any non-soluble carbon. Soluble TOC was calculated by measuring the total carbon (TC) and the inorganic carbon (IC) and subtracting IC from TC readings. The TC standard was made by dissolving 2.125 g potassium hydrogen phthalate in 1 litre of RO water (15.2 MΩ). The IC standard was made by dissolving 1.750 g sodium hydrogen carbonate in 500 mL RO water and adding this to a solution of sodium carbonate (2.205 g) dissolved in 500 mL of RO water. The standards produced had a concentration of 1000 mg L^{-1} and working standards were diluted accordingly with 15.2 MΩ RO water.

6.2.2.7 Bromate and bromide

Analysed using Methods 2, 3 and 5, as outlined in Chapter 5. Methods 2 and 3 only utilised for samples prior to IC system installation, with chemostat phase I analysed by a combination of Method 2 (80 mg L^{-1} bromate samples) and Method 3 (0.2 and 1.4 mg L^{-1} bromate samples). Duplicate analytical runs were completed on all samples, with the average value of these two results used. Whenever possible, samples were analysed on the day of collection. Where this was not possible, samples were frozen at -20°C on the day of collection for later analysis. Values for bromate are given as 'concentration as BrO_3^-', and bromide as 'concentration as Br^-' throughout the text, unless otherwise indicated.

6.2.2.8 Nitrate, nitrite and sulphate

Analysed by Method 5 (IC-CD) simultaneously with bromate and bromide as described in Chapter 5. Duplicate analytical runs were completed on all samples, with the average value of these two results used. Phase I chemostat samples were analysed for nitrate and nitrite using Merck 'Spectroquant' test kits (VWR International, Lutterworth, UK):

- Nitrate – Measured by Merck 'Spectroquant' test kit no. 14563 (VWR International, Lutterworth, UK). A 1.0 mL sample was pipetted into the supplied reaction cell, followed by 1.0 mL of reagent NO_3-1K. The cell was left to stand for 10 minutes, and the completed test measured spectrophotometrically at 338 nm using a Merck Nova 60 spectrophotometer (VWR International, Lutterworth, UK). Calibration range of test was 0.5 – 25.0 mg L^{-1} NO_3^--N. Values obtained were converted to mg L^{-1} NO_3^- prior to interpretation.

- Nitrite – Measured by Merck 'Spectroquant' test kit no. 14547 (VWR International, Lutterworth, UK). A 5 mL sample was pipetted into the supplied reaction cell and the cell shaken vigorously until the solid reagent within the cell dissolved. Following a reaction time of 10 minutes, the completed test was measured spectrophotometrically at 525 nm using a Merck Nova 60 spectrophotometer (VWR International, Lutterworth, UK). Calibration range of test was 0.01 – 0.7 mg L^{-1} NO_2^--N. Values obtained were converted to mg L^{-1} NO_2^- prior to interpretation.

Values for nitrate are given as 'concentration as NO_3^-', nitrite as 'concentration as NO_2^-', and sulphate as 'concentration as SO_4^{2-}' throughout the text unless otherwise indicated.

Samples were analysed immediately and, where necessary, subsequently frozen at -20°C in a domestic freezer on day of sampling for long-term storage. Immediate anion and TOC analysis was not possible for all samples due to periodic unavailability of analytical equipment. Analysis on these occasions was completed following freezing.

6.2.3 Mathematical analysis

6.2.3.1 Mass balance

Mass balances for bromine were completed with each groundwater bioremediation trial where possible to calculate stoichiometry of bromate reduction to bromide, and therefore investigate the possible stable formation of intermediates such as bromite and hypobromite which could not be analysed due to a lack of commercially-available analytical standards for calibration. A mass balance for carbon was also completed for batch trial G (Section 6.4.3.4) using comparable calculations. For each bromine mass balance, total influent and effluent bromine content were calculated, using Equation 6.4 for converting bromate as BrO_3^- to bromate as Br, with Equation 6.5 then giving overall bromine recovery for the system.

$$Bromate\,(as\,Br) = Bromate\,(as\,BrO_3^-) \times \frac{Atomic\,Mass\,(Br)}{Molecular\,Mass\,(BrO_3^-)}$$

$$= Bromate\,(as\,BrO_3^-) \times \frac{79.9}{127.9} = Bromate\,(as\,BrO_3^-) \times 0.625$$

Equation 6.4

$$\text{Br recovery}\,(\%) = \frac{\text{Total effluent bromine (as Br)}^{1}}{\text{Total influent bromine (as Br)}^{2}} \times 100$$

Equation 6.5

[1] Total effluent bromine calculated as sum of bromate (as Br) and bromide in supernatant samples (continuous flow) or at end of the trial period (batch culture)

[2] Total influent bromine calculated as sum of bromate (as Br) and bromide in influent samples (continuous flow) or at start of the trial period (batch culture)

6.2.3.2 Reduction rate

Reduction rates were calculated for bromate and nitrate in both batch and continuous flow groundwater bioremediation trials, and are reported with units of $\mu g\ L^{-1}\ BrO_3^-\ hr^{-1}$ or $\mu g\ L^{-1}\ NO_3^-\ hr^{-1}$ respectively. Specific bromate and nitrate reduction rates (qBr and qN) were calculated for continuous flow trials where biomass

concentrations were analysed, and are given with units of μmol Br (or N) g dry wt^{-1} hr^{-1}. Equation 6.6 was used for calculation of bromate reduction rates, with equations 6.7 – 6.9 utilised for calculation of specific bromate reduction rates. Nitrate reduction and specific reduction rates were calculated in a similar manner. The relative rate of nitrate to bromate removal was calculated from continuous flow data using Equation 6.10.

$$\text{Reduction rate}\,(\mu g\,L^{-1}\,hr^{-1}) = \frac{(\text{Initial bromate}\,(\mu g\,L^{-1})^{a} - \text{Final bromate}\,(\mu g\,L^{-1}))^{b}}{\text{Time}\,(hr)^{c}}$$

Equation 6.6

[a] Initial bromate - Bromate concentration (as BrO_3^-) in influent sample (continuous flow) or at the start of trial period (batch)

[b] Final bromate - Bromate concentration (as BrO_3^-) in supernatant sample (continuous flow) or at the end of trial period (batch)

[c] Time – Reactor retention time (continuous flow) or experimental period (batch)

$$qBrO_3\left(\frac{mgBrO_3}{g\,dry\,wt\,hr}\right) = \frac{(Influent\,bromate - Supernatant\,bromate) \times dilution\,rate}{Biomass\,concentration}$$

Equation 6.7

$$qBr\left(\frac{mg\,Br}{g\,dry\,wthr}\right) = qBrO_3 \times \frac{Atomic\,mass\,Br}{Molecular\,mass\,BrO_3}$$

Equation 6.8

$$qBr\left(\frac{\mu molBr}{g\,dry\,wt\,hr}\right) = \frac{qBr\,(as\,mg\,Br) \times 1000}{Atomic\,mass\,Br}$$

Equation 6.9

$$\text{Relative rate} = \frac{qN}{qBr}$$

Equation 6.10

6.2.3.3 Statistical analysis and calculation of error

Statistical analysis was performed using the non-parametric Kruskal-Wallis and Mann-Whitney U-tests. Non-parametric tests, which compare median instead of mean values and may be used with smaller and uneven sample sizes than parametric

equivalents (Fowler and Cohen, 1995), were preferred due to the small sample size of some observations and were utilised throughout for continuity. The Kruskal-Wallis test was used to compare averages of three or more samples, and the Mann-Whitney U-test for comparison of two datasets. Analysis was completed using the Analyse-it add-on (Analyse-it software Ltd, Leeds, UK) to Microsoft Excel. In all cases, significance was determined at the 95% confidence level ($p < 0.05$).

Averages within datasets were calculated as mean values, with error calculated using the standard deviation function of Microsoft Excel. Reported data is presented in this study as the mean value, or the mean value ± one standard deviation.

6.3 BROMATE CONTAMINATION WITHIN WASTEWATER

6.3.1 Wastewater sludge biomass respiration inhibition

A wide range of methodologies are available for measurement of respiration inhibition (examples include British Standards Institute, 1995; Mayhew and Stephenson, 1998; Burgess, 1999; Gendig *et al.*, 2003), with differences between methods including variations in sludge preparation and biomass feeding. Run times for these methodologies also vary widely, with intervals between 3 hours and 5 days suggested. The methodology chosen for these trials was based on that outlined in Mayhew and Stephenson (1998) which, as in this trial, was utilised for initial toxicity screening. A run time of 27-hours was used, which was recommended in the method advanced by Gendig *et al.* (2003).

A 20-channel aerobic respirometer system (Co-ordinated Environmental Services Ltd, Sittingbourne, UK) was used for the trials. This system is based on the coupling of an electrolytic oxygen generation process to a sensitive manometric system within a closed cell at constant temperature. Following sealing of the cell from the outside environment, any biodegradation within a sample causes a decrease in oxygen concentration coupled with production of carbon dioxide. Use of an alkali carbon dioxide trap leads to a net reduction in gas pressure within the cell, which is detected by the manometric system and triggers electrolysis of an aqueous copper sulphate solution (25%). Electrolysis produces oxygen, which replenishes that utilised by the biomass. In

this way, magnitude and duration of the electrolytic current required are together directly proportional to the amount of oxygen consumed by microorganisms within the sample, and it is this parameter that is logged as oxygen consumption. A schematic diagram of one cell is given in Figure 6.2.

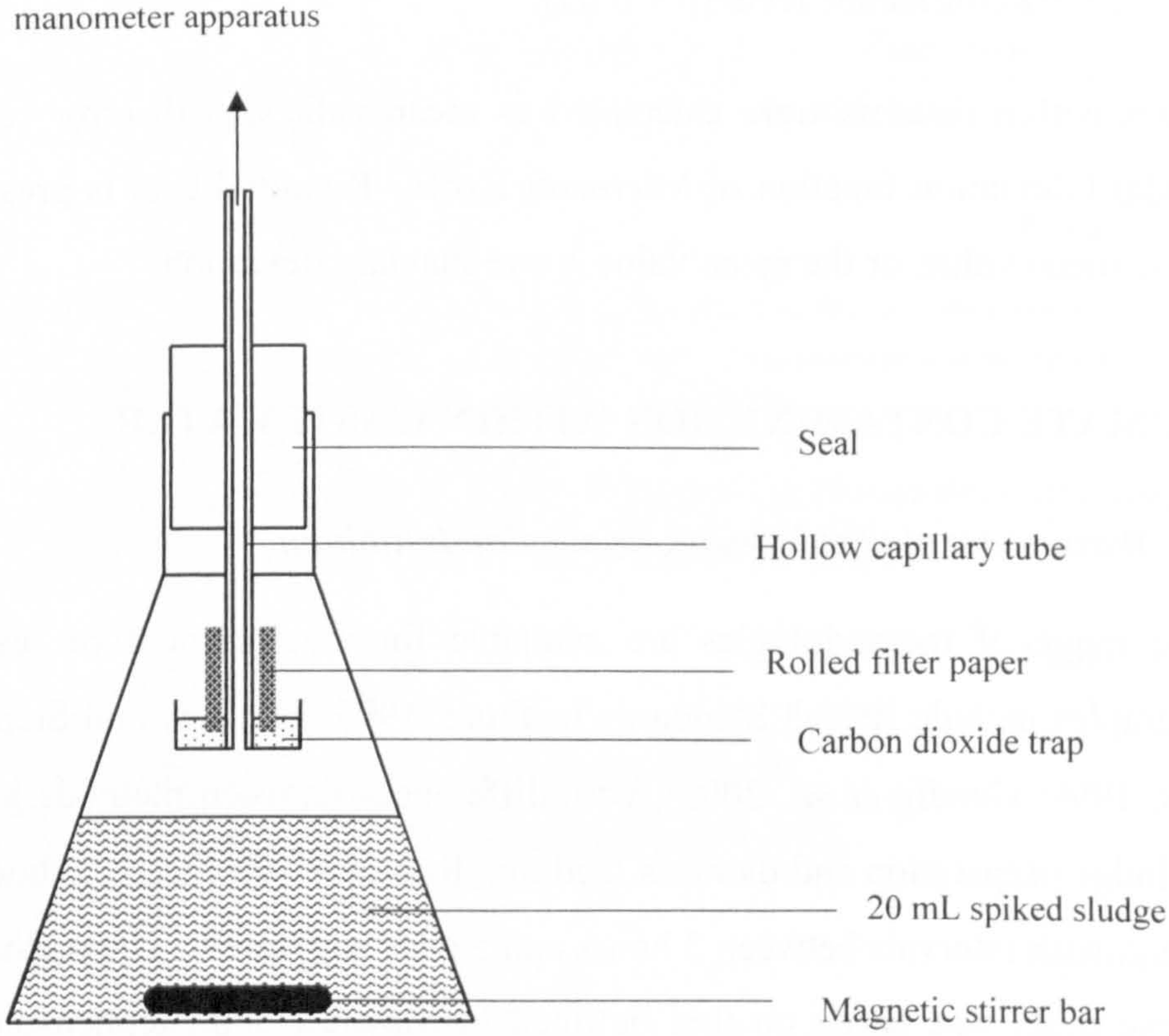


Figure 6.2 - Schematic diagram of one respirometer cell

A total sample volume of 20 mL in each cell was used during all runs, with a magnetic stirrer bar added to ensure uniform mixing during the experimental period. The carbon dioxide trap was installed by inserting a rolled rectangle of filter paper onto the capillary tube to increase surface area available for carbon dioxide absorption, and then filling the trap with 1 mL of 10 Molar sodium hydroxide. The cell was assembled, joints being smeared with a thin film of paraffin grease to prevent air ingress, and the completed cell was attached to the manometric cell/electrolysis apparatus, placed in a water bath set at 20°C and continuously stirred. Once all 20 cells were assembled, they

were initialised to balance gas pressures, and oxygen consumption was then logged at 5 minute intervals using dedicated respirometry control software (Version 1.5, Co-ordinated Environmental Services Ltd, Sittingbourne, UK), using a 27 hour run time. Results were outputted as oxygen uptake (mg O_2), with oxygen uptake rate (OUR; mg O_2 min^{-1}) and specific oxygen uptake rate (SOUR, mg O_2 mg $MLSS^{-1}$ min^{-1}) subsequently calculated. For each trial, an average MLSS value for all cells was calculated and used to produce the SOUR results.

Trials were undertaken to ascertain overall effects of bromate and bromide contamination on respiration within MBR sludge. Four trials were completed, trials 1 and 2 investigating bromate and bromide spiking respectively, trial 3 a combination of bromate and bromide contamination, and trial 4 two bromate concentrations but with greater replication. Trial 1 was carried out twice (Trials 1a and 1b), but inconclusive results required the subsequent completion of trial 4. The four trials, with spiking concentrations utilised, are outlined in Table 6.2. For each of the 20 channels, 29 mL MBR sludge was initially added to the cell, along with 1 mL of bromate/bromide contamination. Sludge samples were obtained from the pilot-scale MBR described in Section 6.3.3, and were used immediately following collection. Bromate and bromide contamination was added using solutions of potassium bromate and potassium bromide (Fisher Scientific, Loughborough, UK), concentrated 30 times from the required final concentration. 10 mL sludge was removed for determination of MLSS concentration using the procedure outlined in Section 6.2.1.4, to account for differences in sludge strength. The remaining spiked sludge sample was then subjected to the respiration inhibition test.

Table 6.2 - Bromate and bromide concentrations for respiration inhibition trials

Trial		Bromate concentrations	Bromide concentrations	Replicates
1	a	No bromate 20; 200; 2000 µg L^{-1} 2; 20; 200 mg L^{-1} 2 g L^{-1}	n/a	3 (2 g L^{-1} = 2)
	b	No bromate 20; 200; 2000 µg L^{-1} 2; 20; 200 mg L^{-1} 2 g L^{-1}	n/a	3 (2 g L^{-1} = 2)
2	a	n/a	No bromide 10; 200; µg L^{-1} 20 mg L^{-1}	5
	b	n/a	No bromide 20 µg L^{-1} 2; 200 mg L^{-1}	5
3		2.5 mg L^{-1} 200 µg L^{-1} 30 µg L^{-1}	5 mg L^{-1} 800 µg L^{-1} 180 µg L^{-1}	5
4	a	0; 200 mg L^{-1}	n/a	5
	b	0; 2 g L^{-1}	n/a	10

6.3.2 Wastewater sludge biomass sorption

Trials were based on OECD Guideline for the Testing of Chemicals number 106, 'Adsorption-Desorption using a Batch Equilibrium Method' (OECD, 2000). This Guideline uses known volumes of the test substance at known concentrations, in this case bromate, which are added to soil samples of known dry weight and agitated. At specified times the mixture is centrifuged, and the supernatant analysed for bromate concentration, with the amount of bromate adsorbed on the soil sample calculated as the difference between bromate initially present in solution and the amount remaining following the trial period. Detection of any increase in bromide concentration within the supernatant solution would provide evidence for bromate reduction by the sludge instead of reversible adsorption processes. 'Soil' samples for the purposes of this trial were samples of biomass from the pilot-scale MBR, which were prepared by freeze-drying using a Savant

Modulyo freeze-drier (Savant Instruments Inc, Holbrook, USA) and subsequently stored until use within a dessicator.

Trials were carried out within 250 mL centrifuge bottles, which allowed centrifugation during the trial without removing samples from the test vessel. Stock bromate solutions of 10 times final required concentrations were prepared using AnalaR grade potassium bromate (Fisher Scientific, Loughborough, UK) dissolved in a 0.01 Molar solution of calcium chloride ($CaCl_2$). Use of $CaCl_2$ solution as the aqueous solvent phase was advised by OECD Guidelines to improve centrifugation and minimise cation exchange (OECD, 2000).

The appropriate mass of freeze-dried biomass was measured into a 250 mL centrifuge bottle and equilibrated by agitating with a volume of 45 mL or 90 mL 0.01 Molar $CaCl_2$ (depending on biomass:solution ratio required) and leaving overnight. Gentle agitation was provided by a stirrer bar added to each bottle, which were placed in the dark on a magnetic stirrer system (RT15 stirrer; IKA-Werke GMGH, Staufen, Germany). After 12 hours of equilibrium, 5 mL or 10 mL bromate stock solution (depending on $CaCl_2$ volume) was added to give a final volume of either 50 mL or 100 mL. Initial bromate and bromide concentrations were ascertained by measurement of a sample taken immediately from each bottle. Samples for bromate and bromide analysis were obtained by placing the bottles in a centrifuge (Rotanta 96R, Hettich instruments, Tuttlingen, Germany) at 5000 rpm for 5 minutes to separate biomass and aqueous phase (supernatant). A 0.5 mL aliquot of supernatant from each sample was taken for analysis using ion chromatography (IC-CD) with a 10x dilution factor, and the bottles were replaced on the stirrers. Control and blank samples were subjected to the same steps as test bottles. Control samples (no biomass addition) were used to ascertain stability of bromate in $CaCl_2$ solution and its possible adsorption to test vessel surfaces. Blank runs (no bromate addition) were included to check for bromate contamination or interfering compounds within the biomass.

Desorption was investigated in a similar manner to adsorption in trial 2 only. Following completion of the adsorption test, the bottles were centrifuged and supernatant

removed and discarded. The volume of solution removed was replaced by fresh 0.01 Molar $CaCl_2$ and bottles were replaced on the stirrers. After an interval of 72 hours the bottles were centrifuged and 0.5 mL supernatant aliquots taken for IC-CD analysis.

Trial 1 was performed as a preliminary study to ascertain optimal biomass:solution ratios for trial 2. Detection limits for the analytical method should be at least two orders of magnitude lower than initial concentration of the test substance for preliminary studies (OECD, 2000), so an initial bromate concentration of 1 mg L^{-1} was utilised. A range of biomass:solution ratios were investigated, summarised in Table 6.3. Samples for bromate and bromide analysis were taken at 1, 2, 4, 8, 12, 24 and 48 hours. No desorption samples were taken.

Table 6.3 - Sample summary for sorption trial 1

Sample ratio (w:v)	Mass of freeze-dried biomass (g)	Total solution volume (mL)	Nominal bromate addition (mg L^{-1})
1:25	2	50	1
1:50	2	100	1
1:100	1	100	1
1:200	0.5	100	1
n/a (Control)	0	100	1
n/a (Blank)	1	100	0

Trial 2 was carried out using a biomass:solution ratio of 1:100, with 0.5 g biomass per sample and a total solution volume of 50 mL. A range of bromate levels were trialled, with initial concentrations of 30 mg L^{-1}, 5 mg L^{-1}, 3 mg L^{-1} and 0.3 mg L^{-1}. Control and blank samples were also completed as during trial 1, with bromate addition to the control of 3 mg L^{-1}. Samples for bromate and bromide analysis were taken at 1, 2, 4, 8, 24, 48 and 72 hours. Desorption samples were taken after 72 hours only.

Trial 3 was aimed at investigating a possible abiotic bromate reduction process noted in trials 1 and 2, by studying bromate and bromide concentrations following two methods of bacterial inactivation, one physical and one chemical. Four samples were prepared using a soil:solution ratio of 1:100 (0.5 g biomass per sample and a total

solution volume of 50 mL), with a bromate concentration of 5 mg L^{-1}. Two of these samples were autoclaved at 121°C for 15 minutes (Priorclave autoclave, Priorclave, London, UK) prior to acclimation, with sterile bromate solution added at t=0. The other two samples were spiked with a final concentration of 1% of the biocide formaldehyde (Fisher Scientific, Loughborough, UK) prior to bromate addition. In addition, two control samples were prepared, containing no biomass. Samples for bromate and bromide analysis were taken at 24, 48 and 72 hours. No desorption samples were taken.

6.3.3 Wastewater process dosing

For trials investigating dosing of bromate within a wastewater treatment process, a two-chambered pilot-scale MBR was utilised, situated within the test-facility at Cranfield University. Each chamber had a sludge volume of 33 litres and was run in a submerged configuration, incorporating the filtration unit within the body of the bioreactor. A picture of the rig is given in Figure 6.3, and a schematic of one chamber in Figure 6.4. The membranes used in each chamber were two parallel 0.035 m^3 submerged membrane bioreactors containing 0.24 m^2 of commercially available polysulphone flat sheet membrane rated at a pore size of around 0.4 μm (Kubota, Japan). These were operated under constant flux conditions by incorporating a peristaltic pump on the suction (permeate) side of the membrane. Coarse aeration to reduce membrane fouling was introduced at the base of each chamber, with constant volume conditions imposed by a constant level apparatus situated within each chamber. The reactors were operated on a test and control basis under a Hydraulic Retention Time (HRT) of 12 hours and calculated Sludge Retention Time (SRT) of 50 days, and were allowed to stabilise before commencement of the bromate dosing and analysis phase.

Figure 6.3 - Picture of two-chambered membrane bioreactor rig setup

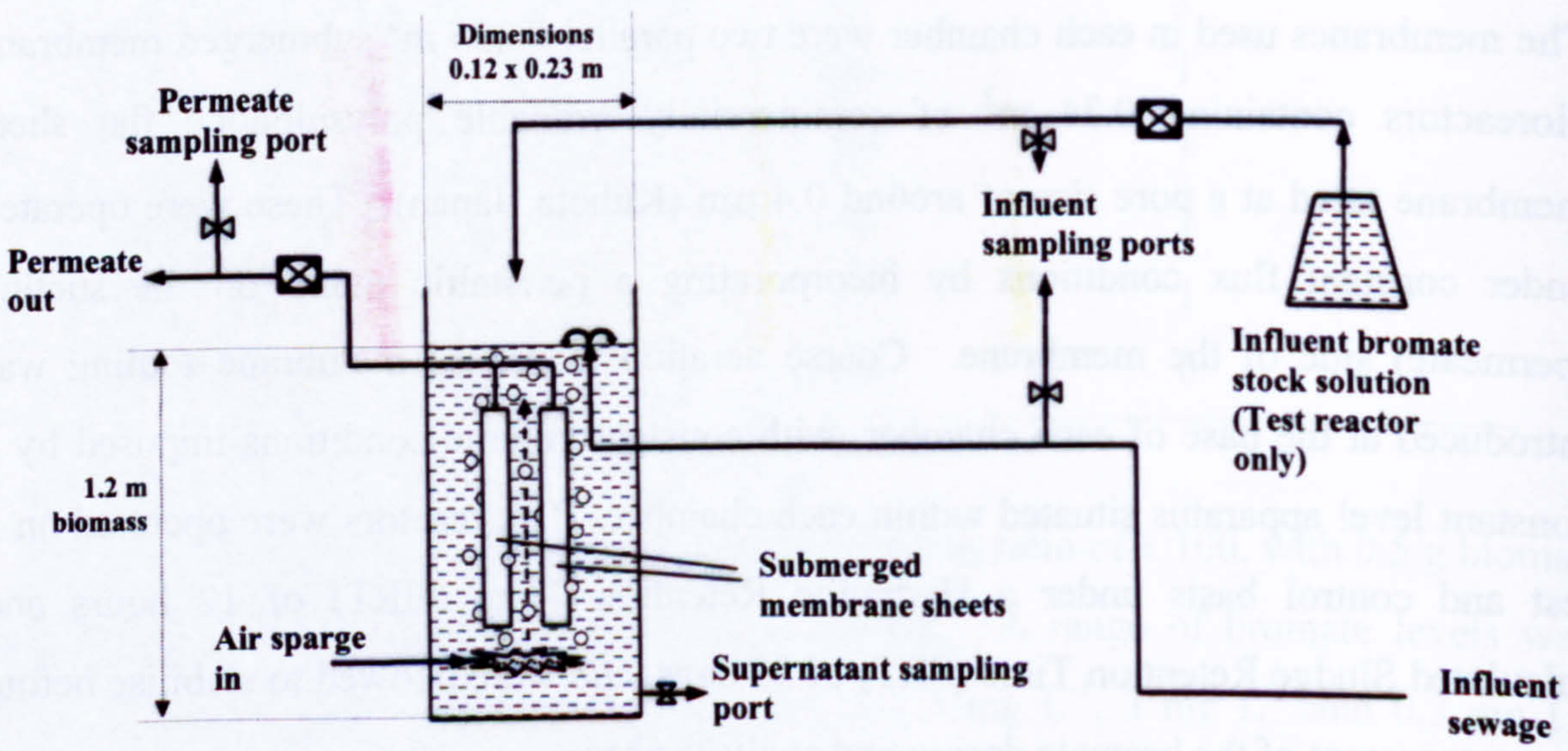


Figure 6.4 - Schematic of pilot-scale membrane bioreactor chamber

Two bromate concentrations were trialled, both contamination levels being introduced by dosing the test reactor with bromate from a concentrated stock solution of laboratory-grade potassium bromate (Fisher Scientific, Loughborough, UK) using a peristaltic pump (Watson Marlow 302S, Falmouth, UK). The two doses selected reflected a typical bromate concentration encountered near the plume source (2 mg L^{-1}) and an extreme event (100 mg L^{-1}) to assess ultimate potential impacts. Prior to the sampling phase in both cases the reactors were stabilised under constant influent conditions.

Samples were collected in 250 mL Nalgene bottles (VWR International, Lutterworth, UK), with analysis carried out the same day. A range of standard wastewater parameters, outlined in Section 6.2.1, were analysed at both bromate dosing levels. Test and control samples were taken daily where possible for a week to give a total of 7 datasets for each dose.

For both datasets (test and control) at each bromate dose, average and standard deviation values were calculated using Microsoft Excel software, with results given as mean value ± one standard deviation. Statistical significance was measured through application of the non-parametric Mann-Whitney *U*-test.

Following completion of sampling for the second (100 mg L^{-1}) bromate contamination level, a respiration inhibition trial was carried out on biomass within the test reactor to investigate whether prolonged exposure to bromate had led to a degree of acclimatisation by the sludge bacteria. Experimental procedure was as described for respiration inhibition trial 4 (Section 6.3.1).

6.4 GROUNDWATER BROMATE BIOREMEDIATION

6.4.1 Laboratory-scale chemostat

A two-vessel anoxic suspended growth chemostat was used to investigate bromate reduction following glucose augmentation of groundwater under continuous culture conditions. Anoxic suspended growth was maintained by slow stirring (approximately 50

rpm) using overhead stirrers, and nitrogen sparging of reactors and influent supply (Oxygen-free nitrogen, BOC gases, Manchester, UK). Temperature control was achieved by use of a submerged heat exchanger within each reactor, with constant temperature water kept within a water bath and circulated by heater/pump apparatus (Haake DC10; Haake, Karlsruhe, Germany). Cooling of water in the water bath was only required during summer months, and was achieved by use of either a Conair 06CTCV (Conair Churchill, Uxbridge, UK) cooler system or a Haake K10 water bath (Haake, Karlsruhe, Germany). Pictures of the rig are given in Figure 6.5, with a schematic of one reactor in Figure 6.6. Trials were completed both with the two reactors operating as separate systems (Figure 6.7a), and also with the reactors in series (Figure 6.7b).

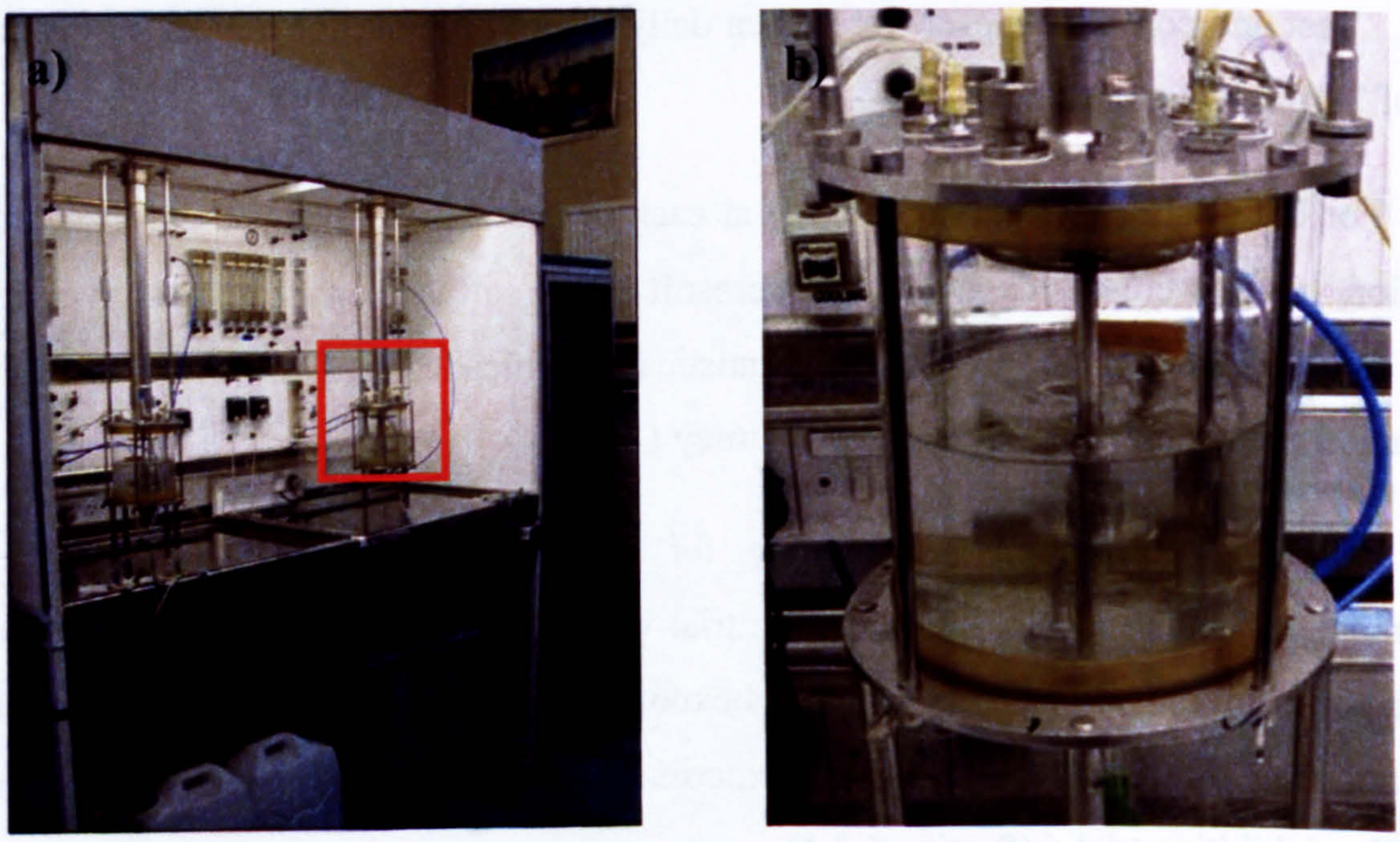


Figure 6.5 – a) Picture of two-chambered anoxic suspended growth chemostat. Red square indicates area covered by b) one reactor chamber.

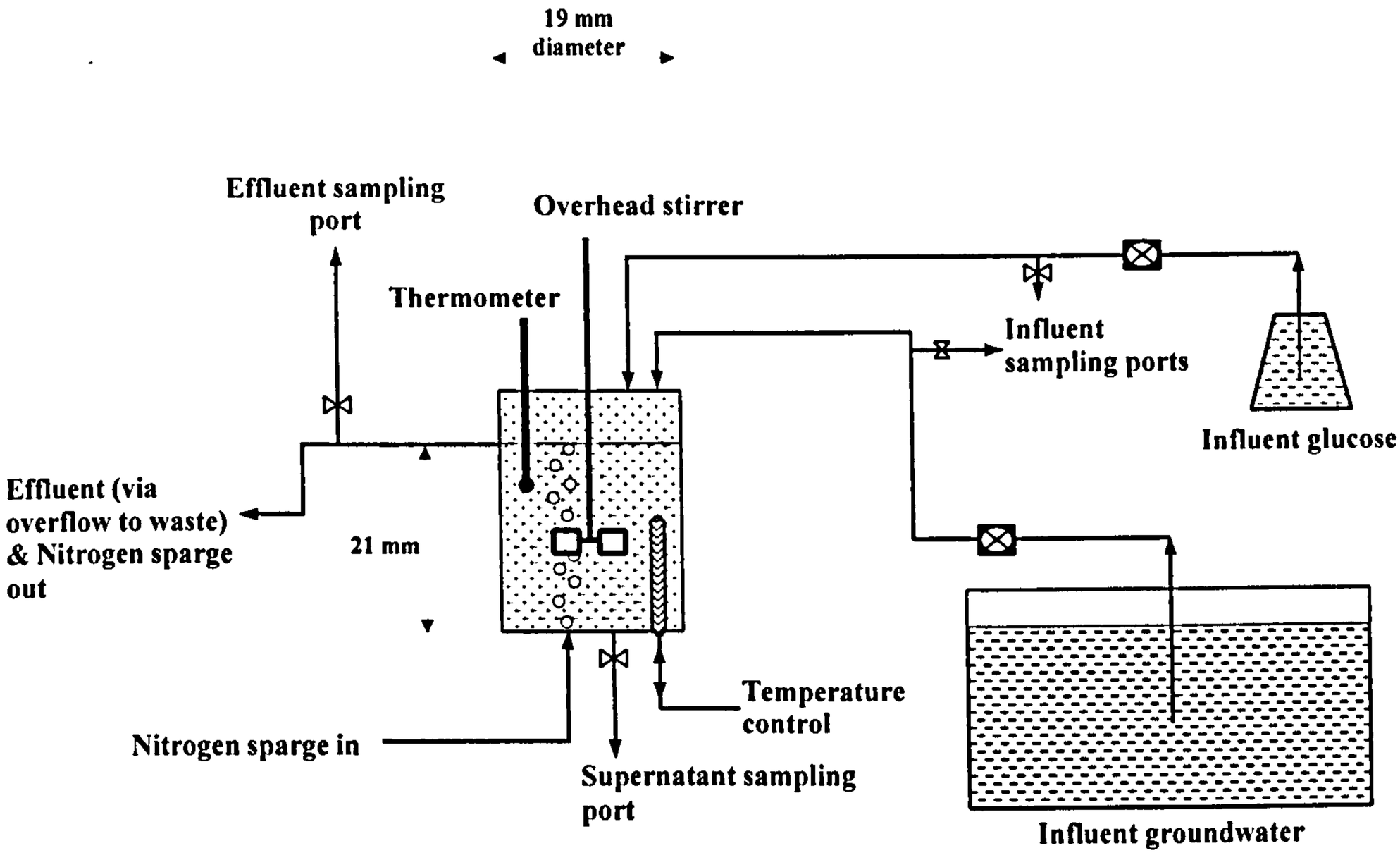


Figure 6.6 - Schematic of chemostat reactor configuration

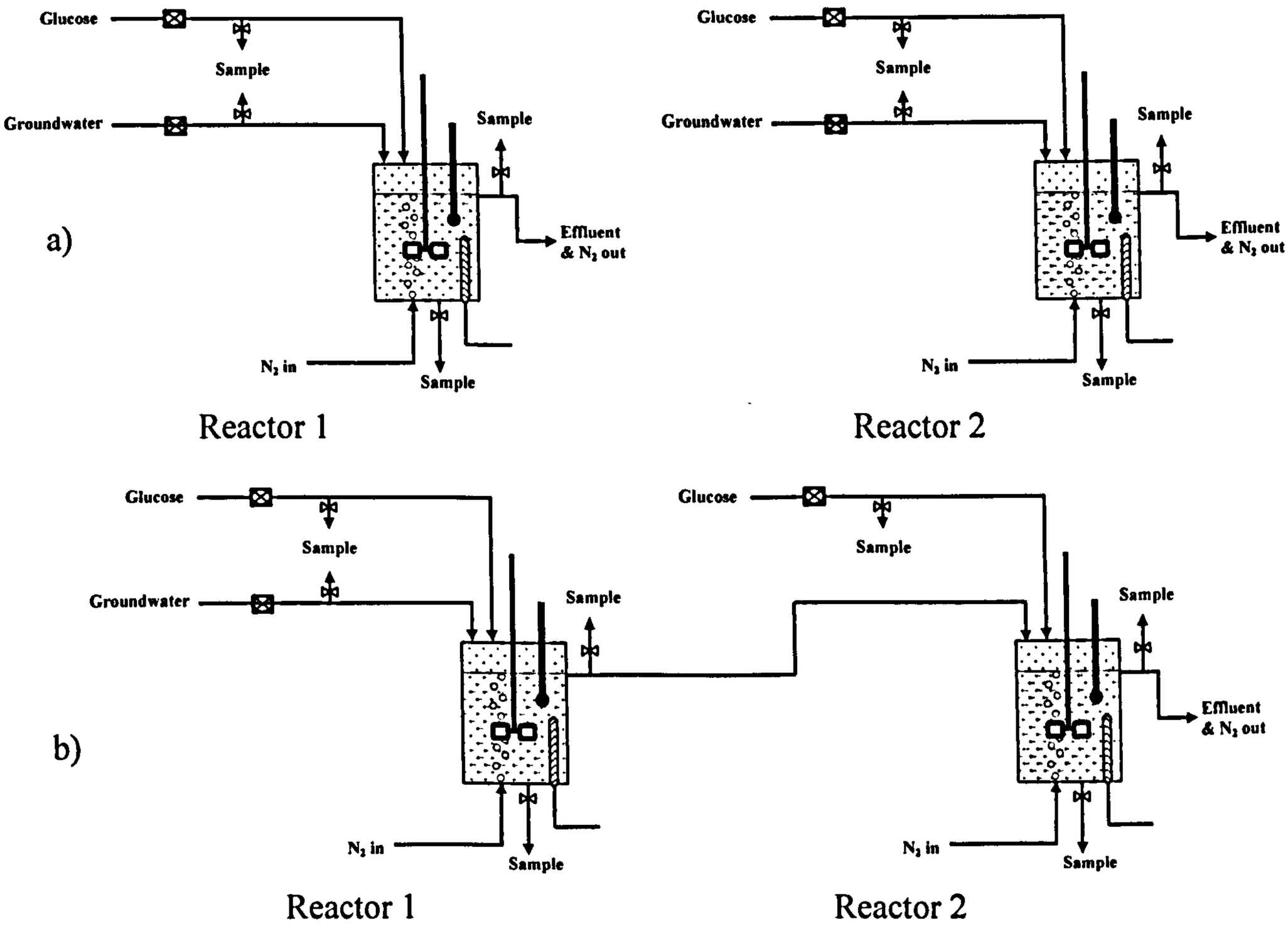


Figure 6.7 – Chemostat reactor configuration for (a) separate and (b) series operation

Experimental setup was designed to allow close control of the major potential variables (including pH, temperature and nitrate concentration), whilst at the same time facilitating precise manipulation of the parameters of interest where necessary. Influent supply was provided by separate groundwater and glucose feeds, to minimise growth in supply lines. Groundwater represented either 90% (Phase I only) or 95% of influent flow, along with glucose solution concentrated by 10x or 20x respectively. Glucose solutions were prepared from laboratory-grade glucose (Fisher Scientific, Loughborough, Leics) and dissolved in RO water (minimum 15.2 MΩ). Glucose solutions were degassed by sonication (Ultrawave U500; Ultrawave Ltd, Cardiff, UK) prior to use to minimise oxygen ingress to the reactors. Influent flow rates were calculated to provide a RT of 20 (Phase I only) or 40 hours in each reactor, both of which had a 2.0 litre capacity. The groundwater feed was supplied by peristaltic pumps (Watson-Marlow 101U; Watson-Marlow, Falmouth, UK), and the concentrated glucose feed via a low-volume peristaltic pump (Gilson Minipuls 2; Gilson, Villiers-le-Bel, France).

Groundwater was sourced from the bromate-contaminated aquifer, with source dependent on the trial phase. Borehole source for each phase is given in Table 6.4, which also outlines major operating parameters. Groundwater supplies were spiked with laboratory-grade potassium bromate solution (Fisher Scientific, Loughborough, UK), apart from during the latter stages of Phase I (0.2 mg L^{-1} and 1.4 mg L^{-1}), to give the required bromate concentrations prior to use. All influent groundwater supplies were augmented with bacterial nutrients, made up as stock solutions in RO water (15.0 MΩ). Nutrient addition was carried out to ensure growth limitation by either carbon or nitrate/bromate only. Phase IIIb also utilised an artificial medium, free from nitrate and sulphate additions. This was made up in 10 litre batches using MilliQ water (18.2 MΩ quality) with defined nutrient additions. Nutrient additions for both groundwater-based and artificial matrices are outlined in Table 6.5. No additional biomass was added, with the aim of the study to investigate the potential for indigenous aquifer microorganisms to carry out bromate reduction in the presence of an augmented carbon source.

Table 6.4 - Operating conditions of the suspended growth chemostat system

Parameter	Trial				
	Phase I	Phase IIa	Phase IIb	Phase IIIa	Phase IIIb
Reactor volume	2.0 litre volume per reactor with slow overhead stirring				
Sparging	Continual nitrogen sparging of influent and supernatant				
Reactor configuration	Two reactors operated as independent units	Two reactors in series		Two reactors operated as independent units	
Water source	Hatfield/Nashes Farm	House Lane		House Lane	Artificial Medium (Test); House Lane (Control)
Temperature*	10°C (11.3 ± 1.6°C; n=44)	20°C (20.4 ± 0.9°C; n=38)		10 / 15 / 20°C (Test) 20°C (Control)	20°C (20.3 ± 0.7°C)
Dilution rate (Retention time)	0.05 hr^{-1} (20 hours)	0.025 hr^{-1} (40 hours – reactor 1); 0.013 hr^{-1} (80 hours – reactors 1 and 2 combined)		0.025 hr^{-1} (40 hours)	
Flow rate	100 mL hr^{-1} to each reactor	50 mL hr^{-1} to combined system		50 mL hr^{-1} to each reactor	
Bromate**	0.2 / 1.4 / 80 mg L^{-1}	1 – 128 mg L^{-1}	32 / 48 mg L^{-1}	32 mg L^{-1}	32 mg L^{-1}
Glucose**	10 – 150 mg L^{-1}	52 mg L^{-1} (both reactors)	100 / 150 mg L^{-1} (reactor 1) 52 mg L^{-1} (reactor 2)	52 – 100 mg L^{-1} (Test) 52 mg L^{-1} (Control)	100 mg L^{-1}
Nitrate**	30 – 40 mg L^{-1}	40 mg L^{-1} (spiked)	12 mg L^{-1}	11 mg L^{-1}	3 mg L^{-1}
Total run time	8 weeks	13 weeks	28 weeks	10 weeks	10 weeks

* Expected temperature – average temperature achieved in brackets (not phase IIIa) ** Approximate/expected concentrations – actual concentrations achieved given in relevant sections of results chapter

Table 6.5 - Chemical additions to chemostat influent supplies

Nutrient	Concentration within groundwater matrices (mg L^{-1})	Concentration within artificial matrix (mg L^{-1})
$NaH_2PO_4.2H_2O$	300	300
$MgSO_4.7H_2O$	10	-
$MgCl_2.6H_2O$	-	8.25
H_3BO_3	3	250
$MnSO_4.H_2O$	2	-
$MnCl_2.4H_2O$	-	2500
$CuSO_4$	0.4	-
$CuCl_2.2H_2O$	-	150
$ZnCl_2$	0.2	250
$CoCl_2.6H_2O$	0.4	250
$Na_2MoO_4.2H_2O$	0.3	5
$FeCl_3.6H_2O$	1	1
$CaCl_2.2H_2O$	-	15
NH_4Cl	300	300
$NiCl_2.6H_2O$	-	226
$NaNO_3$ as NO_3^-	32 (Phase IIa only)	-

Routine sampling was carried out every 2 – 3 days, with one 10 mL sample taken from each reactor via the basal (supernatant) outlet for biomass and pH monitoring. In all cases steady state conditions were deemed to have been reached after a minimum of 5 liquid volume changes, and when biomass and pH readings had stabilised.

Full analysis of samples was undertaken upon attainment of a steady state, or every 1 – 2 weeks during extended steady state phases. A 20 mL (Phase I only) or 10 mL glucose solution sample was taken and made up to 200 mL with groundwater to emulate influent supply, as separate glucose and groundwater influents precluded direct sampling. Effluent samples were collected over a 2-hour period from each reactor, taken over ice to minimise further bacterial activity. A supernatant sample of 100 mL was subsequently taken from each reactor via a basal outlet. Parameters analysed during routine monitoring and full analysis are summarised in Table 6.6.

Table 6.6 - Analytical procedures undertaken during the experimental period

Measurement	Sample types	Analysis type
pH	Supernatant	Routine; Full
	Influent, Effluent	Full
Biomass (optical Density)	Supernatant	Routine; Full
	Influent, Effluent	Full
Temperature	Influent, Supernatant	Routine; Full
Groundwater flow rate	Influent	Routine; Full
Glucose flow rate	Influent	Routine; Full
Dissolved Oxygen	Influent, Supernatant	Full
Total Organic Carbon	Influent, Supernatant, Effluent	Full
Bromate Bromide Nitrate Nitrite Sulphate	Influent, Supernatant, Effluent	Full

6.4.1.1 Phase I (Proof of concept)

A. Reactor startup

Initiation of the chemostat culture commenced in February 2003. Reactor temperature was set at 10°C to emulate conditions commonly found within aquifer systems, as groundwater temperatures generally remain constant at around this temperature (Hiscock *et al.*, 1991). Nashes Farm groundwater was utilised and amended with nutrients (Table 6.5) and approximately 80 mg L^{-1} bromate as BrO_3^- (Laboratory grade potassium bromate, Fisher Scientific, Loughborough, UK). Both reactors were filled and immediately placed under continuous culture conditions, with a 20-hour RT and 52 mg L^{-1} glucose influent. Reactor configuration was as outlined in Figure 6.7a, with a period of 43 days required prior to collection of the first steady state data.

B. Phase I bromate and glucose concentration trials

Three bromate contamination levels were trialled, with seven glucose concentrations studied at each bromate influent concentration giving a total of 21 steady-state conditions. The bromate concentrations were chosen as two contamination levels

typically found within the contamination plume (1.4 mg L^{-1} and 0.2 mg L^{-1}), and a higher concentraton emulating levels identified in trial boreholes at the source site (80 mg L^{-1}). Glucose concentrations studied ranged from 10 – 150 mg L^{-1}. Order of completion for steady state conditions is given in Table 6.7, with higher bromate concentrations trialled first and glucose concentrations assigned randomly to the two reactors, thus minimising any effects of differences between the two reactors. Completion of steady states at the highest bromate concentration (80 mg L^{-1}) was completed in 7 weeks, following which bromate spiking of the Nashes Farm influent was suspended to give the second influent concentration (approximately 1.4 mg L^{-1}). Four weeks was required for completion of steady states at this concentration. The final bromate concentration (approximately 0.2 mg L^{-1}) was achieved by altering influent groundwater supply to that of the Hatfield source, for which steady states were completed within a further four week period.

Table 6.7 - Order of completion for phase I chemostat trials

Bromate concentration (mg L^{-1})	Glucose concentrations (mg L^{-1})	Reactor
80	52, 30, 100, 150	1
	20, 10, 40	2
1.4	100, 40, 20, 150	1
	52, 30, 10	2
0.2	150, 52, 30, 10	1
	100, 40, 20	2

6.4.1.2 Phase II (Two-stage reduction and enrichment)

For phase II, conditions were optimised using operational experience gained during phase I, to maximise possibilities of enriching the culture with bromate reducing bacterial strains. Supernatant temperature was raised from 10°C to 20°C to enhance bacterial growth rates, which may be expected to roughly double with a 10°C increase (Pirt, 1975). Reactor configuration was altered to that outlined in Figure 6.7b, giving series operation of the two units (ie. reactor 1 effluent providing influent to reactor 2). RT was doubled to 40 hours within each reactor, giving a combined retention time of 80 hours. Groundwater source was altered to the House Lane supply. This water is

collected from a sampling point at the boundary of the contaminated site, but contains anomalously low bromate and high bromide concentrations (Table 6.1). This water was therefore utilised as a possible source of acclimatised bacteria amenable to enrichment in a bromate-rich inoculum.

A. Phase IIa – Bromate concentration

A range of bromate concentrations from 1 – 128 mg L^{-1} were trialled. Over the experimental period bromate influent concentrations were increased, doubling each time to give a set of 8 steady-state readings. Glucose concentration to both reactors for phase IIa was set at 52 mg L^{-1}, with influent nitrate augmented using potassium nitrate solution (Laboratory-grade; Fisher Scientific, Loughborough, UK) to give a total nitrate influent of approximately 40 mg L^{-1}. Completion of all steady-state conditions was carried out over a 13-week period.

B. Phase IIb – Steady state enrichment

Following completion of phase IIa, influent bromate concentration to the system was reduced to 32 mg L^{-1}. Nitrate spiking of influent groundwater was discontinued to leave approximately 12 mg L^{-1} as NO_3^- in the supply. Glucose spiking of reactor 2 was left at 52 mg L^{-1} but influent glucose concentration to reactor 1 was increased to 100 mg L^{-1}. Conditions during phase IIb were then kept stable for 28 weeks, with the only alterations being an increase of bromate spiking concentration from 32 mg L^{-1} to 48 mg L^{-1} after 15 weeks and a further increase in reactor 1 glucose concentration to 150 mg L^{-1} after 23 weeks.

6.4.1.3 Phase III (Parameter assessment)

Upon commencement of Phase III, the two reactors were split and reconfigured back to that given in Figure 6.7a, with bromate spiking again reduced to 32 mg L^{-1}. A four-week period was required with reactor 1 glucose influent set at 150 mg L^{-1} to encourage similar bromate reduction capabilities within both reactors. Identical conditions in both reactors were then imposed by reducing reactor 2 glucose supply to 52 mg L^{-1}, and these were kept constant for six weeks prior to commencement of phase IIIa.

A. Phase IIIa – Glucose and temperature manipulation

Effect of glucose concentration was investigated over a three-week period, with reactors used on a test (reactor 2) and control (reactor 1) basis. The test reactor was brought to steady-state with 100, 75 and then 52 mg L^{-1} glucose doses, whilst the control received a constant 52 mg L^{-1}.

Temperature manipulation was achieved by reducing the temperature of water circulating through the heat exchanger. Reactors were again run on a test and control basis, with reactor 1 acting as the test and reactor 2 as control. A period of approximately four weeks was required with reactor 1 set at 15°C prior to attainment of steady state, with reactor 1 subsequently run at a temperature of 10°C for a further three weeks.

B. Phase IIIb – Artificial medium

The final phase of chemostat operation investigated an artificial water influent designed to replace the groundwater-based supply utilised for all previous trials. The artificial medium was made up using RO water (15.2 MΩ), with nutrient additions (Table 6.5) based on the M-R2A agar medium utilised for isolation studies (Section 6.4.2). No nitrate or sulphate was added to the medium, which aimed to investigate bromate reduction by chemostat inoculum at low levels of these alternative electron acceptors.

Influent glucose concentration to both reactors was raised to 100 mg L^{-1} and temperature set at 20°C. Both reactors were run using House Lane water for a period of six weeks, to encourage similar bromate reducing cultures prior to use of the artificial water. Reactors were then run on a test and control basis with reactor 1 acting as test vessel (artificial medium). Conditions were maintained for four weeks prior to steady state sampling.

6.4.2 Groundwater biomass isolation and maintenance

Biomass samples for isolation trials were obtained throughout the project from the chemostat and pilot-scale reactors treating bromate-contaminated groundwater, using supernatant inoculum confirmed by IC analysis to be actively reducing bromate.

Agar medium was based on that used by Kirisits (2000) for isolation of bromate reducing bacteria taken from a BAC filter, and was named M-R2A medium. M-R2A is modified from R2A medium, which is a standard medium utilised for culture of heterotrophic bacteria in water samples (APHA, 1998). Carbon source was added in the form of either sodium pyruvate (300 mg L^{-1} as pyruvate) or glucose (300 mg L^{-1} as glucose), with both carbon sources trialled. Medium was prepared containing no bromate, 25 mg L^{-1} and also 250 mg L^{-1} bromate (both as BrO_3^-). Later studies also challenged promising colonies with a medium further modified from M-R2A to remove salts containing sulphate and nitrate. Two versions of this medium were used, one acting as control and containing sulphate and nitrate added as the sodium salts (both laboratory grade; Fisher Scientific, Loughborough, UK), and one without these additions. These media were labelled ART+ and ART- respectively. Media compositions are given in Table 6.8. The media were made up in 1 litre glass bottles with 15 g agar (Fisher Scientific, Loughborough, UK), pH was adjusted to 7.0 and the bottles autoclaved at 121°C for 15 minutes. Vitamin mixture (Table 6.9; Sigma R7256 (RPMI-1640); Sigma-Aldrich Ltd, Poole, UK) was added to the sterile agar medium by filter sterilisation using sterile 0.2 µm pore size syringe-tip filters (Millex 25 mm filters; Millipore, Carrigtwohill, Co. Cork, Ireland) prior to pouring into 90 mm Petri dishes and cooling within a laminar flow cabinet (Labcaire SC-R Class II cabinet; Labcaire systems, Clevedon, UK).

Table 6.8 - Chemical additions for agar plating trials

Nutrient	Concentration within M-R2A agar (mg L^{-1})	Concentration within ART+ agar (mg L^{-1})	Concentration within ART- agar (mg L^{-1})
KH_2PO_4	250	250	250
K_2HPO_4	400	400	400
$CaCl_2.2H_2O$	15	15	15
$MgCl_2.6H_2O$	20	20	20
$FeSO_4.7H_2O$	7	-	-
$FeCl_3.6H_2O$	-	6.76	6.76
NH_4Cl	800	800	800
Yeast extract	500	-	-
Peptone	500	-	-
Casamino acids	500	-	-
Sodium pyruvate (as pyruvate) or glucose	300	300	300
Bromate (as BrO_3^-)	0, 25 or 250	0, 25 or 250	0, 25 or 250
$MnCl_2.4H_2O$	2500	2500	2500
H_3BO_3	250	250	250
$ZnCl_2$	250	250	250
$CoCl_2.6H2O$	250	250	250
$NiSO_4.6H_2O$	250	-	-
$NiCl_2.6H_2O$	-	226	226
$CuCl_2.2H_2O$	150	150	150
$NaMoO_2.2H_2O$	5	5	5
Na_2SO_4	5	250 (as SO_4^{2-})	-
$NaNO_3$ (as NO_3^-)	-	250	-
Vitamin mixture	1 mL per litre	1 mL per litre	1 mL per litre

Table 6.9 - Vitamin mixture for agar plating trials

Component	Concentration (mg L^{-1})
D-biotin	20
Choline chloride	300
Folic acid	100
Myo-Inositol	3500
Niacinamide	100
p-amino Benzoic acid	100
D-pantothenic Acid.½Ca	25
Pyridoxine.HCl	100
Riboflavin	20
Thiamine.HCl	10
Vitamin B-12	0.5

Also includes inorganic salts KCl (200 mg L^{-1}), KH_2PO_4 (200 mg L^{-1}), NaCl (8000 mg L^{-1}), Na_2HPO_4 (1150 mg L^{-1})

The sterile agar plates were inoculated with 0.1 mL biomass during each trial, spread using a disposable sterile spreader bar (Microspec analytical Ltd, Ellesmere Port, UK). Inoculated plates were then placed in an anaerobic jar (GasPak 100; Becton, Dickinson and Co., Franklin Lakes, USA) or plastic pouch (VWR International Ltd, Lutterworth, UK), and anaerobic conditions introduced using packets containing proprietary anaerobic environment-inducing mixtures (Anaerocult A and Anaerocult C respectively; VWR International Ltd, Lutterworth, UK). Plates were incubated at 37°C in a constant temperature module (Genlab M175CF; Genlab, Widnes, UK) for 8 weeks. Plates were then inspected for growth, and any promising colonies subcultured by picking off a sample of the solid culture using a sterile disposable inoculation loop (Microspec analytical Ltd, Ellesmere Port, UK) and restreaking on fresh plates. In this way isolates could be maintained over the timescale of the project. Agar stabs were also briefly investigated as a means of short-term culture storage. Agar slopes were made in small (26 mm x 44 mm) glass vials using M-R2A agar. Following cooling, the stabs were inoculated using sterile plastic inoculation needles (Microspec analytical Ltd, Ellesmere Port, UK), placed in anaerobic plastic pouches and incubated at 37°C.

Some of the most promising cultures were preserved by freezing onto inoculated beads within small vials using the proprietary 'Protect' bacterial preservation (Technical Service Consultants Ltd, Heywood, Lancs, UK). Solid colonies from the agar plates were

picked off using a sterile disposable inoculation loop and placed in the cryopreservation fluid within a supplied vial, containing around 20 beads. The vial was capped and inverted at least 6 times before being left to stand for a minimum of 30 seconds to allow inoculation of the beads. The cryopreservation fluid was removed using a pipette with a sterile tip and the vials frozen in a low-temperature freezer at -76°C (Sanyo Vip -86°C series freezer; Patterson Scientific, Luton, UK). Efficacy of these frozen colonies following defrosting was subsequently tested in batch culture (Section 6.4.3.6, trial N).

6.4.3 Groundwater bioremediation batch trials

Batch trials were carried out on parameters pertinent to efficient chemostat operation. For each trial the standard method given in Section 6.4.3.1 was followed. Any deviations from this method to investigate specific parameters are outlined in the individual trial descriptions (Sections 6.4.3.2 – 6.4.3.6).

6.4.3.1 Standard batch trial method

Trials were carried out using 100 mL volumetric flasks (Fisher Scientific, Loughborough, UK) as test vessels. House Lane groundwater was utilised as the water source, with no further addition of bacterial nutrients. Groundwater was filtered using a 0.45 μm cellulose nitrate filter (Whatman 47 mm WCN type sterile membrane filter; Whatman laboratory division, Maidstone, UK) and Buchner funnel apparatus prior to use to remove bacteria already present in the water. Glucose and bromate solutions were added to all flasks, and resazurin redox solution (Fisher Scientific, Loughborough, UK) was included to give a visual indicator of redox conditions. A liquid (chemostat or batch flask origin) or solid bacterial inoculum was introduced, with the filtered groundwater added to give a final volume of 100 mL. Standard additions to the volumetric flasks are given in Table 6.10 and a picture of representative batch flasks in Figure 6.8.

Table 6.10 - Standard additions to batch flasks

Parameter		Final concentration or volume	Volume of stock solution used
Glucose		200 mg L^{-1}	5 mL of 4 g L^{-1}
Bromate		10 mg L^{-1}	1 mL of 1 g L^{-1}
Resazurin (Redox indicator)		-	0.5 mL of 0.005%
Inoculum	Liquid	5 mL or 10 mL	n/a
	Solid	Cells harvested from agar plate using a sterile inoculating loop	
House Lane groundwater (0.45 µm filtered)		Added to give 100 mL final volume	

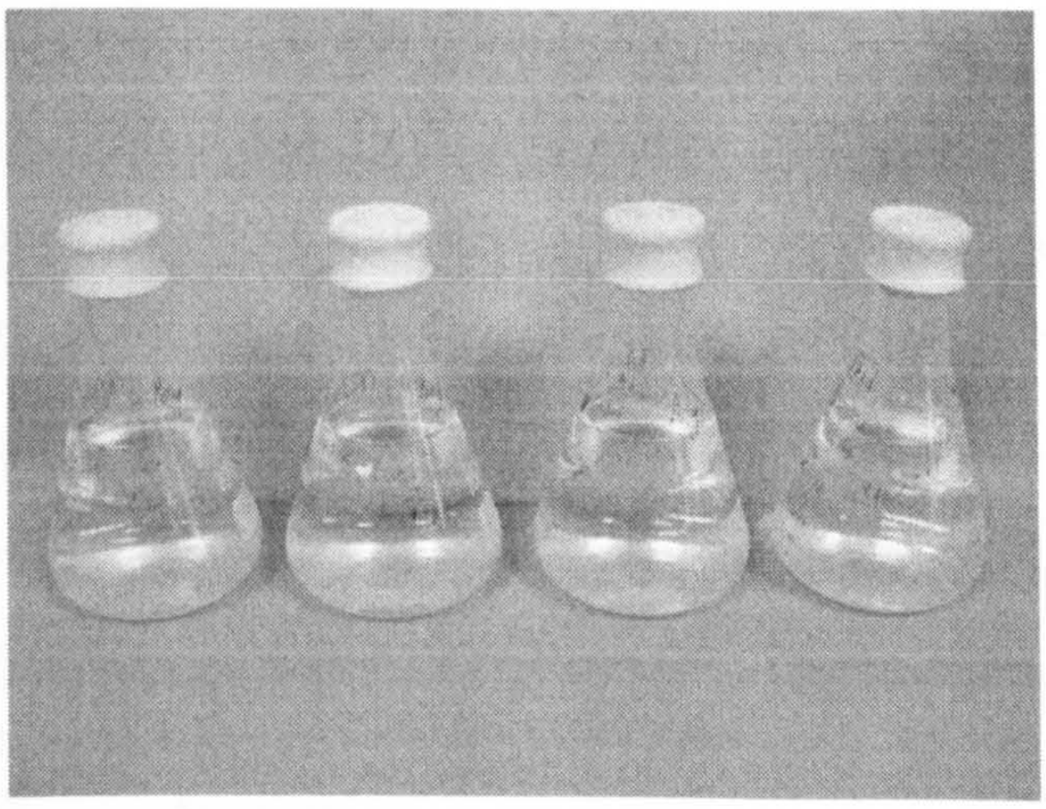


Figure 6.8 - Representative flasks used for batch flask trials

Following addition of all flask constituents a 0.5 mL sample was taken from each flask using a pipette for anion analysis using IC Method 5 (Chapter 5), with duplicate analytical runs completed on all samples and the mean value of these two results recorded. Each flask was sparged with oxygen-free nitrogen (BOC gases, Manchester, UK) for 30 seconds to replace air headspace, and flasks were immediately sealed using 'Suba-seal' type rubber turnover stoppers (Fisher Scientific, Loughborough, UK) to provide an airtight seal.

Flasks were incubated in a constant temperature room at 17°C to emulate conditions within the chemostat environment (Phases II and III). Samples for anion analysis were taken after 4 – 10 days once growth had commenced, and also after 30 – 40 days upon completion of the trial. Samples at each timepoint were taken using a syringe (Fisherbrand; Fisher Scientific, Loughborough, UK) and hypodermic needle (Terumo Neolus; Terumo Europe NV, Leuwen, Belgium) combination, puncturing the stopper and withdrawing approximately 0.6 mL sample. Integrity of the 'Suba-seal' type sealing system is not compromised by puncturing in this way. 0.5 mL of each sample collected was subsequently taken using a pipette for anion analysis by IC Method 5.

6.4.3.2 Parameter assessment

Three parameter assessment trials were completed, investigating bromate concentration (Trial A), carbon source (Trial B) and temperature (Trial C). Flasks were set up according to Section 6.4.3.1 (5 mL liquid chemostat inoculum), although filtering of groundwater was not undertaken for these trials, with the aim only to assess overall bromate degradation ability of the water/inoculum mix present within the chemostat upon manipulation of individual parameters. Trials undertaken are outlined in Table 6.11.

Table 6.11 - Summary of parameter assessment batch trials

Trial	Parameter	Conditions trialled (Units)	Replicates per condition	Analysis (Days from start)
A	Bromate concentration	100, 10, 2 (mg L^{-1} as BrO_3^-)	2	0, 4, 35
B	Carbon source	Glucose, acetate, ethanol	2	0, 4, 35
C	Temperature	6, 17, 27, 37, 44 (°C)	3	0, 2, 4, 8, 16, 32

Bromate levels were altered in trial A by utilising stock solutions of corresponding concentrations. Glucose stock solution in trial B was as outlined in Table 6.10; 4 g L^{-1} acetate stock solution was made up from laboratory-grade sodium acetate (Fisher Scientific, Loughborough, UK); 4 g L^{-1} ethanol stock solution was made by diluting absolute ethanol (Laboratory grade; Fisher Scientific, Loughborough, UK) in RO water (15.2 MΩ). Temperature manipulation in trial C was achieved by use of temperature controlled rooms (6°C and 17°C), a water bath (27°C), and temperature controlled incubators (37°C and 44°C).

6.4.3.3 Biotic versus abiotic reduction

Abiotic reduction of bromate by iron species is known to occur (Section 2.7.2), and one previous brief study (Fielding, 2002) had suggested bromate could be abiotically reduced by glucose, although limited supporting evidence was given in this case. All other, more comprehensive bioreactor trials have shown bromate reduction within both batch and continuous flow bioreactors to be microbially mediated (Hijnen *et al.*, 1995; Kirisits and Snoeyink, 1999; Kirisits *et al.*, 2002; van Ginkel *et al.*, 2005b). Therefore, a requirement for confirmation of the biotic nature of bromate removal in the current study was identified. It was also necessary to experimentally verify the efficacy of House Lane groundwater filtration using 0.45 45 µm pore size filters. Although over 90% of heterotrophic plate count (HPC) bacteria are removed on 0.45 µm filters, studies have identified a small population of filterable bacteria able to pass through a 0.45 µm filter but caught on a filter of 0.22 µm pore size (Lillis and Bissonnette, 2001). Batch trial D undertook to confirm both these points. Finally, competition from facultative anaerobes present on the glassware and other apparatus could possibly affect proliferation of bromate reducers within the flasks. Therefore, batch trial E compared bromate reduction between uninoculated sterile and unsterile flasks, both containing 0.45 µm pore size filtered groundwater. All sampling for IC analysis using IC method 5 (Chapter 5) was using the hypodermic syringe technique outlined in Section 6.4.3.1.

Filters used for batch trial D were Durapore PVDF 0.45 µm filter units (Millipore, Carrigtwohill, Co. Cork, Ireland) and Millex PVDF 0.22 µm 25 mm syringe-tip filters

(Millipore, Carrigtwohill, Co. Cork, Ireland). Flasks were set up as according to Section 6.4.3.1 and uninoculated to act as comparative controls. Separate flasks containing water filtered using the Durapore 0.45 µm pore size filter were inoculated with liquid inoculum from the chemostat system. Flasks containing unfiltered House Lane water were also trialled. Sampling was carried out at 0, 7 and 36 days.

Trial E used identical flasks prepared under sterile and unsterile conditions. No inoculum was added to any flasks. Flasks were set up with four replicates per treatment. Sterile groundwater medium was prepared using the standard batch trial method, and autoclaved at 121°C (Priorclave autoclave; Priorclave, London, UK) for 15 minutes prior to pouring into sterile 100 mL volumetric flasks within a laminar flow cabinet (Labcaire SC-R Class II microbiological safety cabinet; Labcaire systems, Clevedon, UK), sparging with oxygen-free nitrogen to replace air headspace using a sterile hose and sealing with a sterile 'Suba-seal'-type turnover stopper. Sampling of sterile flasks was carried out using sterile needles, with the stopper flamed prior to sampling to prevent any ingress of bacterial contamination. Sampling was carried out at 0, 8 and 36 days.

6.4.3.4 Electron acceptor and donor usage

Trial F investigated order of anion utilisation over a 32-day period within chemostat culture samples, and also samples of culture 35 which was previously isolated by plating studies (Section 6.4.2). Flasks were set up in triplicate according to Section 6.4.3.1, with addition of 5 mL of the relevant inoculum. Flasks were sampled using the hypodermic needle technique at 1, 2, 3, 4, 7, 8, 16 and 32 days, with samples undergoing anion analysis using IC method 5.

Trial G also investigated anion utilisation but, in addition carbon usage was monitored over the experimental period. Five flasks were set up according to Section 6.4.3.1, with 5 mL inoculum from chemostat reactor 2 added to two flasks, 5 mL inoculum from chemostat reactor 1 added to a further two flasks and 5 mL RO water (15.2 MΩ) added to the fifth flask as a control. At the time of trial commencement (January 2005), chemostat reactor 1 was being fed with artificial (low nitrate/sulphate) water and reactor 2 the House Lane-based supply. However, both reactors were

exhibiting active and similar levels of bromate reduction. Following sealing of the flasks, samples were taken by hypodermic needle at 4 and 8 hours, and 1, 2, 3, 9, 19, 27, 36 and 46 days. All samples taken were analysed for anion concentrations using IC method 5. The two flasks with chemostat reactor 2 inoculum plus the control flask were also analysed for glucose, lactate, ethanol and acetate using proprietary test kits (VWR International, Lutterworth, UK; R-Biopharm Rhône Ltd, Glasgow, UK) and a Merck RQFlex reflectometer (VWR International, Lutterworth, UK), as detailed in Appendix A. As a consequence of the additional carbon analysis, a total volume of approximately 1 mL was withdrawn from these flasks at each sampling point. Time constraints of the carbon analysis method at the start of the trial precluded carbon analysis of the two flasks containing reactor 1 inoculum.

6.4.3.5 Dilution-to-extinction

In addition to isolation of strains within plate cultures (Section 6.4.2), a dilution-to-extinction trial (Trial H) was also completed, consisting of progressive inoculum dilutions within batch culture flasks. Procedure was as outlined in Section 6.4.3.1, with 10 mL supernatant inoculum added at the following dilutions: 1:100, 1:1000, $1:10^4$, $1:10^5$, $1:10^6$, $1:10^7$, $1:10^8$. One set of dilutions (single replicate per dilution) was completed using inoculum from chemostat reactor 1 and another set with reactor 2 inoculum. Flasks were incubated at 17°C and samples analysed for anion concentration using IC method 5 after 30 days incubation. Flasks of the lowest two dilutions exhibiting bromate reduction after 30 days incubation were subsequently used as inoculum (10 mL per flask) for a further series of batch trials (Trial I), where flasks were set up in triplicate and samples taken for anion analysis after 8 and 36 days.

6.4.3.6 Bromate removal by isolates

Strains isolated using plate culture techniques (Section 6.4.2) were investigated to confirm their bromate reducing capabilities. Trials undertaken are summarised in Table 6.12. In each trial flasks were set up as outlined in Section 6.4.3.1, apart from Trials L and N. Trial L was aimed at investigating the performance of three isolates and chemostat inoculum within the artificial medium outlined in Section 6.4.1.3. Samples

were therefore inoculated into flasks containing the artificial medium, made up without addition of nitrate and sulphate as given in Table 6.13. Trial N investigated efficacy of cultures previously placed in storage using the 'Protect' bacterial preservation system (Section 6.4.2), and for this trial three frozen beads were used as inoculum in each flask instead of a liquid or solid culture inoculum.

Table 6.12 - Samples undertaken during batch flask trials J – N

Trial	Number/identity of isolates trialled	Replicates per isolate	Analysis (Days from start)
J	10 isolates from plating trial (32 – 41) Liquid chemostat inoculum (labelled 17)	1	0, 8, 30
K	7 liquid cultures subcultured from batch trial J Liquid chemostat inoculum	2	0, 8, 36
L	3 isolates from liquid stock subcultures (31, 33, 35) Liquid chemostat inoculum	1	0, 8, 30
M	40 isolates from plating trials (A – H; M – X; A1 – T1)	1	0, 40
N	6 stored isolates (17, 31, 33, 35, 59, 60) (3 'Protect' beads per flask)	1	0, 30

Table 6.13 - Chemical additions for batch artificial medium (Trial L)

Nutrient	Concentration within artificial matrix (mg L^{-1})
$NaH_2PO_4.2H_2O$	300
$MgCl_2.6H_2O$	8.25
$\pm MnCl_2.4H_2O$	2500
$CuCl_2.2H_2O$	150
$ZnCl_2$	250
$CoCl_2.6H_2O$	250
$Na_2MoO_4 2H_2O$	5
$FeCl_3.6H_2O$	1
$CaCl_2.2H_2O$	15
NH_4Cl	300
$NiCl_2.6H_2O$	226

6.4.4 Pilot-scale groundwater bioremediation

Pilot-scale trials were conducted using two identical bioreactors (Reactors 1 and 2) operated as separate, parallel systems to treat bromate-contaminated groundwater. A picture of the rig is given in Figure 6.9, with a schematic of one reactor in Figure 6.10. The experimental rig was housed in an unheated indoor test facility at Cranfield University. The two cylindrical reactors had a height of 1.4 m and internal diameter of 0.2 m. For continuous flow operation they were packed with Etapak 210 (Koch-Glitsch UK, Stoke-on-Trent, UK), a random plastic media with a diameter of 63 mm, surface area of 200 m^2 m^{-3} and voidage of 96%. The media was packed within each reactor to give a bed height of 1.2 m and volume of 36 litres.

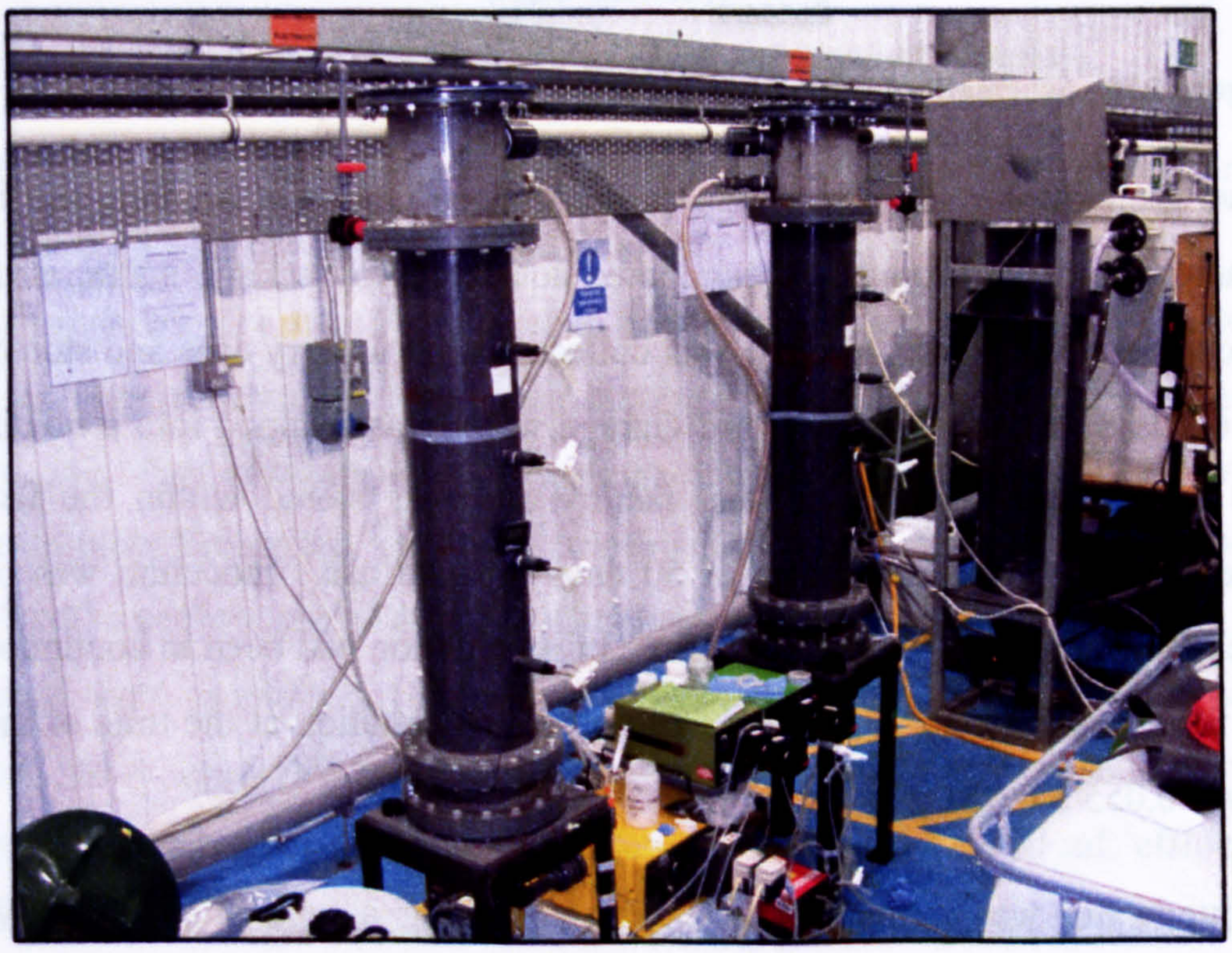


Figure 6.9 - Picture of pilot-scale bromate bioremediation rig

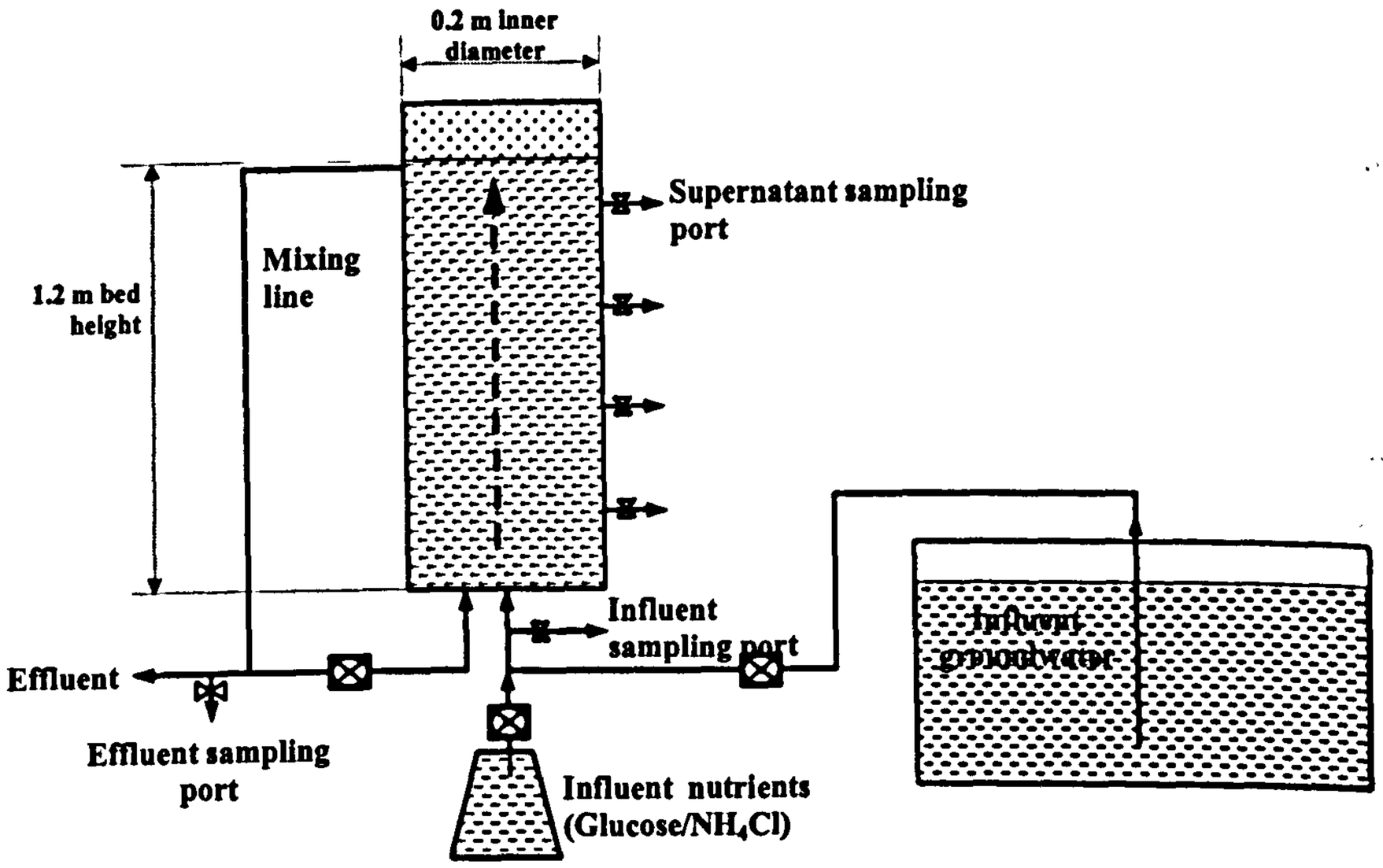


Figure 6.10 - Schematic of upflow bioreactor

Two groundwater supplies with elevated concentrations of bromate and bromide were obtained from the bromate-contaminated aquifer. House Lane water, utilised for initial batch operation (Phase A) only, was collected in 25 L jerry cans and stored at 7°C for up to 60 days prior to use. Orchard Garage water was pumped into a holding tank (1.05 m^3) for transportation to the test facility and was stored within the facility at ambient temperature for a maximum of 50 days prior to use. Inoculum was obtained from the chemostat system which, at the time of inoculation had been in continuous flow operation for approximately 15 months. Biomass concentration at the time of inoculum addition was 0.055 ± 0.021 g dry wt L^{-1}.

Groundwater was pumped into the reactor using a peristaltic pump (Watson-Marlow 302S/503S; Watson-Marlow, Falmouth, UK) at flow rates of 7.1 – 57.0 mL min^{-1}, with nutrient stock solution added at 0.38 – 3.0 mL min^{-1} via a separate peristaltic supply pump (Watson-Marlow 501U; Watson-Marlow, Falmouth, UK). The nutrient stock solution contained 2 g L^{-1} glucose as carbon source and 2 g L^{-1} ammonium chloride (NH_4Cl) as nitrogen source (both laboratory grade; Fisher Scientific, Loughborough, UK). This was added at a rate calculated to give a final

groundwater:amendments dilution ratio of 20:1, and thus a 100 mg L^{-1} final influent concentration of both glucose and NH_4Cl (40.0 mg L^{-1} as C and 26.2 mg L^{-1} as N). It was assumed trace metals were present in the groundwater at sufficient concentrations for bacterial requirements. No attempt was made to optimise addition of carbon (which was added in excess), with the aim of the study focussing on ability and performance of the reactors in removing bromate contamination.

Influent flow rates gave reactor retention times of 10 – 100 hours, with mixing provided by a peristaltic pump (Watson-Marlow 623S; Watson-Marlow, Falmouth, UK) which continuously recirculated the reactor contents at a flow rate of 0.7 L min^{-1}. Effluent flow was via an overflow with one-way valve on the mixing line. No backwashing facility was present within the reactor setup. Four sampling ports were spaced evenly up the side of each reactor but, due to the recirculation leading to mixing within the reactors, only the top port was used for supernatant samples.

Routine monitoring was carried out every 1 – 2 days, with one 10 mL sample taken from each reactor via the top sampling port on each reactor for biomass (measured spectrophotometrically at 600 nm) and pH monitoring. In all cases steady state conditions were deemed to have been reached after a minimum of 3 liquid volume changes, and when biomass and pH readings had stabilised. Groundwater and nutrient solution influent flow rates were also monitored every 1 – 2 days. Influent, supernatant and effluent samples were taken for full analysis every 5 – 7 days and/or upon attainment of a steady state condition. During full analysis, a 5 mL nutrient solution sample was taken and made up to 100 mL with groundwater to emulate influent supply, as separate nutrient and groundwater influents precluded direct sampling. 100 mL effluent samples were collected from the effluent sampling ports, with time required to collect the effluent sample therefore dependent on reactor retention time. A supernatant sample of 100 mL was subsequently taken from both reactors. Parameters analysed during routine monitoring and full analysis were identical to those investigated during laboratory-scale chemostat trials (Section 6.2.2). TOC analysis during these trials was only possible during phase Bii.

The period of reactor operation was split into two phases, with an initial 23-day start-up period of batch configuration identified as Phase A (Days A0 – A22), and the subsequent 217-day continuous flow operation given as Phase B (Days 0 – 216). Phase A was run as a suspended growth system using reactor 2 only. Plastic media was added to both reactors for fixed-film operation on the first day of Phase B (Day 0). Within Phase B an acclimation period (Phase Bi) of 63 days (Days 0 – 62) was followed by two experimental periods investigating effects of retention time (Days 63 – 117) and then performance during an extended experimental run (Days 117 – 216). These periods were designated phases Bii and Biii respectively.

6.4.4.1 Phase A - Inoculation and batch suspended growth period

On day A0, Reactor 2 was completely filled with House Lane groundwater plus 25 mg L^{-1} bromate (laboratory grade potassium bromate; Fisher Scientific, Loughborough, UK) and 100 mg L^{-1} glucose (laboratory grade; Fisher Scientific, Loughborough, UK). 200 mL chemostat inoculum was added on days A1, A2 and A5. The reactor was then left under suspended growth conditions as a batch system to allow acclimation and growth for a total of 23 days. Additional glucose solution was added on days A15, A19, A20 and A21, to give a final reactor concentration of 100 mg L^{-1} in each case. The reactor was continuously sparged with oxygen-free nitrogen (BOC gases, Manchester, UK). On the final day of Phase A (Day A22), 50% of reactor 2 contents were transferred to reactor 1, with both reactors then filled with plastic media on day 0 (start of Phase B), sealed and set into continuous flow using Orchard Garage groundwater and an RT of 80 hours. Groundwater was not further amended with bromate or bromide over that already present as contamination. Sparging with nitrogen was discontinued with completion of Phase A.

6.4.4.2 Phase B – Continuous flow fixed-film reactor operation

Upon commencement of Phase B, reactor 1 was designated the control reactor and operated at an 80-hour RT until day 97, when it was increased to a 100-hour RT for the remaining experimental period. Reactor 2 was operated at an 80-hour RT during the acclimation period (Phase Bi; Days 0 – 62), followed by a series of steady states at

retention times of 80, 60, 40, 20 and 10 hours plus subsequently 100 hours (Phase Bii; Days 63 – 117). Both reactors were operated as batch systems with manual glucose addition on days 45 – 56, due to a nutrient pump failure on day 42 which led to partial washing out of the biomass. Reactor 1 was operated only intermittently in continuous flow mode until day 74, due to an observed susceptibility of the biomass to wash out under these conditions. Steady state conditions were recorded in reactor 1 at an 80-hour RT between days 81 – 97 and at a 100-hour RT (Phase Biii) between days 117 – 216.

7 RESULTS

Bromate behaviour and potential remediation techniques were investigated within wastewater and groundwater matrices using a variety of trials during the current project. In this chapter results of all studies are outlined, commencing with data obtained from trials involving bromate within wastewater samples. Results of investigations into groundwater bromate bioremediation are then set out and examined.

7.1 BROMATE CONTAMINATION WITHIN WASTEWATER

Results from the three wastewater trials are detailed in this section, with trials investigating respiration inhibition outlined (Section 7.1.1), followed by data obtained during sludge biomass sorption trials (Section 7.1.2). Finally, results gained from the pilot-scale MBR dosing trials are given (Section 7.1.3).

7.1.1 Wastewater sludge biomass respiration inhibition

A range of bromate and bromide concentrations between 10 μg L^{-1} and 2 g L^{-1} were trialled for respiration inhibition within an MBR sludge matrix, to ascertain any overall inhibitory effects of contamination on wastewater processes. Trials 1 and 4 both investigated effects of bromate addition, with trial 4 utilising greater replication of each condition to provide greater statistical accuracy. Trial 2 studied the effect of bromide spiking on biomass, and trial 3 investigated combinations of both bromate and bromide spiking. In all four trials at least one cell was spoilt, generally due to equipment failure, but data for 2 or more replicate cells for each treatment were available for every run. Results for all trials were analysed by converting to Specific Oxygen Uptake Rate (SOUR) values and plotting on a graph against time. During each trial a 'settling' period of 1 – 2 hours was noted where the SOUR value fluctuated prior to settling down at a steady value after around 4 hours. Upon completion of the 27-hour run, trials 2, 3 and 4 showed similar SOUR values, with an average of 4.14×10^{-5} mg O_2 mg $MLSS^{-1}$ min^{-1} (n=5) obtained. This result agrees with the value of 3.5×10^{-5} mg O_2 mg $MLSS^{-1}$ min^{-1} reported by Mayhew and Stephenson (1998) using similar apparatus and activated sludge samples, although in that case the steady value was not reached until around 50 hours

after commencement of the trial. Another comparable trial produced a SOUR value approximately 5 times lower than that obtained in the current study (Burgess, 1999). The two runs of trial 1 showed varying final SOUR values of 6.24 mg O_2 mg $MLSS^{-1}$ min^{-1} and 2.34 mg O_2 mg $MLSS^{-1}$ min^{-1}. Sludge MLSS values were carried out for each respirometry trial and, and are used here to give a general indication of operational stability (Figure 7.1). It can be seen that biomass concentrations were still rising from 1.6 to 3.8 g L^{-1} during the trial 1 period, indicating biomass was acclimatising within the MBR at the time of this trial. Trials 2 – 4, by contrast, were all completed during a relatively steady period of MBR operation, indicated by MLSS values which only fluctuated within the range 3.8 – 5.6 g L^{-1}. However, as the current trial was only investigating respiration inhibition (ie. alterations in respiration rate over the timescale of each individual trial) and not absolute SOUR values, it is unlikely magnitude of the SOUR would affect results obtained in any of these trials.

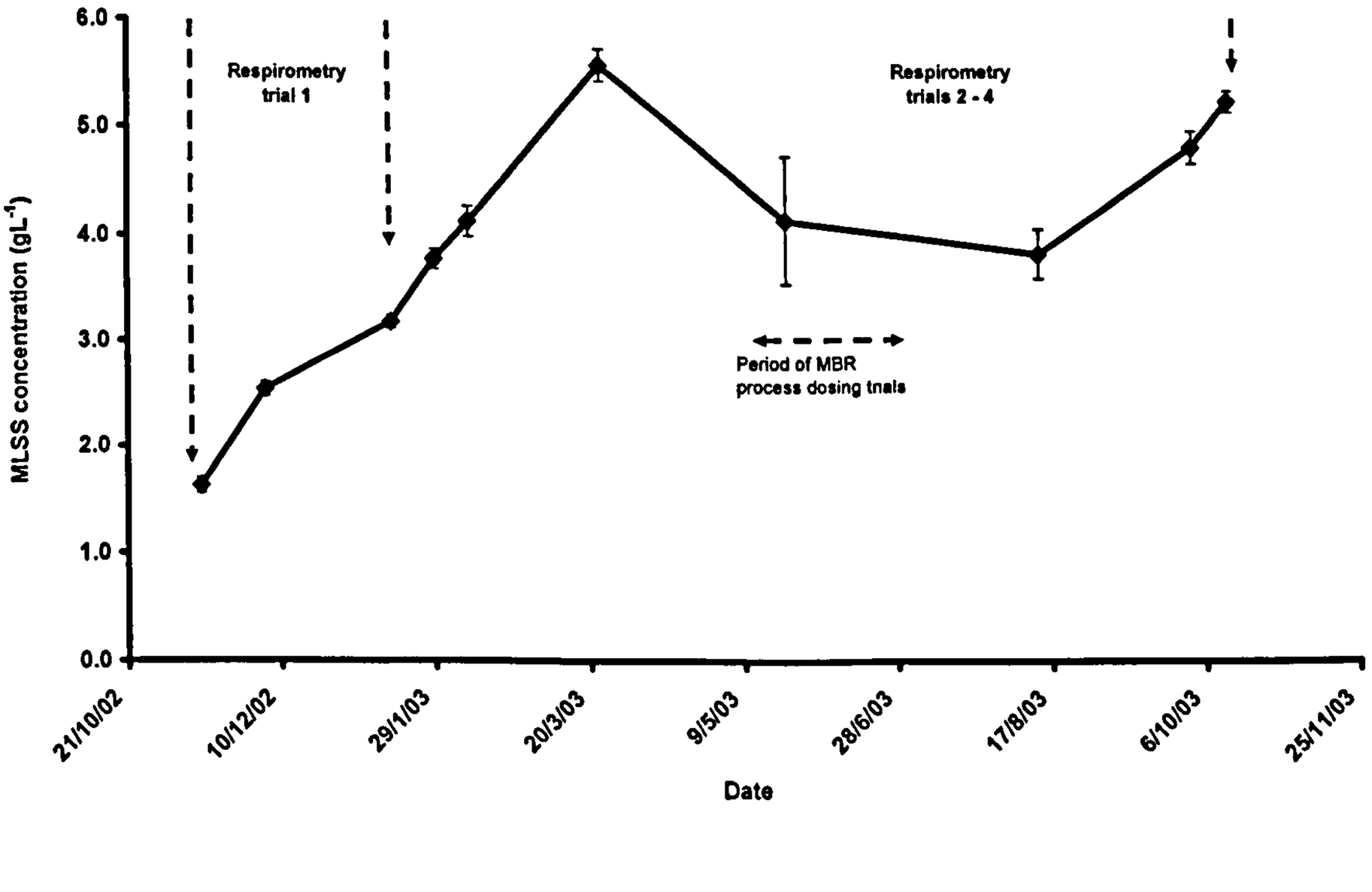


Figure 7.1 - MLSS concentrations for source sludge during respiration inhibition trials

Trial 1 investigated the full range of bromate concentrations (20 μg L^{-1} – 2 g L^{-1}), with duplicate runs carried out as trials 1a and 1b. In trial 1a there was good agreement between replicates at each bromate concentration, and no significant difference from

control (Kruskal Wallis, p = 0.7298) was noted at any contamination level. Trial 1b showed greater variation between replicates and, although there was no significant difference between treatments (Kruskal Wallis, p = 0.3041), visual inspection of the data revealed an apparent lowering of SOUR between control and 2 g L^{-1} bromate spiked samples. This suggested the 2 g L^{-1} bromate concentration was inhibiting respiration. However, the lack of replication (n = 2 for control and 2 g L^{-1}) precluded any further statistical analysis and it was not possible to draw firm conclusions without repeating the study. Repetition was completed during trial 4, using fewer bromate concentrations but with greater replication. Results recorded as SOUR values over the 27-hour run time for trials 1a and 1b are given in Figure 7.2 and Figure 7.3 respectively. Traces for 20 $\mu g\ L^{-1}$, 2 mg L^{-1} and 20 mg L^{-1} have been omitted from Figure 7.3 due to large variations between sample values.

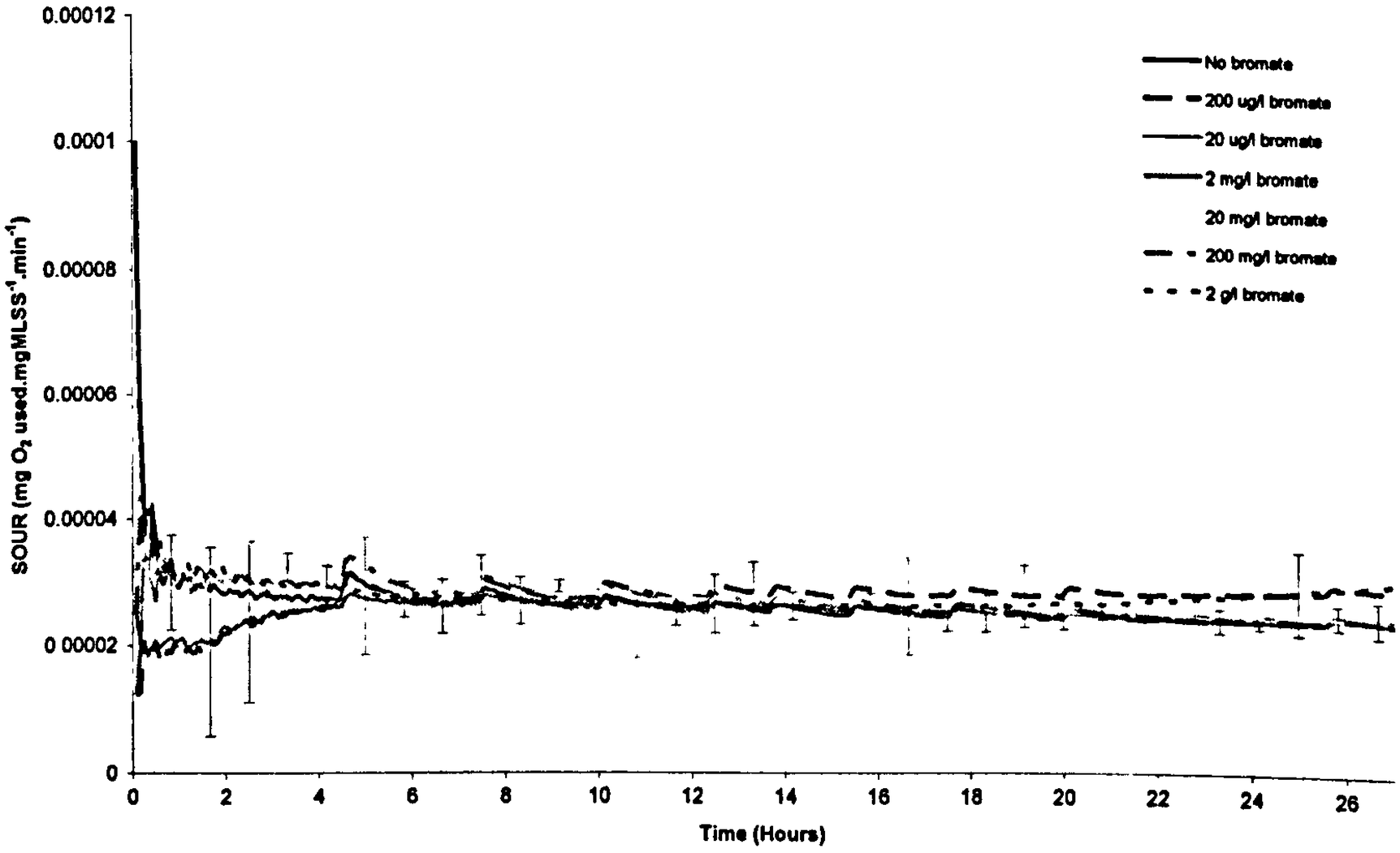


Figure 7.2 - Specific oxygen uptake rate (SOUR) of MBR sludge spiked with a range of bromate concentrations (Trial 1a)

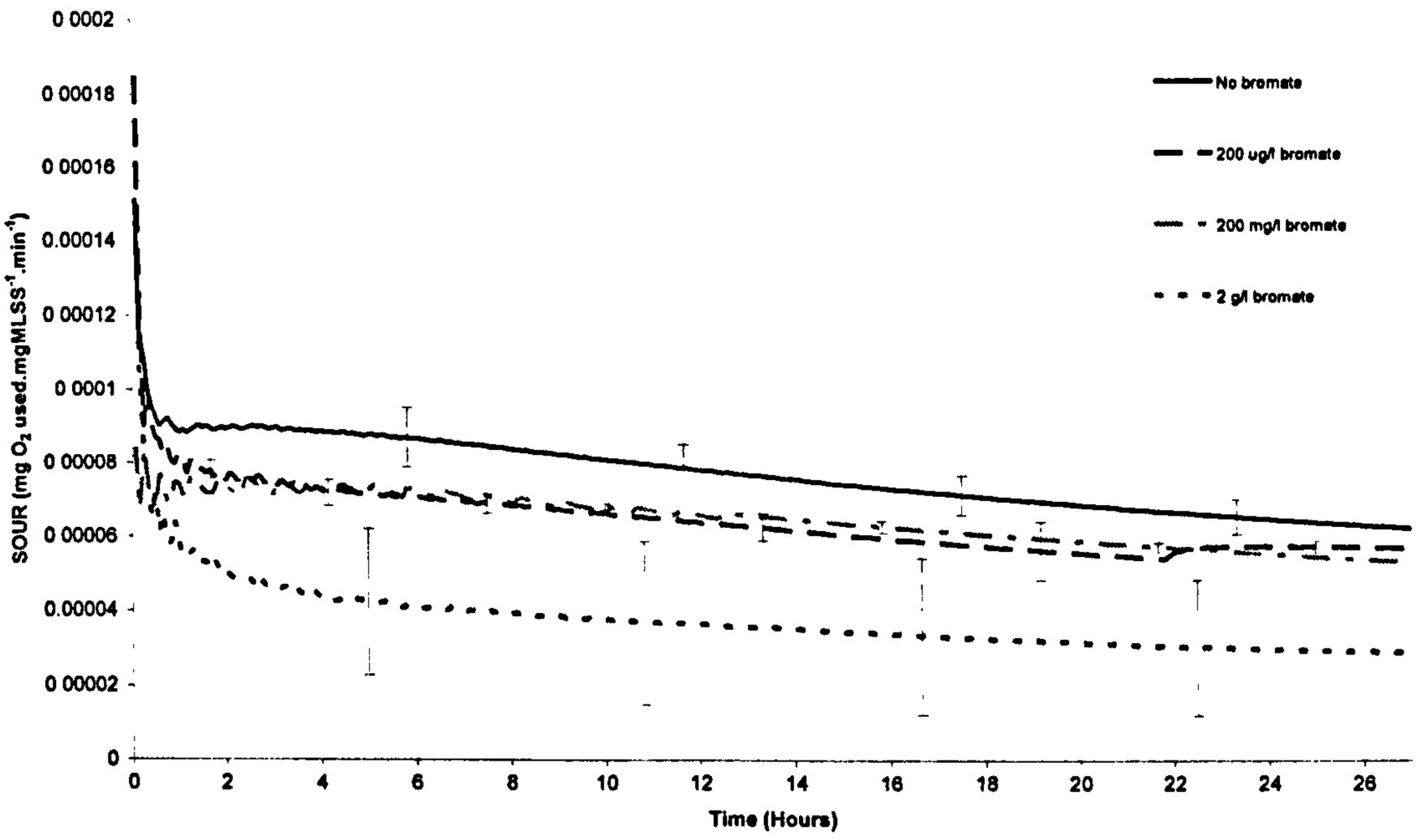


Figure 7.3 - Specific oxygen uptake rate (SOUR) of MBR sludge spiked with a range of bromate concentrations (Trial 1b)

Trial 2 repeated the methodology of trial 1 with bromide instead of bromate spiking, and an increase in replication (5 replicates per sample) was used to improve resolution of the results. Thus two trials were completed to investigate the entire range of concentrations required. Bromide concentrations trialled were between 10 μg L^{-1} and 200 mg L^{-1}. In neither trial 2a or 2b was a significant alteration in bacterial respiration noted (Kruskal Wallis, $p = 0.7831$ (Trial 2a), $p = 0.5460$ (Trial 2b)). Results recorded as SOUR values over the 27-hour run time are given in Figure 7.4 and Figure 7.5 for trials 2a and 2b respectively.

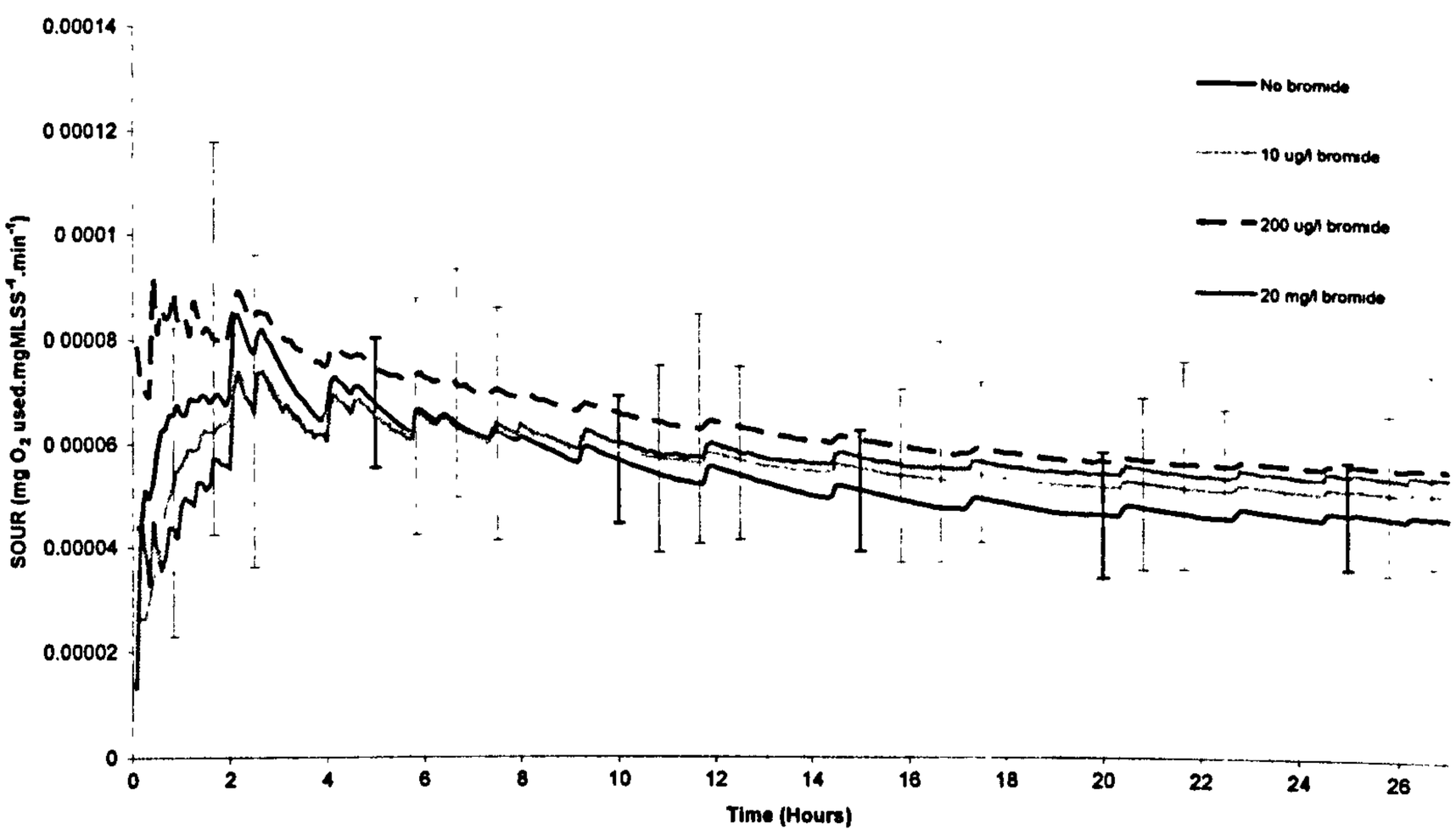


Figure 7.4 - Specific oxygen uptake rate (SOUR) of MBR sludge biomass spiked with 10 µg L^{-1}, 200 µg L^{-1} and 20 mg L^{-1} bromide concentrations (Trial 2a)

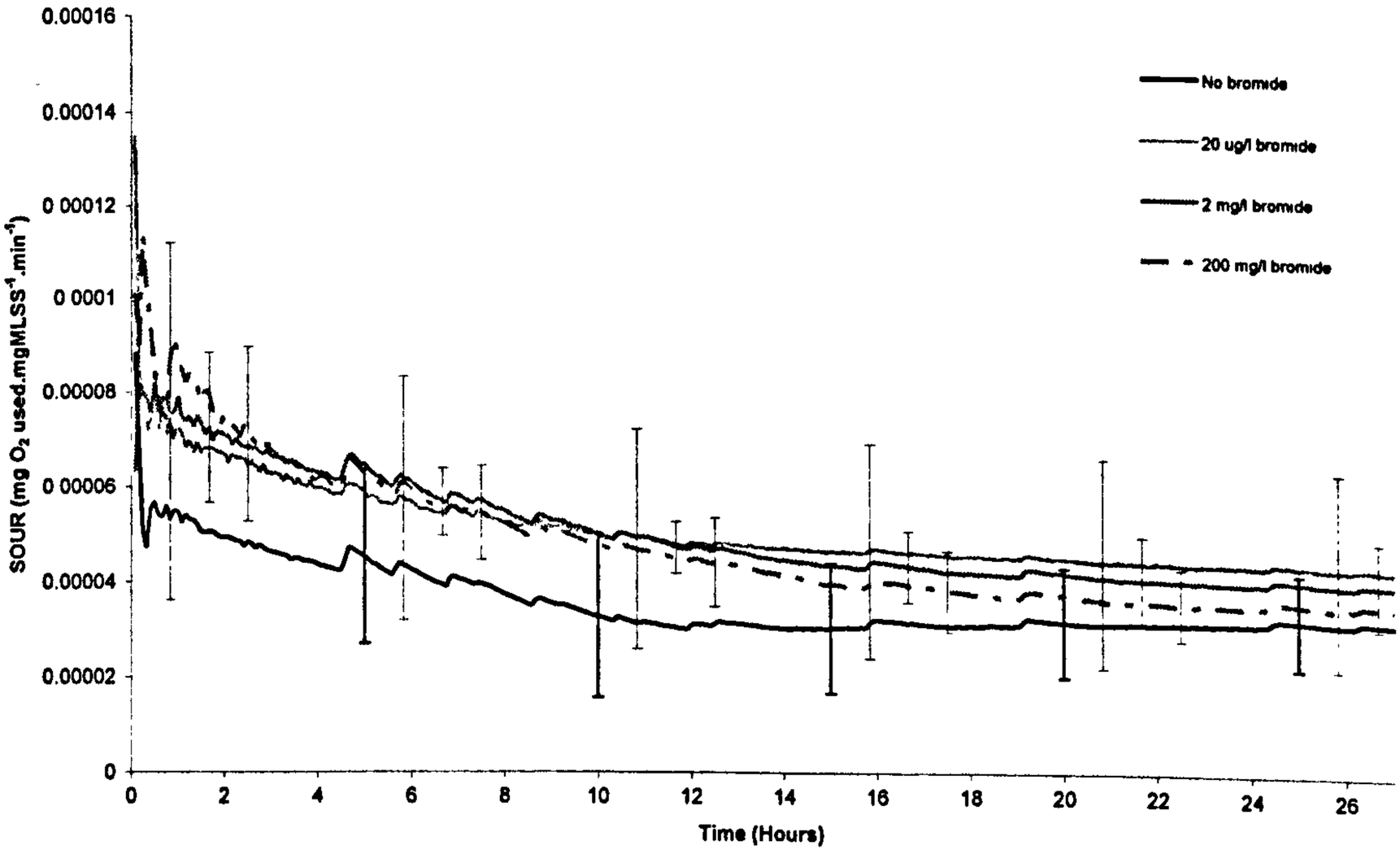


Figure 7.5- Specific oxygen uptake rate (SOUR) of MBR sludge biomass spiked with 20 µg L^{-1}, 2 mg L^{-1} and 200 mg L^{-1} bromide concentrations (Trial 2b)

Trial 3 investigated potential interactions between bromate and bromide on respiration rate, as any ingress of contaminated water to a treatment process would be likely to contain both constituents. Bromate:bromide ratios of 1:2, 1:4 and 1:6 were selected, dosed as bromate:bromide contamination levels of 2.5:5 mg L^{-1}, 200:800 µg L^{-1}, and 30:180 µg L^{-1} respectively. These concentrations were selected as they roughly corresponded with contamination levels encountered within three boreholes at the time of the trial. No significant differences over the control treatment were noted (Kruskal Wallis, p = 0.9749). Results recorded as SOUR values over the 27-hour run time are given in Figure 7.6.

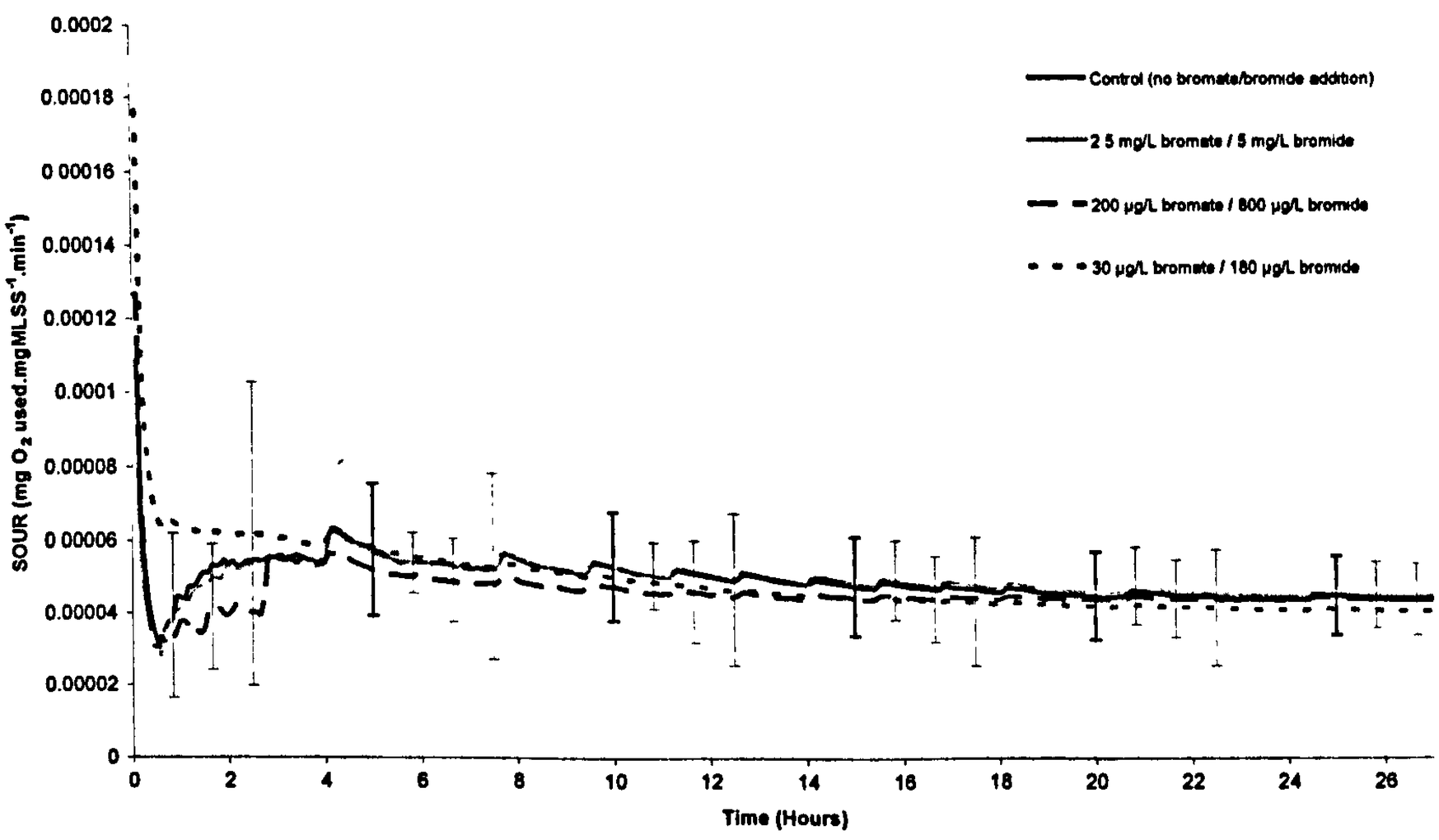


Figure 7.6 - Specific oxygen uptake rate (SOUR) of MBR sludge biomass spiked with three bromate: bromide ratios (Trial 3)

Trial 4 reinvestigated the non-significant result of 2 g L^{-1} bromate contamination noted in trial 1b. Trial 4a was aimed at confirming a lack of respiration inhibition at the medium bromate concentration of 2 mg L^{-1}, and trial 4b re-examined effects of the 2 g L^{-1} contamination level. With a dose of 2 mg L^{-1} no effects of bromate dosing were noted (Mann Whitney, p = 0.9654), but with the 2 g L^{-1} bromate concentration a significant effect was observed (Mann Whitney, p = 0.0159) at the 95% confidence level, thus confirming the inhibitory effects noted in trial 1b. Results recorded as SOUR values over the 27-hour run time are given in Figure 7.7 and Figure 7.8 for trials 4a and 4b respectively.

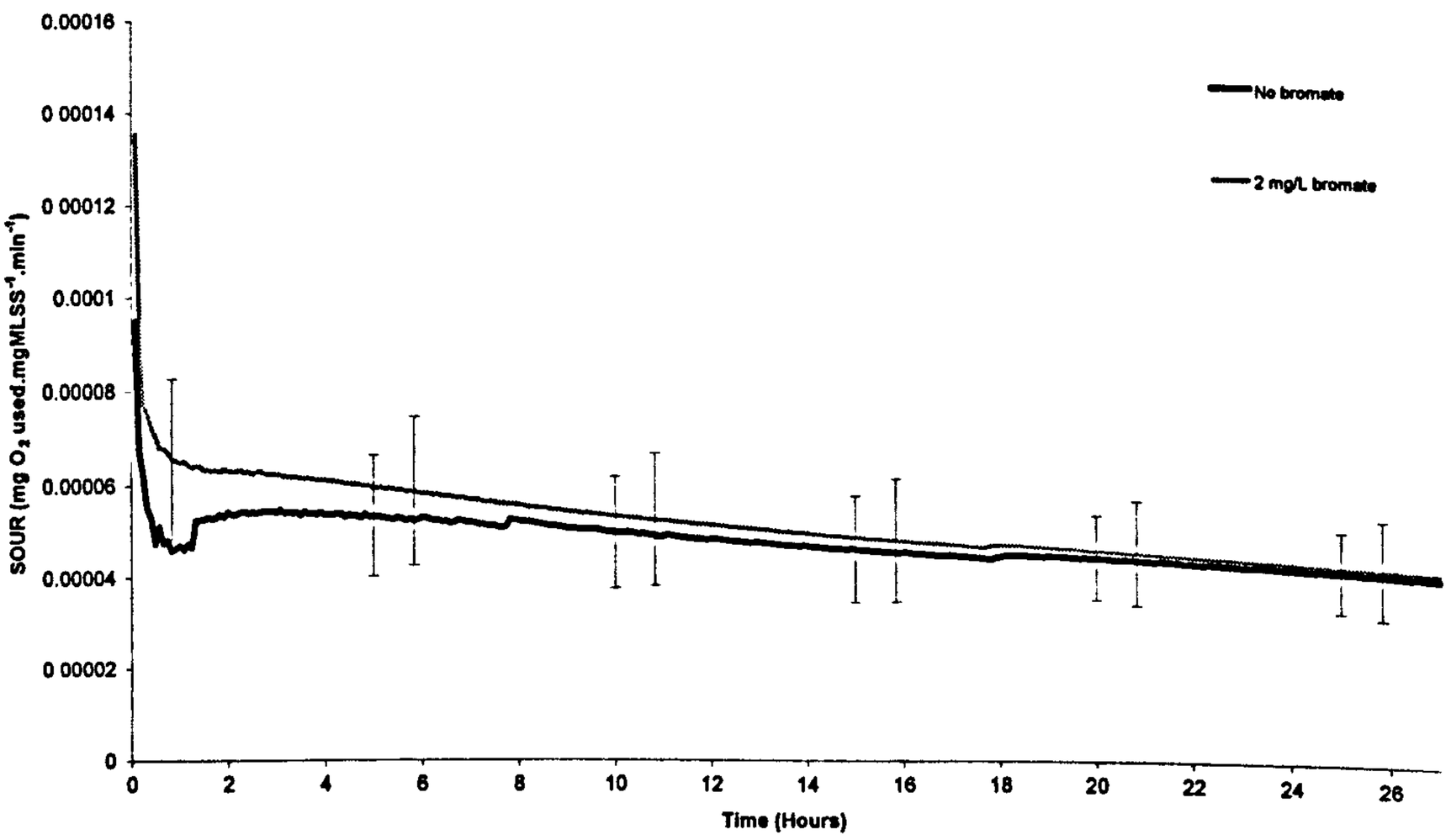


Figure 7.7 - Specific oxygen uptake rate (SOUR) of MBR sludge biomass spiked with 2 mg L^{-1} bromate (Trial 4a)

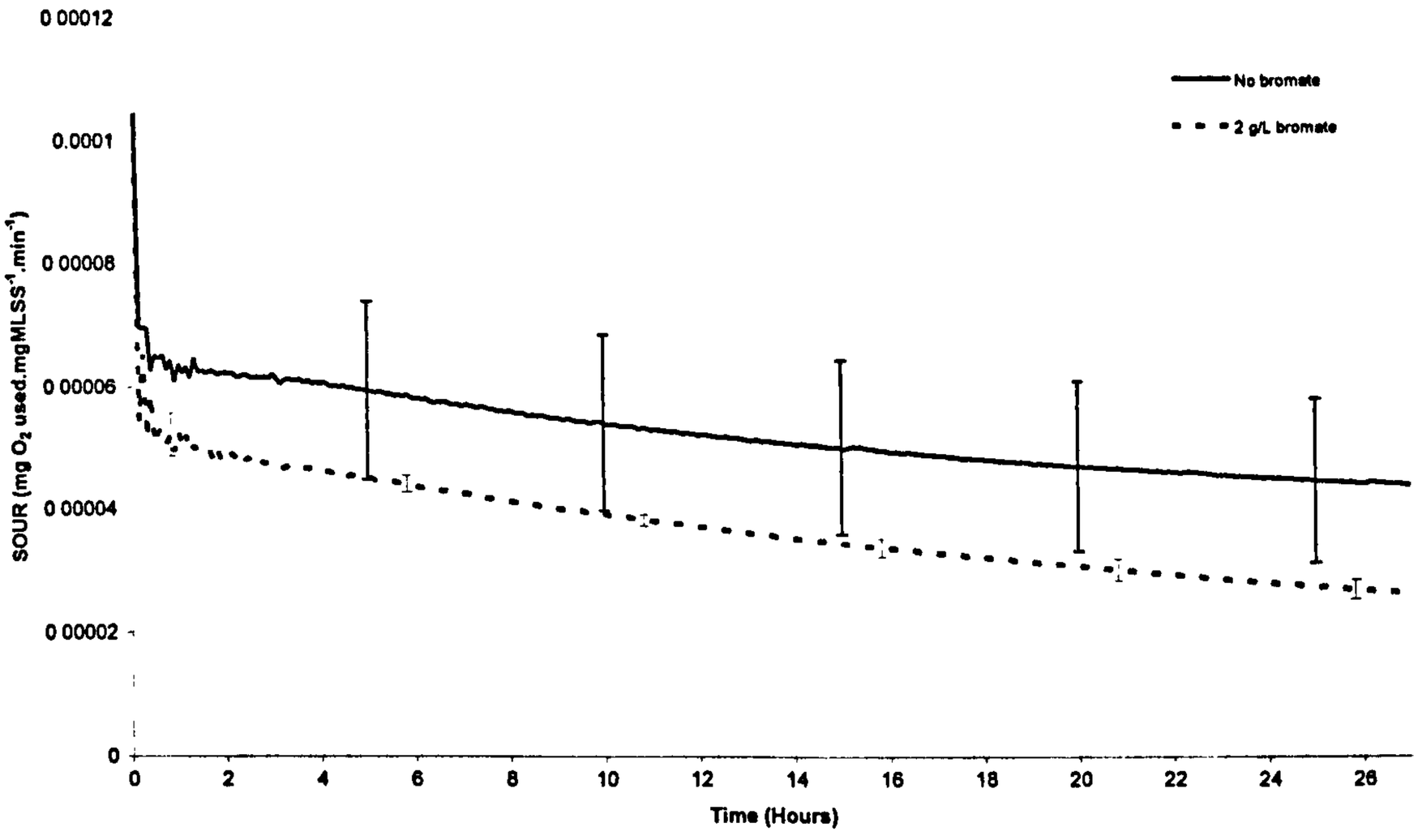


Figure 7.8 - Specific oxygen uptake rate (SOUR) of MBR sludge biomass spiked with 2 g L^{-1} bromate (Trial 4b)

7.1.2 Wastewater sludge biomass sorption

Sorption of bromate to sludge biomass was investigated to ascertain whether bromate would be reversibly retained within wastewater processes. Sorption trial 1 investigated bromate and bromide concentrations at a range of biomass:solution ratios between 1:25 and 1:200 using a constant initial bromate concentration, of approximately 1 mg L^{-1}. Figure 7.9 and Figure 7.10 show bromate and bromide concentrations for each treatment over the 48-hour experimental period.

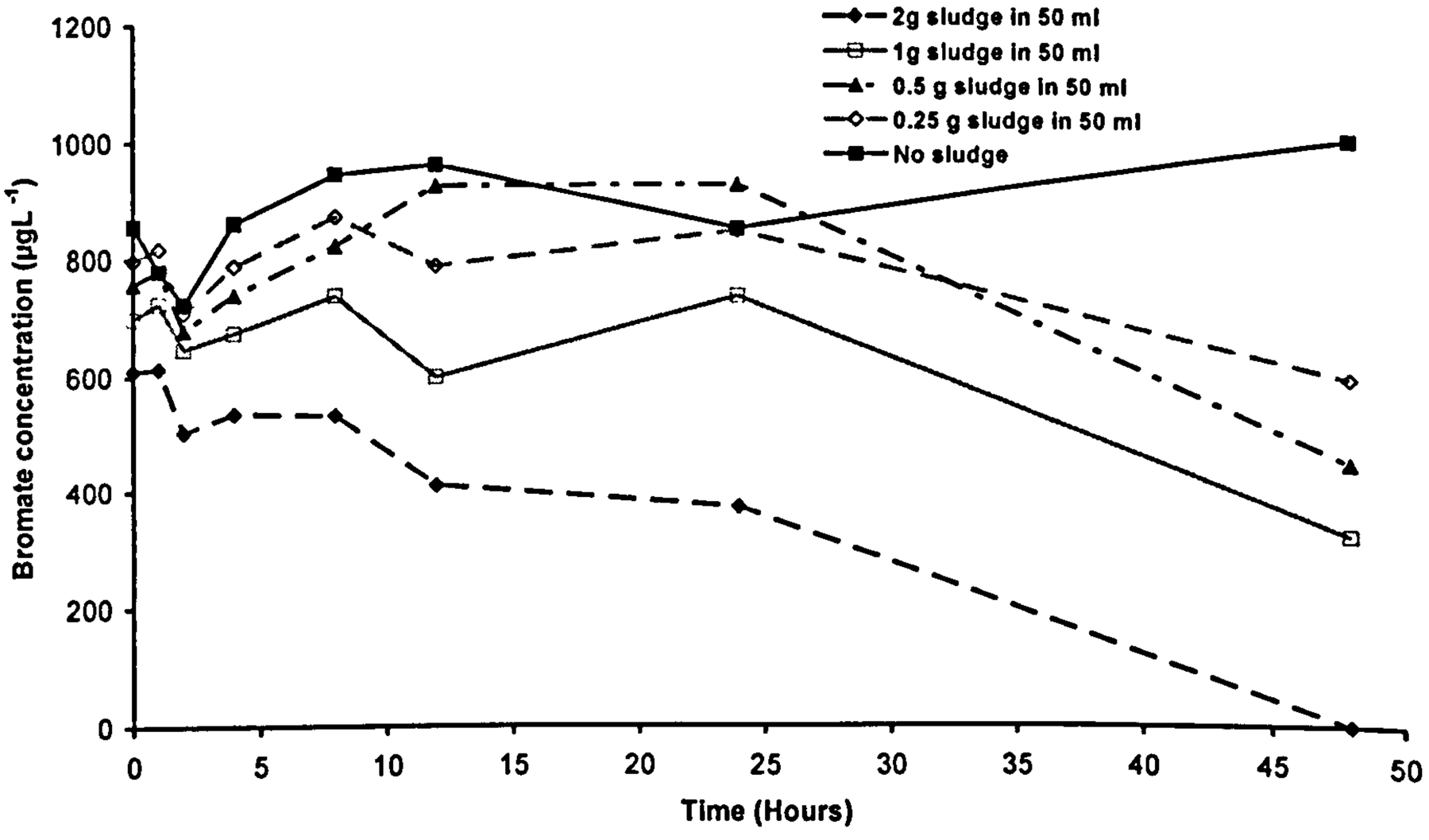


Figure 7.9 - Bromate concentrations during sorption trials at a range of biomass:solution ratios

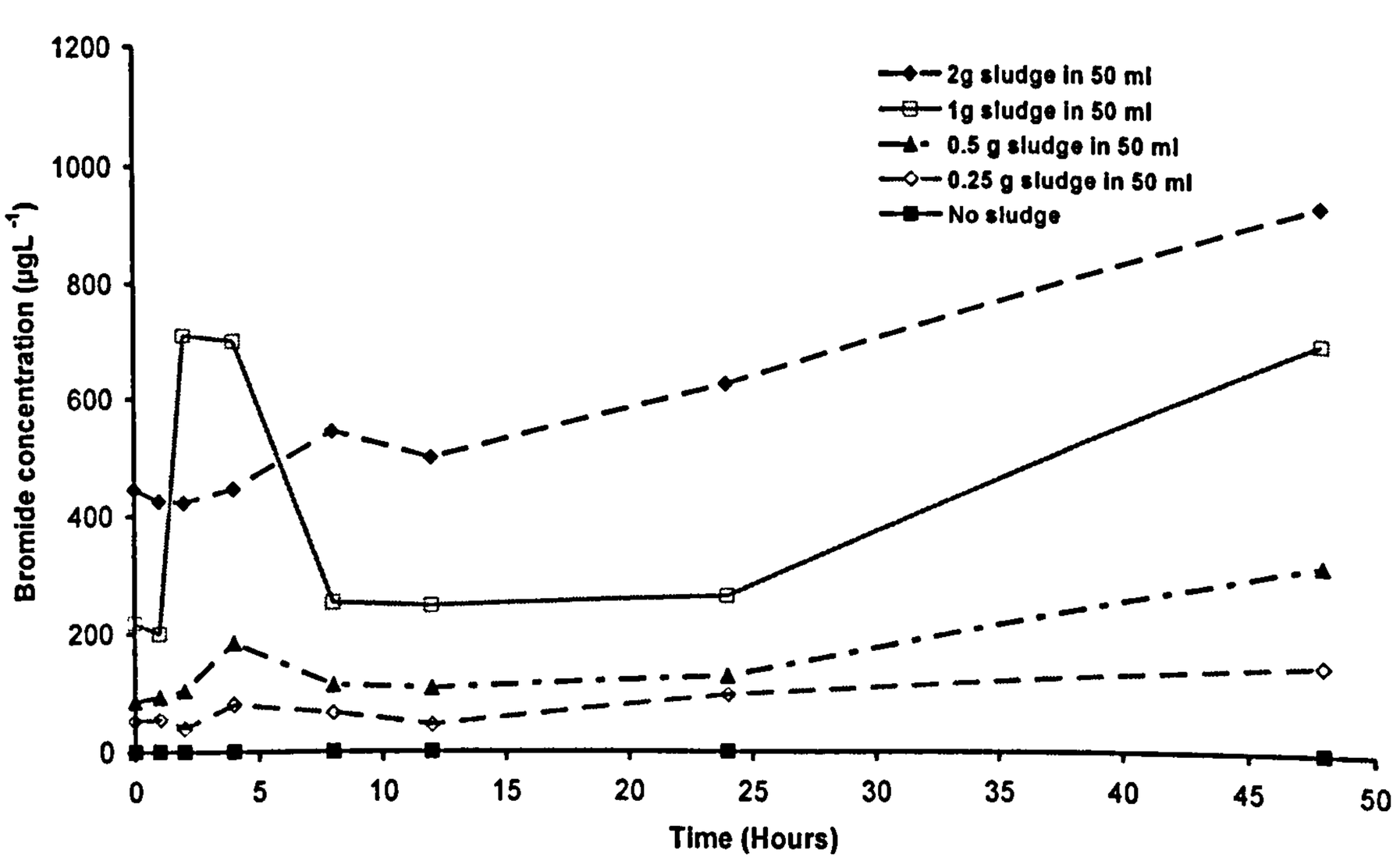
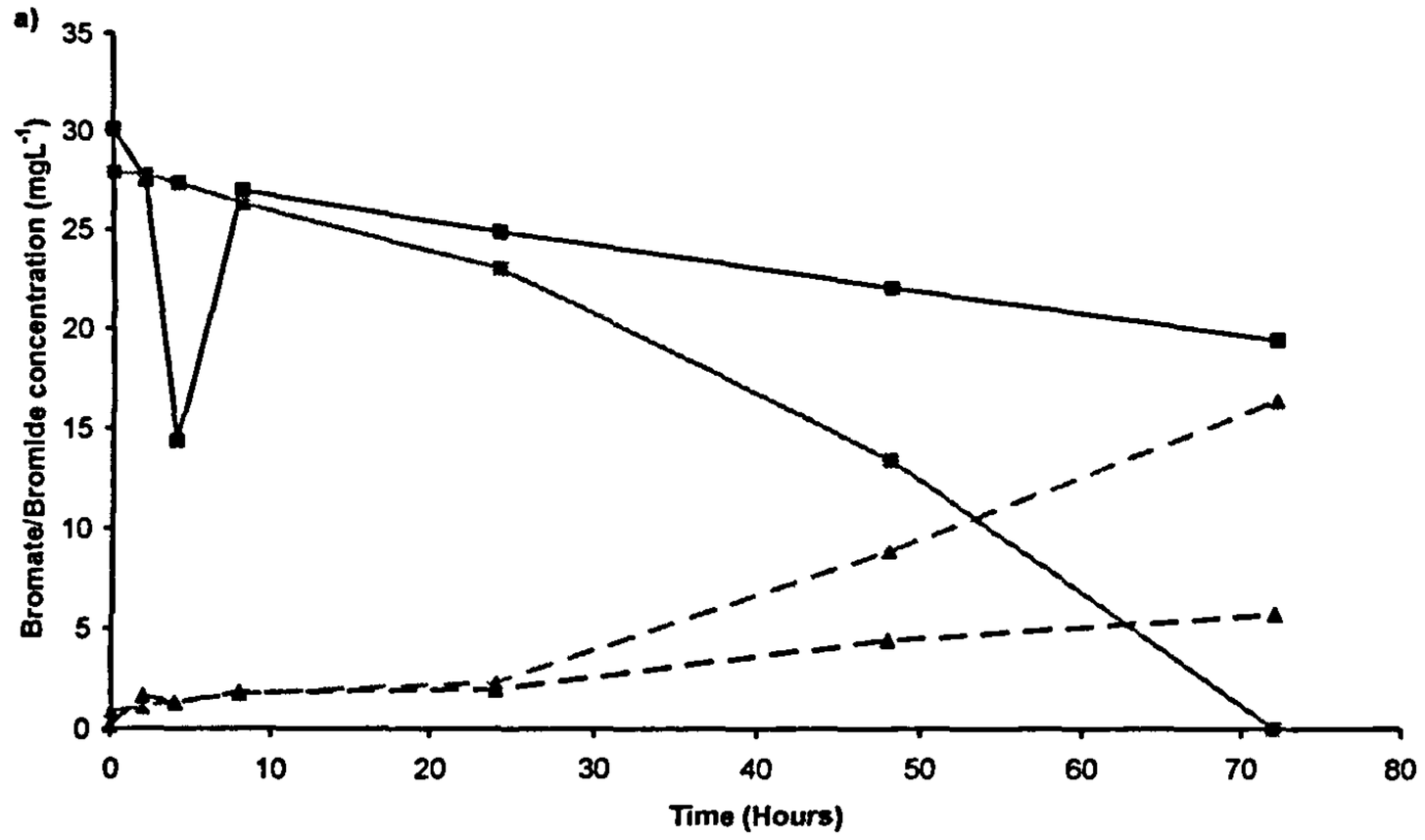


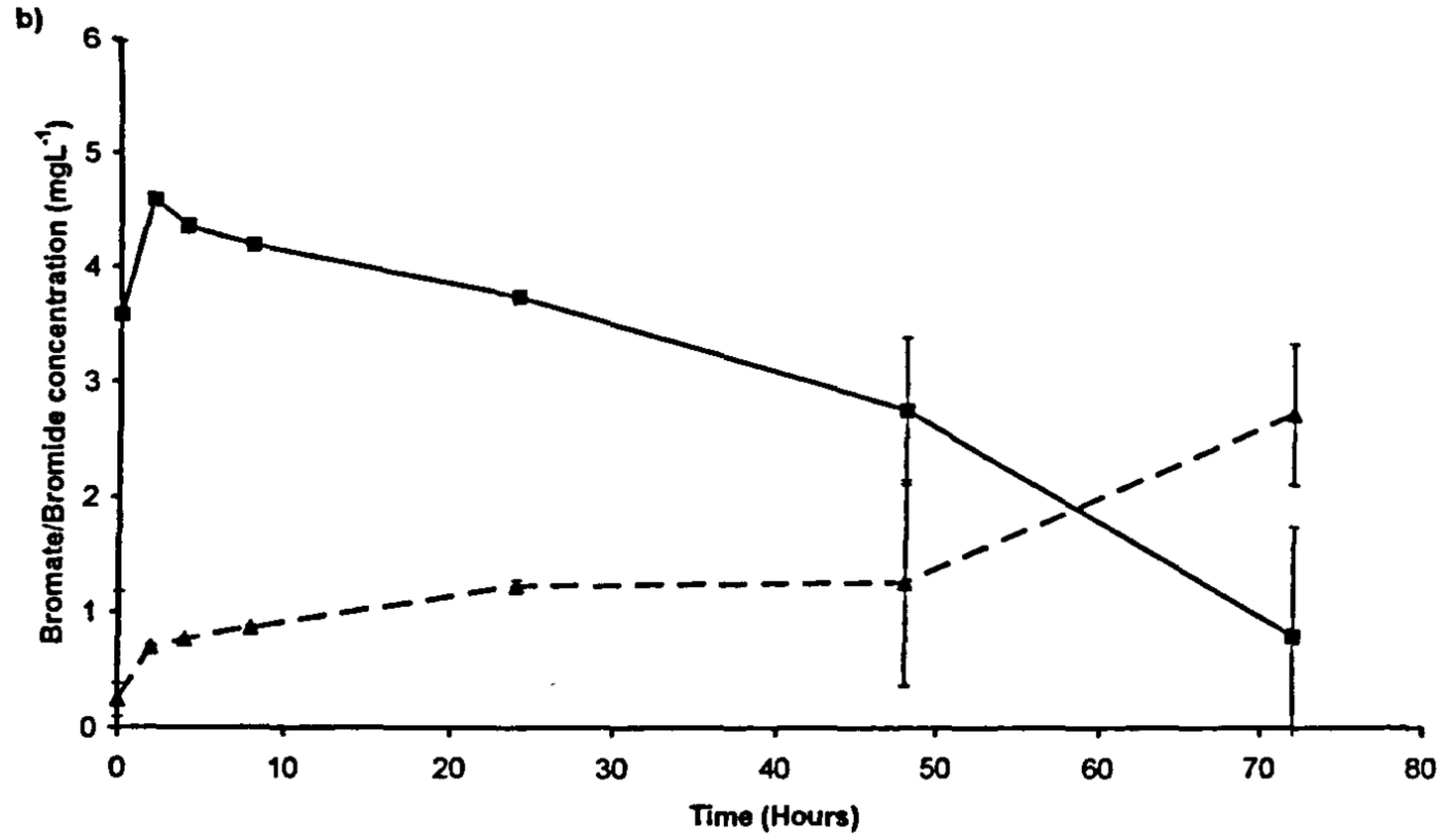
Figure 7.10 - Bromide concentrations during sorption trials at a range of biomass:solution ratios

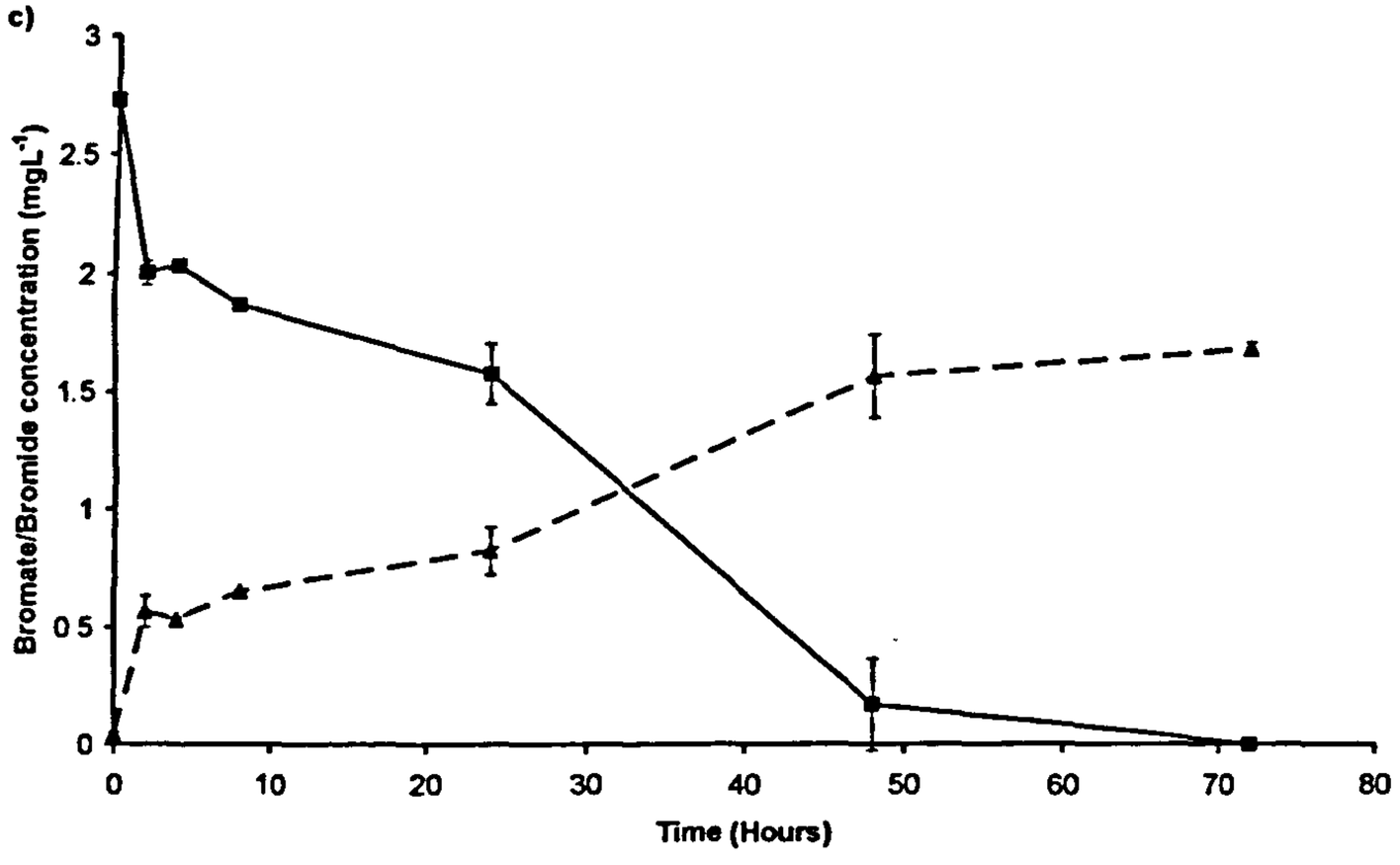
Figure 7.9 shows that bromate concentrations decreased over the 48-hour experimental period, with a greater decrease at higher biomass concentrations. However, bromide analysis revealed that bromide concentrations increased concurrently with the bromate decrease (Figure 7.10), with a greater increase at higher biomass concentrations, thus suggesting bromate reduction instead of reversible sorption. The control sample showed some deviation from the initial value during the experimental run, possibly due to the lack of replication which precluded elimination of sampling error, but no overall decrease in bromate concentration was observed and no bromide was detected at any time. This result confirmed that bromate was not adsorbed onto the test vessel surfaces and did not react with the $CaCl_2$ solution. No bromate was detected in the blank sample, confirming that bromate was not reversibly sorbed to the sludge biomass prior to commencement of the trial. Bromide concentrations in the blank sample suggested the presence of background bromide in the biomass, with an initial concentration of 0.088 mg L^{-1}. A slight increase in bromide concentration (0.036 mg L^{-1}) was noted after 48 hours, but this increase was lower than any of the test samples, and may be explained by limited desorption of naturally-occurring bromide from the biomass. Bromide has been reported to undergo reversible adsorption, but has a very low adsorbability to GAC. Only 381 $\mu g\ g^{-1}$ was adsorbed when 1 mg L^{-1} bromide was contacted with 1 g L^{-1} activated carbon for 48 hours (Asami *et al.*, 1999). A separate trial also suggested that bromide adsorbs to GAC, using a process of physical sorption (Marhaba, 2000). It is possible a similar process leading to weak reversible bromide adsorption occurs onto biomass within the MBR, that this is still present on the dried sludge, and that desorption of this bromide occurred during the course of the trial period.

Trial 2 was completed to further investigate the observation that bromate reduction and not adsorption may have occurred during trial 1. The biomass:solution ratio of 1:100 was utilised as this ratio produced around 50% reduction of initial bromate concentrations in trial 1, allowing both upper and lower limits to be further explored. For each initial bromate concentration in the range 0.3 – 30 mg L^{-1}, two replicate samples were analysed. All duplicate samples recorded similar results, apart from the highest (30 mg L^{-1}) bromate concentration. Results are therefore recorded as averages apart from at this

concentration. All initial bromate concentrations were reduced, with only one sample at the highest bromate concentration still recording residual bromate after 72 hours. An increase in bromide concentration was also noted in all test samples. Figure 7.11a shows bromate and bromide concentrations for the two duplicate samples with an initial bromate spike of 30 mg L^{-1}, with average readings for 5 mg L^{-1}, 3 mg L^{-1}, and 0.3 mg L^{-1} bromate spikes given in Figure 7.11b, Figure 7.11c and Figure 7.11d respectively.







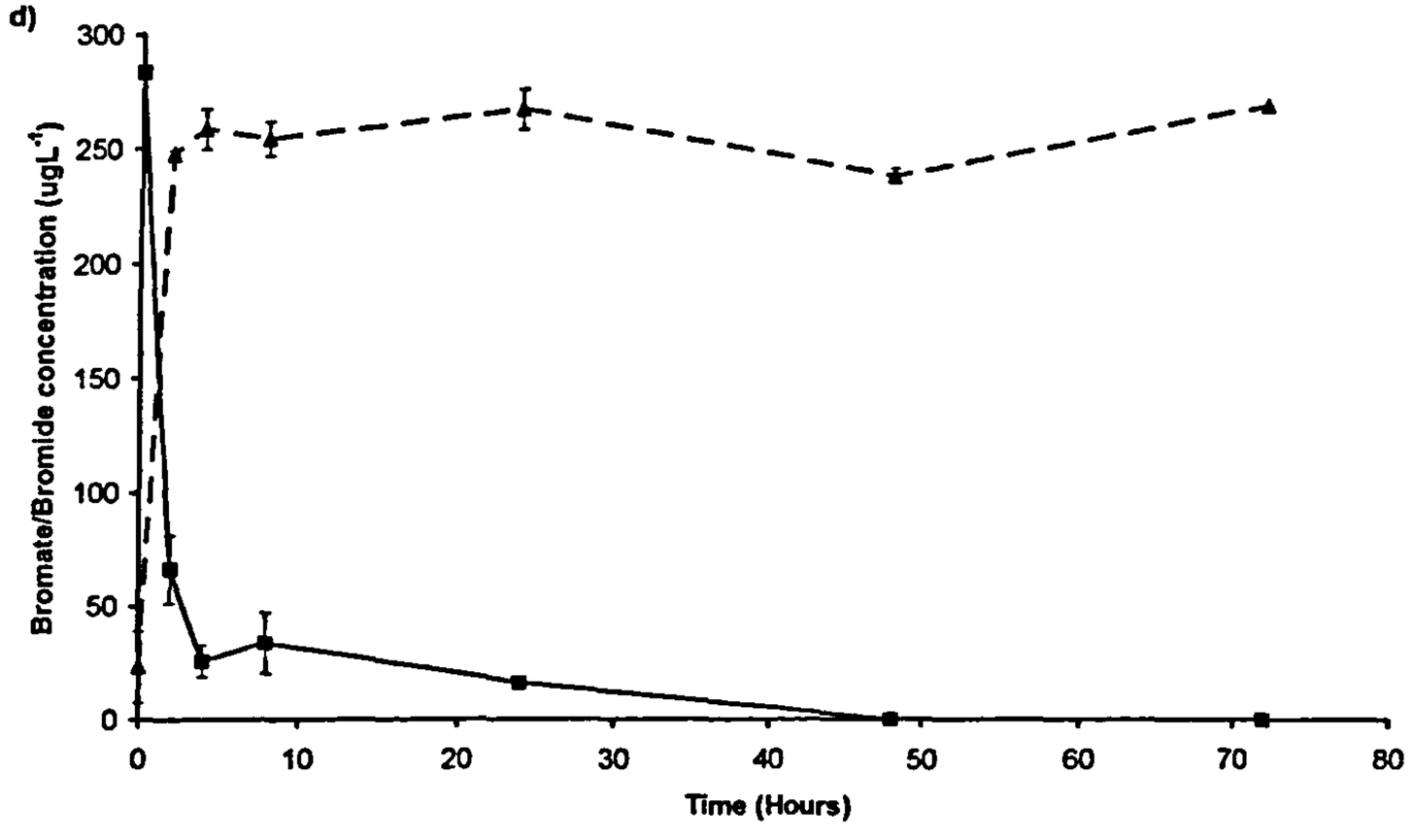


Figure 7.11 - Bromate (■) and bromide (▲) concentrations during trial 2 sorption trials, at initial nominal bromate concentrations of (a) 30 mg L^{-1} (Two replicate samples indicated by black and grey traces); (b) 5 mg L^{-1}; (c) 3 mg L^{-1}; (d) 0.3 mg L^{-1}. Error bars indicate ± one standard deviation.

Table 7.1 shows recovery of bromide species within samples over the total 72-hour adsorption and subsequent 72-hour desorption periods in trial 2. No bromate was desorbed from the sludge during this time, but low concentrations of bromide (0.064 – 1.50 mg L^{-1}) were detected in all desorption samples. This sorbed bromide has been taken into account, with total bromide calculated as the sum after both sorption and desorption periods. Due to an anomalously low initial bromate concentration reading with the 5 mg L^{-1} samples, this value has been taken from the 2 hour bromate reading. The figures in brackets indicate recovery with use of the t=0 bromate reading.

Table 7.1 - Bromine recovery during sorption trials

Nominal initial bromate (mg L^{-1})		Total influent bromine[1] (mg L^{-1} as Br^-)	Effluent bromine[2] (mg L^{-1} as Br^-)	Bromide desorption[3] (mg L^{-1})	Total effluent bromine[4] (mg L^{-1} as Br^-)	Recovery[5] (%)
30	a	18.94	16.43	1.50	17.93	94.6
	b	18.33	17.86	0.34	18.20	99.3
5		3.11 (2.48)	2.21	0.19	3.40	109.3 (137.2)
3		1.75	1.68	0.09	1.77	101.4
0.3		0.20	0.27	0.064	0.28	138.0

[1]Sum of influent bromate (as Br^-) and bromide
[2]Sum of effluent bromate (as Br^-) and bromide
[3]Bromide desorption after 72 hours
[4]Sum of effluent bromine and desorbed bromide

$$^{5}\,Br^- \text{ recovery}\,(\%) = \frac{\text{Total effluent bromine (as } Br^-)}{\text{Total influent bromine (as } Br^-)} \times 10$$

Trials 1 and 2 investigated different sludge:solution ratios and bromate concentrations respectively. With both variables, bromate reduction (given as mg L^{-1} reduction after 48 hours) increased roughly linearly within the ranges trialled, suggesting that both were limiting reaction rate under the conditions used, although the preliminary nature of these trials means that further studies would be necessary to confirm a relationship. Figure 7.12a and Figure 7.12b show bromate reduction after 48 hours over the experimental timespan under varying sludge:solution ratios and bromate concentrations respectively.

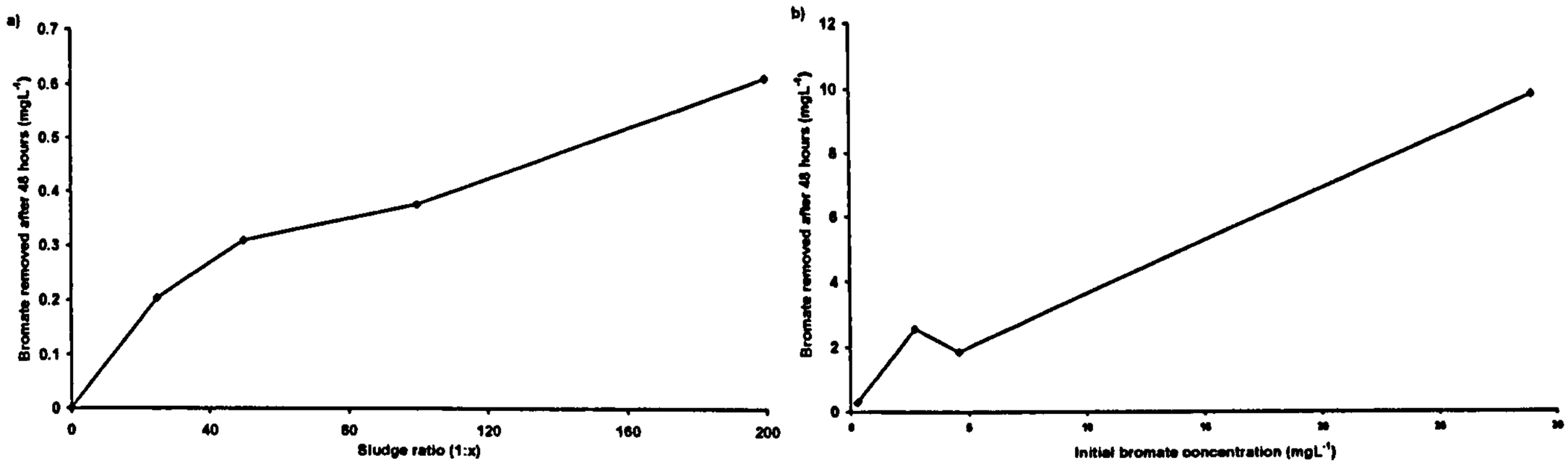
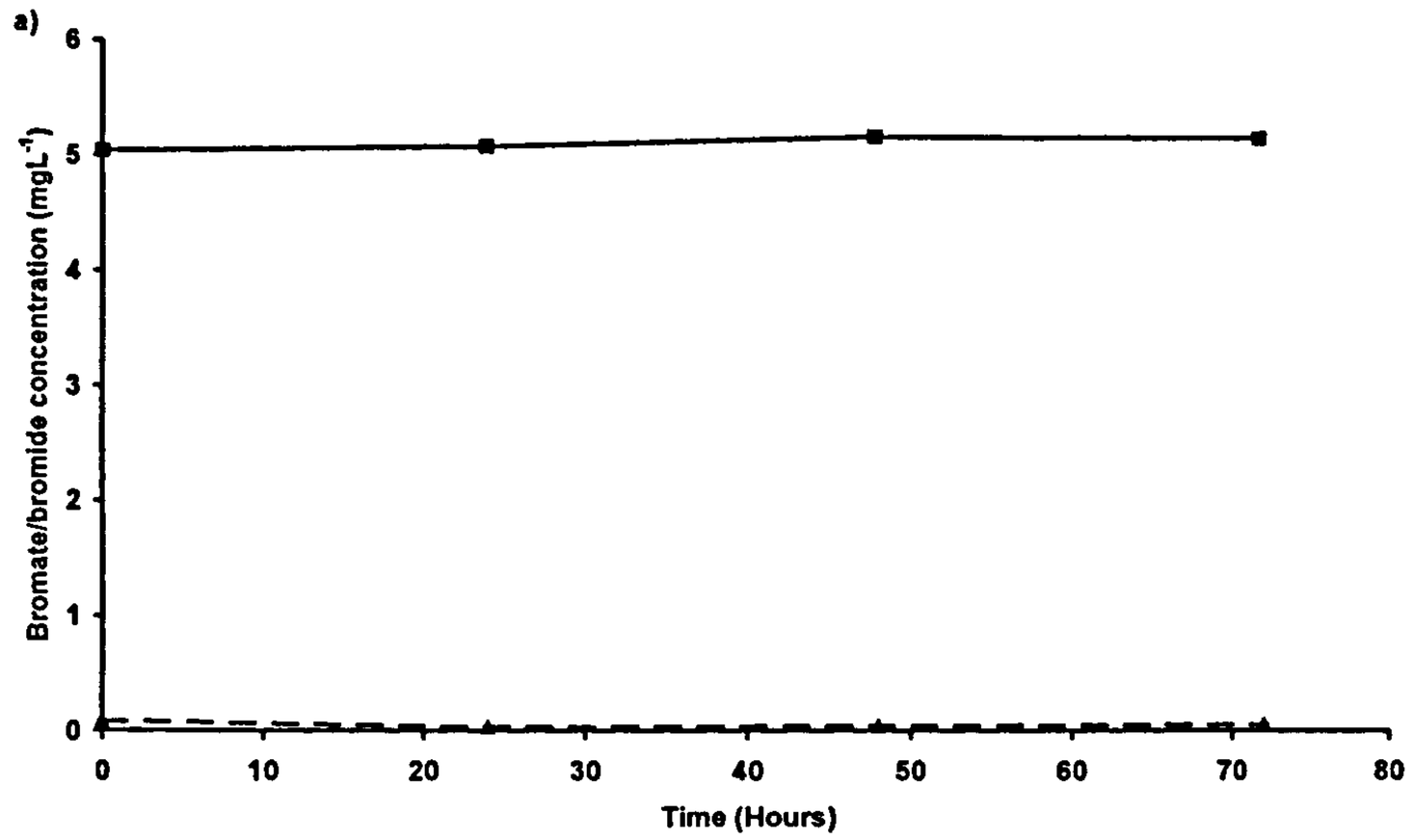
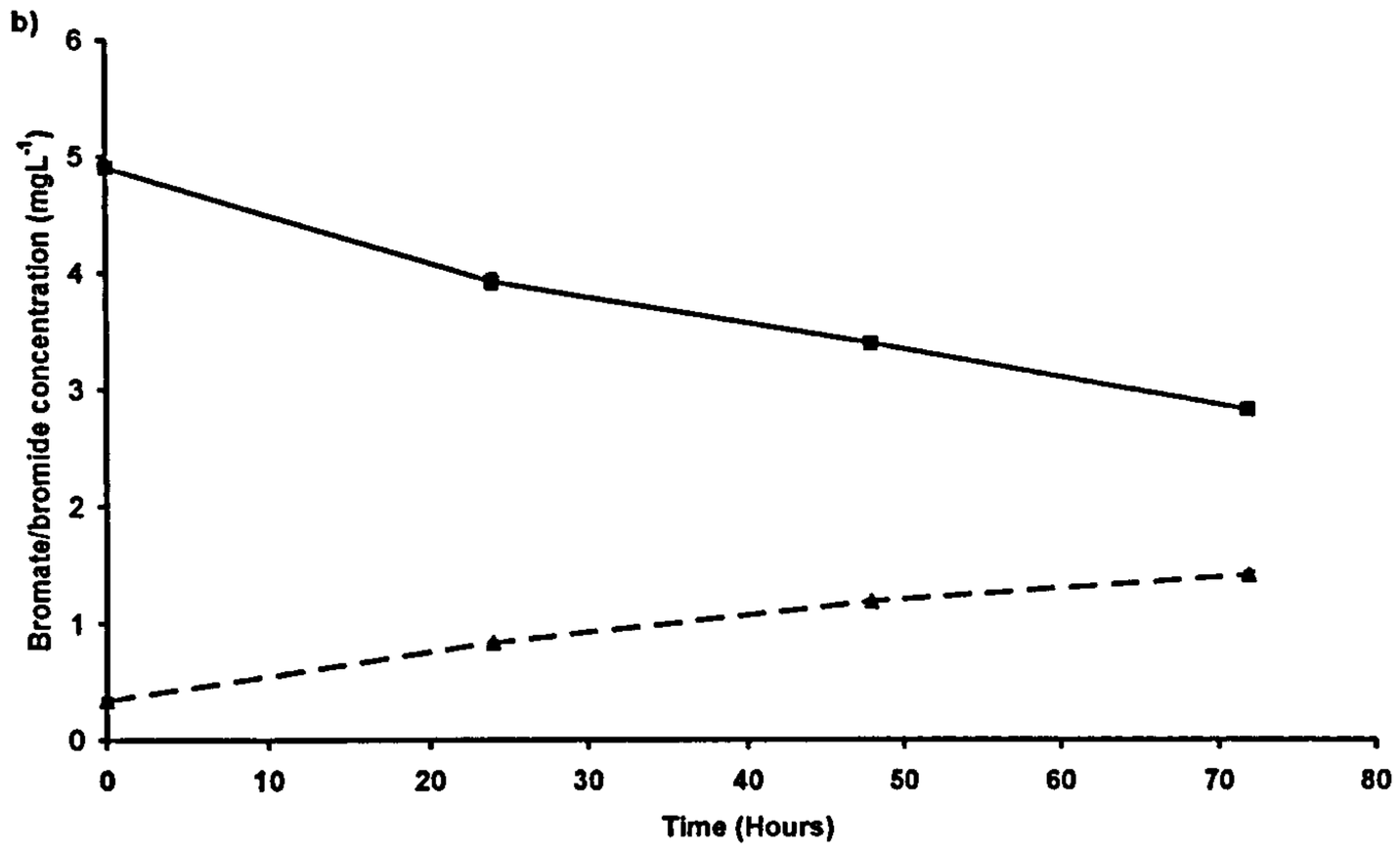


Figure 7.12 - Effect of (a) sludge:solution ratio and (b) initial bromate concentration on bromate reduction over the experimental timespan during sorption trials

Following analysis of trial 1 and 2 results, which strongly suggested a reduction process was occurring instead of reversible adsorption, the nature of this reduction mechanism was investigated in trial 3, using sludge sterilised by both autoclaving and addition of formaldehyde. No alteration of initial bromate or bromide concentrations was noted with an absence of biomass in the control sample after the 72-hour trial period, confirming the trial 1 control result. However, following sample sterilisation by both chemical and physical methods a reduction in bromate concentration was still recorded. Initial values of 4.9 ± 0.03 and 4.4 ± 0.03 mg L^{-1} for autoclaved and chemically sterilised samples respectively were reduced to 2.8 ± 0.05 and 2.1 ± 0.01 mg L^{-1} after a 72-hour period Concurrent bromide increases of 1.1 and 1.2 mg L^{-1} respectively led to bromine recoveries of 98.9 % and 99.9 %. Bromate and bromide concentrations during trial 3 are given in Figure 7.13a – 7.13c.





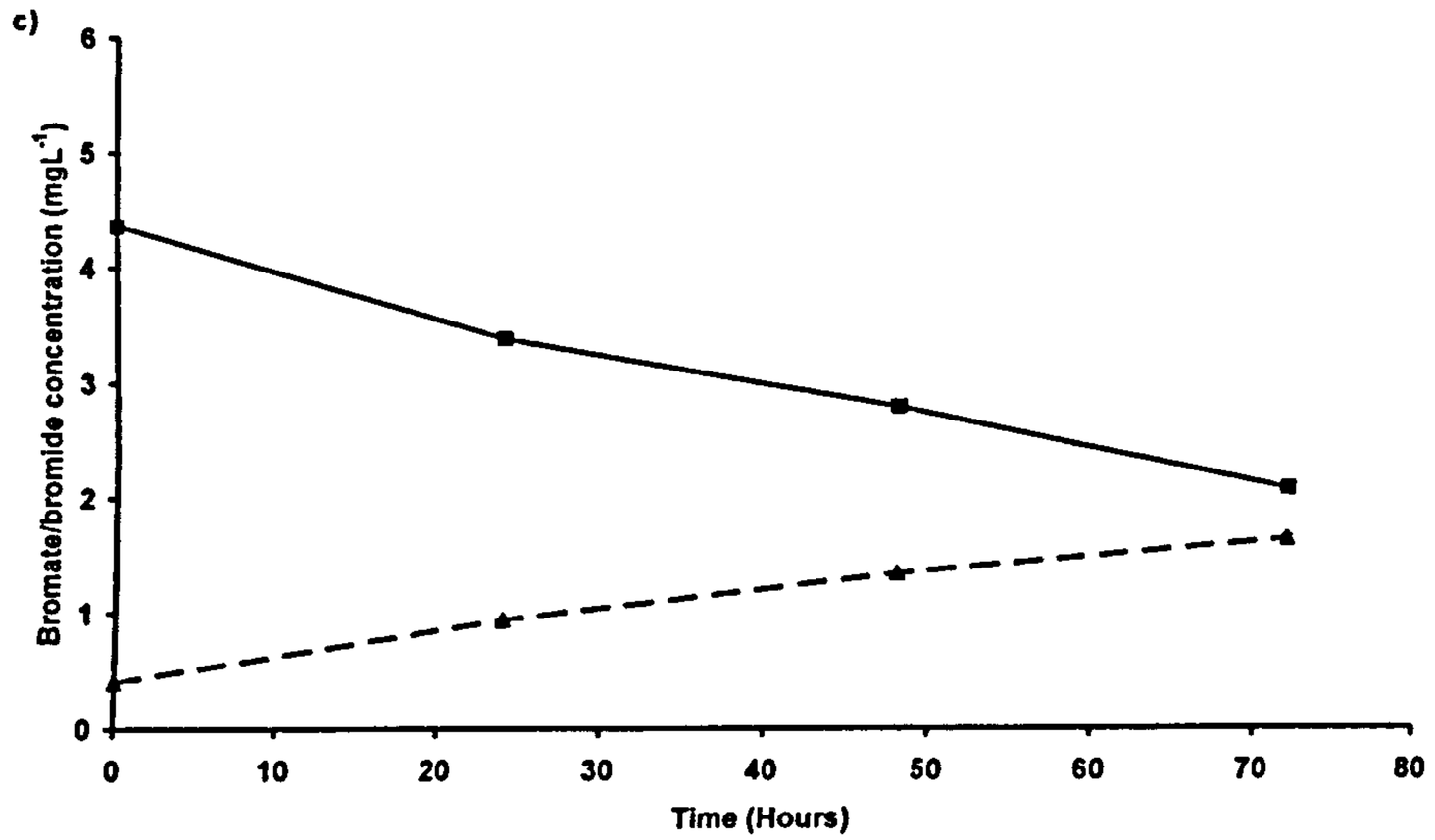


Figure 7.13 - Bromate (■) and bromide (▲) concentrations during trial 3 sorption trials. (a) Control (no sludge); (b) autoclaved sludge; and (c) sludge with formaldehyde addition. Error bars (where visible) indicate ± one standard deviation.

7.1.3 Wastewater process dosing

Wastewater process dosing trials were operated on a test and control basis with one continuous-flow reactor dosed with bromate at 2 mg L^{-1} and subsequently 100 mg L^{-1}, the aim being to complement results obtained during respiration inhibition trials on effects of bromate dosing within wastewater systems. The two reactors were operated under continuous conditions for the duration of the trial, to allow achievement and maintenance of steady-state conditions. Over the study timescale, periodic operational difficulties beyond the control of the trial such as feed and air outages led to occasional slight perturbations in operational conditions, but these were minimised wherever possible. Sludge was not wasted at any point as a regular procedure, but samples were removed periodically for use within respirometry trials. The reported sludge age of 50 days was estimated from the sludge volumes removed in this manner. Sludge age within the range 5 – 3500 days (the latter having effectively no sludge wastage) has been reported to have little influence on effluent quality (Stephenson *et al.*, 2001), and it is unlikely that sludge age would have a significant effect on this test and control trial.

Prior to commencement of bromate dosing, an acclimatisation period of approximately 6 months was allowed to increase sludge strength to within normal operating parameters. Figure 7.1 shows MLSS concentrations over the entire period of operation. After around 3 months of acclimatisation MLSS values appeared to stabilise, and during the following period of operation remained within the range 3.8 – 5.6 g L^{-1}. These values are lower than reported values of 10 – 20 g L^{-1} for MBRs receiving municipal wastewater treatment, being more typical of activated sludge systems (0.2 – 10 g L^{-1}) or MBRs for industrial waste treatment (2 – 40 mg L^{-1}) (Metcalf and Eddy, 1991; Stephenson *et al.*, 2001). However, a previous trial utilising a similar experimental setup also reported relatively low MLSS values, within the range 2.5 – 10 g L^{-1} (Gander *et al.*, 2000), and it is possible MLSS values obtained may simply be a function of influent sewage strength. Other averaged standard control reactor parameters were also similar to comparable studies utilising submerged membranes, suggesting the MBR was operating normally within reasonable operational limits.

Following dosing at both 2 and 100 mg L^{-1} bromate concentrations, all parameters analysed were compared and tested for significant differences between test and control reactors using the Mann-Whitney U-test. Comparison of results gained from test and control reactors are given in Table 7.2, with parameters showing significant differences (95% confidence level) highlighted in bold. Errors are shown as ± one standard deviation. Parameters are grouped into three categories:

- Hydraulic parameters (including operating conditions)
- Macroscopic performance
- Biomass properties

No differences were observed between the test and control reactors in terms of all **hydraulic parameters** measured. MLSS concentrations increased slightly between analytical phases of the two bromate doses, but this was noted in both test and control reactors and is likely to be due to continued acclimation of the biomass. **Macroscopic performance** of the reactors was also similar for both test and control at both bromate doses, with bromate dosing showing no signs of effecting overall reactor operational efficiency. Total COD removal efficiencies of 68.4 ± 4.0% and 68.9 ± 7.4% were measured respectively for the control and the test reactors during the 2 mg L^{-1} bromate dosing trials. Similarly during the 100 mg L^{-1} bromate dosing trials COD removals of 65.7 ± 10.6% and 67.4 ± 17.8% respectively were obtained. COD removal was lower than that found in comparable pilot-scale trials where 92.4% removal has been achieved (Gander *et al.*, 2000). Organic carbon is generally the limiting substrate in a wastewater treatment system, with full-scale MBR systems consequently removing 90 – 98% influent COD (Stephenson *et al.*, 2001), so the reason for the lower COD removal efficiency noted in the current study is unknown. However, it is possible another substrate was limiting COD removal in this case. Ammonia removal remained above 99% throughout all the trials. Ammonia removal values in both reactors were comparable with previous studies, with Cote *et al.* (1997) obtaining 99% removal with a 50-day sludge age.

Table 7.2 - Summary of parameters analysed during MBR bromate dosing trials. Significant differences (95% confidence level) shown in bold type.

	Parameter	Bromate dose			
		2 mg L^{-1}		100 mg L^{-1}	
		Control	Test	Control	Test
Operating conditions / hydraulic parameters	HRT (hours)	12	12	12	12
	SRT (days)	50	50	50	50
	Flux (LMH)	10.25	10.25	10.25	10.25
	TMP (mbar)	4.2 ± 2.3	4.2 ± 2.3	6.5 ± 4.4	6.5 ± 4.4
	Temperature (°C)	18.6 ± 0.6	17.9 ± 0.6	18.8 ± 1.4	19.1 ± 1.1
	DO (mg L^{-1})	9.2 ± 0.6	9.6 ± 1.0	11.7 ± 2.7	10.9 ± 2.5
	MLSS (g L^{-1})	4.5 ± 0.5	4.5 ± 0.1	5.2 ± 0.5	5.1 ± 0.5
	MLVSS (g L^{-1})	4.0 ± 0.2	3.9 ± 0.2	n/d	n/d
Macroscopic performance	COD in (mg L^{-1})	209.1 ± 32.4	209.1 ± 32.4	231.3 ± 50.0	231.3 ± 50.0
	COD out (mg L^{-1})	65.7 ± 10.8	67.0 ± 17.2	81.7 ± 23.5	73.7 ± 30.0
	COD removal (%)	68.4 ± 4.0	68.9 ± 7.4	65.7 ± 10.6	67.4 ± 17.8
	Ammonia in (mg L^{-1})	18.5 ± 0.6	18.5 ± 0.6	18.7 ± 4.7	18.7 ± 4.7
	Ammonia out (mg L^{-1})	0.05 ± 0.04	0.07 ± 0.08	0.12 ± 0.06	0.13 ± 0.1
	Ammonia removal (%)	99.7 ± 0.2	99.6 ± 0.5	99.3 ± 0.4	99.6 ± 0.4
Biomass properties	$SMP_{Protein}$ (mg g_{SS}^{-1})	6.1 ± 4.3	8.4 ± 8.6	8.8 ± 4.4	11.7 ± 8.7
	SMP_{Carb} (mg g_{SS}^{-1})	1.7 ± 0.7	1.9 ± 1.2	**1.8 ± 0.7**	**8.2 ± 3.1**
	SMP_{COD} (mg g_{SS}^{-1})	14.1 ± 2.3	17.2 ± 4.8	**17.6 ± 4.7**	**34.3 ± 3.7**
	$EPS_{Protein}$ (mg g_{SS}^{-1})	117.1 ± 23.5	106.3 ± 10.2	92.4 ± 34.2	76.5 ± 15.4
	EPS_{Carb} (mg g_{SS}^{-1})	27.8 ± 8.4	26.0 ± 10.6	19.8 ± 5.0	22.9 ± 6.2
	EPS_{COD} (mg g_{SS}^{-1})	198.0 ± 23.4	179.7 ± 14.2	162.2 ± 8.0	166.5 ± 13.0
	d(0.1) (µm)	21.3 ± 1.5	20.8 ± 1.9	14.8 ± 3.3	13.0 ± 1.6
	d(0.5) (µm)	62.9 ± 9.6	59.7 ± 5.4	47.0 ± 8.2	41.7 ± 8.3
	d(0.9) (µm)	157.3 ± 44.7	129.8 ± 13.1	118.5 ± 15.1	92.8 ± 17.1
	µ at 100 rpm (mPa s^{-1})	**2.9 ± 0.3**	**2.4 ± 0.2**	2.4 ± 0.2	2.8 ± 0.4
	µ at 60 rpm (mPa s^{-1})	**3.3 ± 0.4**	**2.7 ± 0.3**	2.7 ± 0.2	3.1 ± 0.5
	µ at 50 rpm (mPa s^{-1})	**3.5 ± 0.4**	**2.8 ± 0.3**	2.9 ± 0.2	3.3 ± 0.6
	µ at 30 rpm (mPa s^{-1})	**4.2 ± 0.6**	**3.3 ± 0.4**	3.4 ± 0.3	3.7 ± 0.8
	µ at 20 rpm (mPa s^{-1})	**4.9 ± 0.7**	**3.7 ± 0.6**	3.8 ± 0.3	4.2 ± 1.0
	CST (s)	**27.9 ± 3.3**	**18.2 ± 3.0**	33.6 ± 11.9	91.0 ± 61.0
	α (m kg^{-1})	**$1.3x10^{13} \pm 5.1x10^{12}$**	**$4.9x10^{13} \pm 2.3x10^{12}$**	$1.4x10^{13} \pm 6.7x10^{11}$	$1.9x10^{14} \pm 7.3x10^{13}$
	HPSEC	Graphs shown in Figure 7.14 and Figure 7.15			

n/d – no data

Biomass properties accounted for the majority of parameters investigated. Comparison of SMP and EPS in the two reactors at both bromate dosing concentrations revealed no statistically significant differences with the exception of carbohydrate and COD measurements of extracted SMP following dosing of 100 mg L^{-1} bromate. The carbohydrate fraction of the SMP was measured at 8.2 ± 3.1 mg g_{ss}^{-1} and 1.8 ± 0.7 mg g_{ss}^{-1} for test and control biomasses, with SMP COD measurements of 34.3 ± 3.7 mg g_{ss}^{-1} and 17.6 ± 4.7 mg g_{ss}^{-1} respectively. Similar investigations across a range of pilot- and full-scale MBR treating municipal sewage (without bromate dosing) have shown the carbohydrate fraction of the SMP to vary between 0.25 and 9.8 mg g_{ss}^{-1} based on the same method of analysis (Brookes *et al.*, 2003). Levels of protein in the SMP were not statistically different at either bromate dosing concentration, but were higher than normally recorded during biomass measurements in MBRs (Brookes *et al.*, 2003). However, this could be explained by periodic operational difficulties experienced during the trials. Variations such as feed and air cessation periods can potentially cause the release of organics including proteins from sludges into the soluble phase (Laffray *et al.*, 2003), which could lead to the observed higher SMP protein concentrations. No statistical differences were observed in any of the EPS parameters during either dosing trial. High EPS concentrations are generally accepted as the main foulant in aerobic MBRs (Stephenson *et al.*, 2001), with reductions in EPS correlating with reductions in hydraulic resistance and therefore fouling potential (Chang and Lee, 1998; Nagoaka *et al.*, 1999). The lack of effect on EPS by bromate dosing suggests this would not affect fouling potential of the membranes. Concentrations of proteins and carbohydrates in biomass EPS and SMP samples in the current study were generally slightly higher than those found in previous similar studies (Brookes *et al.*, 2003), but were largely comparable to a trial utilising the same sewage influent at a similar MLSS concentration (LeClech, 2002). Table 7.3 compares EPS and SMP values for the protein and carbohydrate fractions in these studies.

Table 7.3 - Comparison of selected biomass properties with previous studies

MLSS ($g\ L^{-1}$)	CST (s)	d(0.5) (μm)	$EPS_{Protein}$ ($mg\ gSS^{-1}$)	EPS_{Carb} ($mg\ gSS^{-1}$)	$SMP_{Protein}$ ($mg\ gSS^{-1}$)	SMP_{Carb} ($mg\ gSS^{-1}$)	Ref.
4.5 ± 0.5 – 5.2 ± 0.5	27.9 ± 3.3 – 33.6 ± 11.9	47.0 ± 8.2 – 62.9 ± 9.6	92.4 ± 35 – 117.1 ± 23.5	19.8 ± 5.0 – 27.8 ± 8.4	6.1 ± 4.3 – 8.8 ± 4.4	1.7 ± 0.7 – 1.8 ± 0.7	Current study[1]
4.6 ± 0.7 – 12.2 ± 0.7	63 ± 21 – 154 ± 304	38.8 ± 1.6 – 86.7	31.3 ± 9.5 – 116	5.7 ± 10.4 – 15.4 ± 2.7	4.5	0.5 ± 0.2 – 9.8	Brookes *et al.*, 2003[2]
4.4 ± 0.9	29 ± 11	n/d	102 ± 21	28 ± 9	n/d	1.7 ± 1.7	LeClech, 2002

[1]Control reactor conly [2]Review of a range of pilot and full-scale MBR systems

HPSEC analysis examined the molecular size profile of biomass EPS and SMP samples to investigate apparent molecular weight (AMW) in kiloDaltons (kDa) of molecules contained within the two fractions. Calibration of these HPSEC profiles is dependent on the nature of organics within the water (Laffray *et al.*, 2003). For example, calibration using the same column and equipment setup produced a retention time of 9.1 – 10.4 minutes for an upland surface water fraction containing molecules of AMW between 1 – 3.5 kDa (Goslan, 2003), and 10 – 12 minutes for wastewater EPS and SMP fractions with similar AMW values (1 – 3 kDa) (LeClech, 2002). In addition wide and overlapping peaks are often observed, which makes it hard to characterise the nature and concentration of all individual components. Therefore, whilst HPSEC cannot be used to assign absolute AMW values, it is useful for relative comparisons (Goslan, 2003) such as completed in the current study. For this trial the calibration completed by LeClech (2002) on wastewater samples was used to give approximate molecular weight bands. This calibration was carried out according to the method of Logan and Jiang (1990) using ultrafiltration membranes with a range of Molecular Weight Cut-Offs (MWCO), with analysis on identical equipment and conditions to the current study. Size fractions obtained by LeClech (2002) are given in Table 7.4.

Table 7.4 - Assigned molecular weights for each peak (from LeClech, 2002)

Retention time range (minutes)	Apparent Molecular Weight (kDa)
5.0 – 6.0	> 10
6.0 – 8.3	3 – 10
8.3 – 10.0	1 – 3
> 10.0	< 1

Repeat samples taken over the course of both bromate dosing concentrations were analysed and compared using HPSEC. Traces revealed consistent data over both sampling periods, suggesting the production of SMP and EPS had in part stabilised and that any differences observed between test and control were likely to be significant. For each bromate dose a representative SMP and EPS trace are given. Examination of SMP traces showed similar size distributions between test and control reactors at both bromate dosing concentrations (Figure 7.14), with peak elution time towards the longest elution times measured (8 – 11 minutes) and hence smaller molecular weights (< 3kDa). A peak in distribution on all SMP traces was noted at an elution time of approximately 10.5 minutes, which correlates with a molecular weight of < 1kDa. This peak has also been observed in SMP samples from other MBR samples treating municipal waste (Brookes *et al.*, 2003) suggesting it to be a common component of MBR SMP samples, although comparison between MBRs is difficult as SMP profiles can be highly variable between reactors (Laffray *et al.*, 2003). The height of all peaks in the test reactor was greater than those of the control, suggesting a generally higher concentration of organics. This effect has been noted previously as an effect of different MLSS concentrations (LeClech, 2003), but MLSS values at both bromate doses were comparable in the current trial. It was also apparent that test reactor samples contained a wider range of molecular weights, with elution over the period 5.0 – 13.4 min and 7.8 – 10.6 minutes for test and control reactors respectively. However, traces for the test reactor at both bromate doses had similar peak heights and a similar spread of molecular weights, suggesting bromate dosing was not the cause and that the two reactors had developed different molecular weight compositions prior to commencement of trials. EPS traces revealed a broader distribution of molecular weights than with SMP, a low broad band apparent at elution times of 5 – 9 minutes (2 – >10 kDa), and five distinct peaks between 9 – 13 minutes (< 2 kDa). (Figure 7.15). However, little difference between molecular weight distributions of test and control reactors was observed overall, suggesting the addition of bromate was not significantly affecting the production of either SMP or EPS by sludge bacteria.

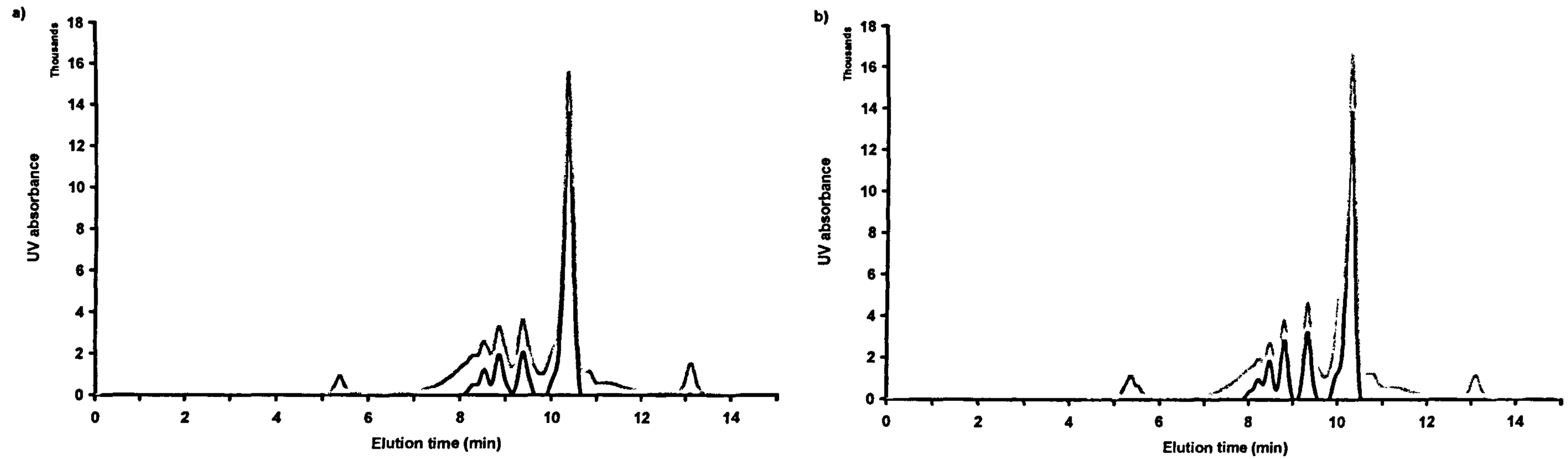


Figure 7.14 - HPSEC profile of SMP for test (grey lines) and control (black lines) reactors during a) 2 mg L^{-1} and b) 100 mg L^{-1} dosing trials

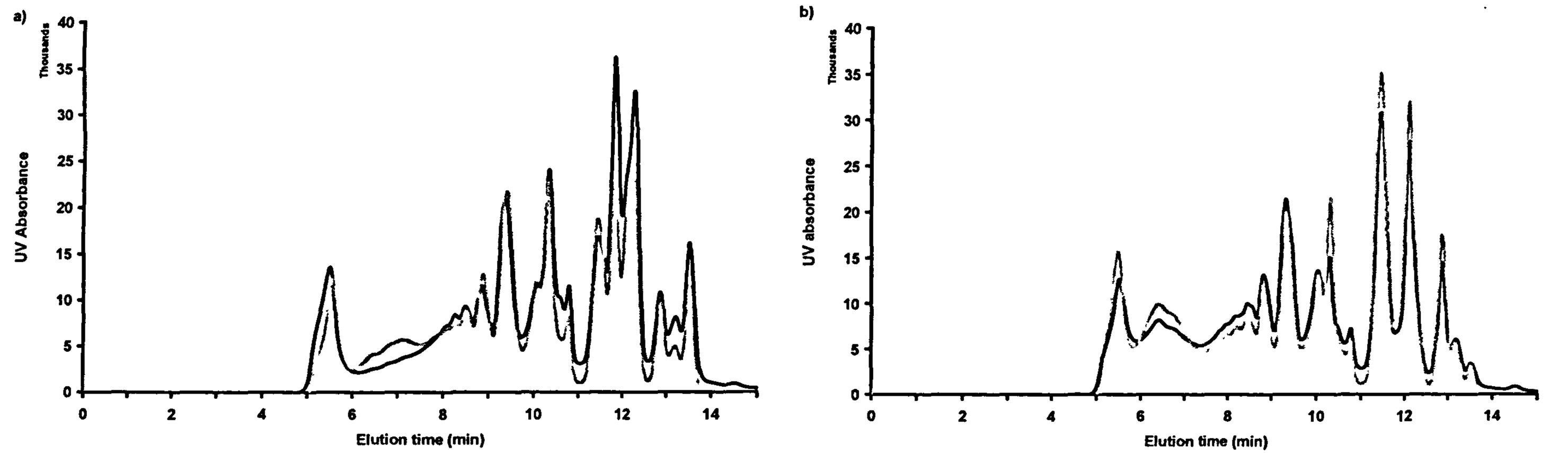


Figure 7.15 - HPSEC profile of EPS for test (grey lines) and control (black lines) reactors during a) 2 mg L^{-1} and b) 100 mg L^{-1} dosing trials

The sludge exhibited pseudoplastic behaviour during all viscosity measurements, where a viscosity decrease is apparent at increased shear stress (Stephenson *et al.*, 2001). For example, the test biomass dosed with 100 mg L^{-1} bromate decreased viscosity (μ) from 4.2 ± 1.0 mPa s^{-1} at a shear rate of 20 rpm to 2.8 ± 0.4 mPa s^{-1} at a 100 rpm shear rate. No difference in viscosity was observed between test and control biomass during the 100 mg L^{-1} dosing trial, but a significant decrease was noted following dosing with 2 mg L^{-1} bromate. Other differences in sludge properties of the biomass were also noted during the 2 mg L^{-1} bromate dosing trial, but results were slightly contradictory. CST values of 27.9 ± 3.3 seconds and 18.2 ± 3.0 seconds were recorded for the control and test biomass samples respectively, suggesting an increase in dewaterability following bromate dosing. However, corresponding specific cake resistances (α) showed an opposite trend, with average values of 1.3 x 10^{13} and 4.9 x 10^{13} m kg^{-1} respectively. No significant difference between these parameters was noted at the higher bromate dose.

A respiration inhibition run using bromate acclimatised biomass from the test reactor immediately following cessation of spiking and carried out during the respiration inhibition trials (Figure 7.16), produced no significant difference in SOUR (Mann Whitney; $p = 0.056$) between biomass dosed with 2 g L^{-1} bromate and control samples. This contrasts with respiration inhibition trial 4b (Figure 7.8), which showed that a significant lowering of SOUR values was apparent with use of unacclimatised biomass (Mann Whitney; $p = 0.0159$).

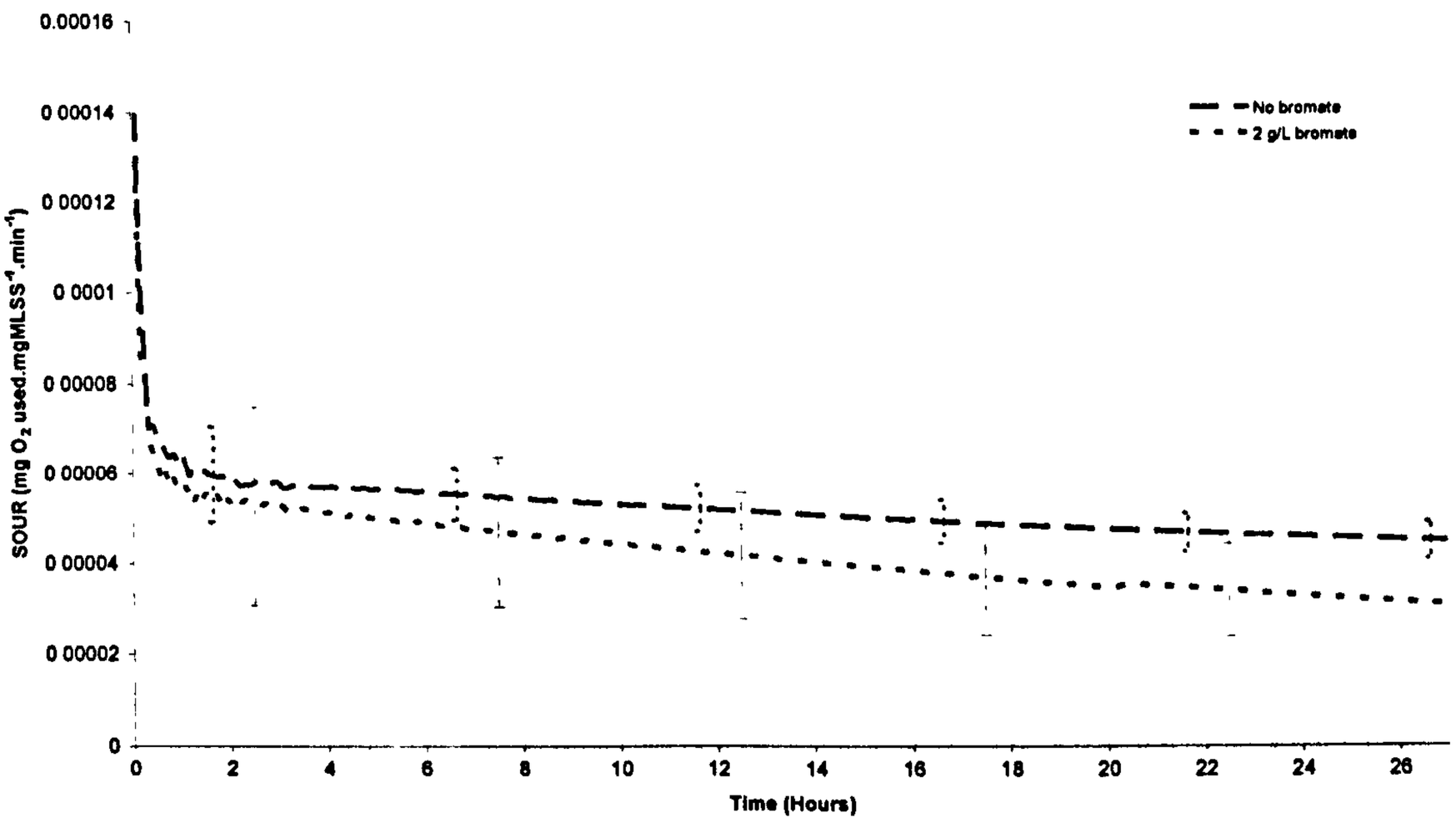


Figure 7.16 - Specific oxygen uptake rate (SOUR) of bromate-acclimated biomass spiked with 2 g L^{-1} bromate

7.2 GROUNDWATER BROMATE BIOREMEDIATION

Results from the four groundwater bioremediation trials are outlined here in Sections 7.2.1 – 7.2.4.

7.2.1 Laboratory-scale chemostat

The continuous-flow chemostat system was utilised for investigations of bromate bioremediation ability by indigenous groundwater bacteria. The two reactors were running for approximately 26 months under continuous-flow conditions with a bromate-contaminated groundwater influent supply and glucose addition as carbon source. During this extended period, occasional short interruptions or perturbations (generally less than 24 hours) to influent water or glucose supplies were caused by influent pipe blockage or peristaltic pump failure. On all occasions this was quickly rectified upon discovery, and at no point was batch operation required to regenerate biomass. A single groundwater feed failure over a period of 6 days during phase II caused the most serious perturbation to operation. Effects of this failure are covered in Section 7.2.1.2.

7.2.1.1 Phase I (proof of concept)

A. Reactor startup

Biomass concentration within influent Nashes Farm water was 0.003 – 0.004 g dry wt L^{-1} at the time of startup. Following commencement of continuous flow conditions at an RT of 20 hours, a lag phase of around 9 days was observed in both reactors, where biomass measurements (with one exception) were < 0.01 g dry wt L^{-1}. On day 10, biomass concentrations within both reactors began to increase more rapidly but fluctuated around 0.02 – 0.06 g dry wt L^{-1} until day 38, when concentrations appeared to stabilise with reactor 2 having a lower biomass content (0.022 g dry wt L^{-1}) than reactor 1 (0.047 g dry wt L^{-1}). Samples for full analysis were taken on day 43 from both reactors. pH values during this startup period fluctuated between 6.0 – 8.5, with addition of ≤ 1.5 mL 1.17 Molar laboratory-grade HCl or ≤ 1.5 mL 1.0 Molar laboratory-grade NaOH (both Fisher Scientific, Loughborough, UK) on an almost daily basis. The initial aim of these additions was to stabilise pH at approximately 7.0, but subsequent operation without acid/alkali additions showed this to not be necessary. pH stabilisation was carried on intermittently, with ≤ 1.0 mL 1.17 Molar HCl added on 9 further occasions. However, with stabilisation found to be decreasingly necessary, it was subsequently discontinued on day 88. Reactor temperature remained constant throughout the phase, with an average for both reactors of 11.3 ± 1.6°C. Anion analysis was not carried out during the startup phase, with biomass and pH stabilisation identified as priorities during this initial period. Biomass and pH readings during phase I are given in Figure 7.17 and Figure 7.18. pH readings following acid or alkali additions are not given.

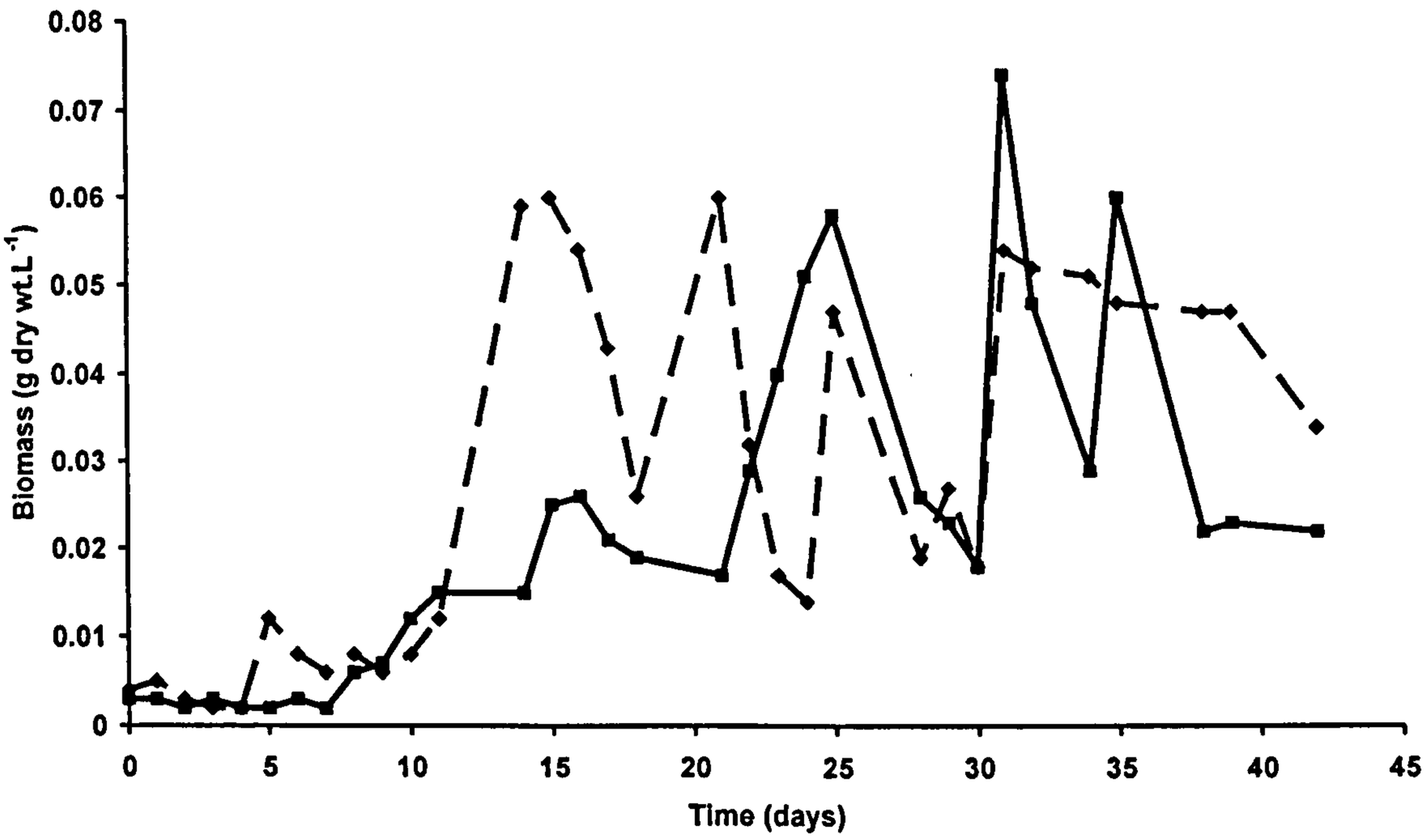


Figure 7.17 - Biomass monitoring during initial chemostat startup for reactors 1 (■) and 2 (♦)

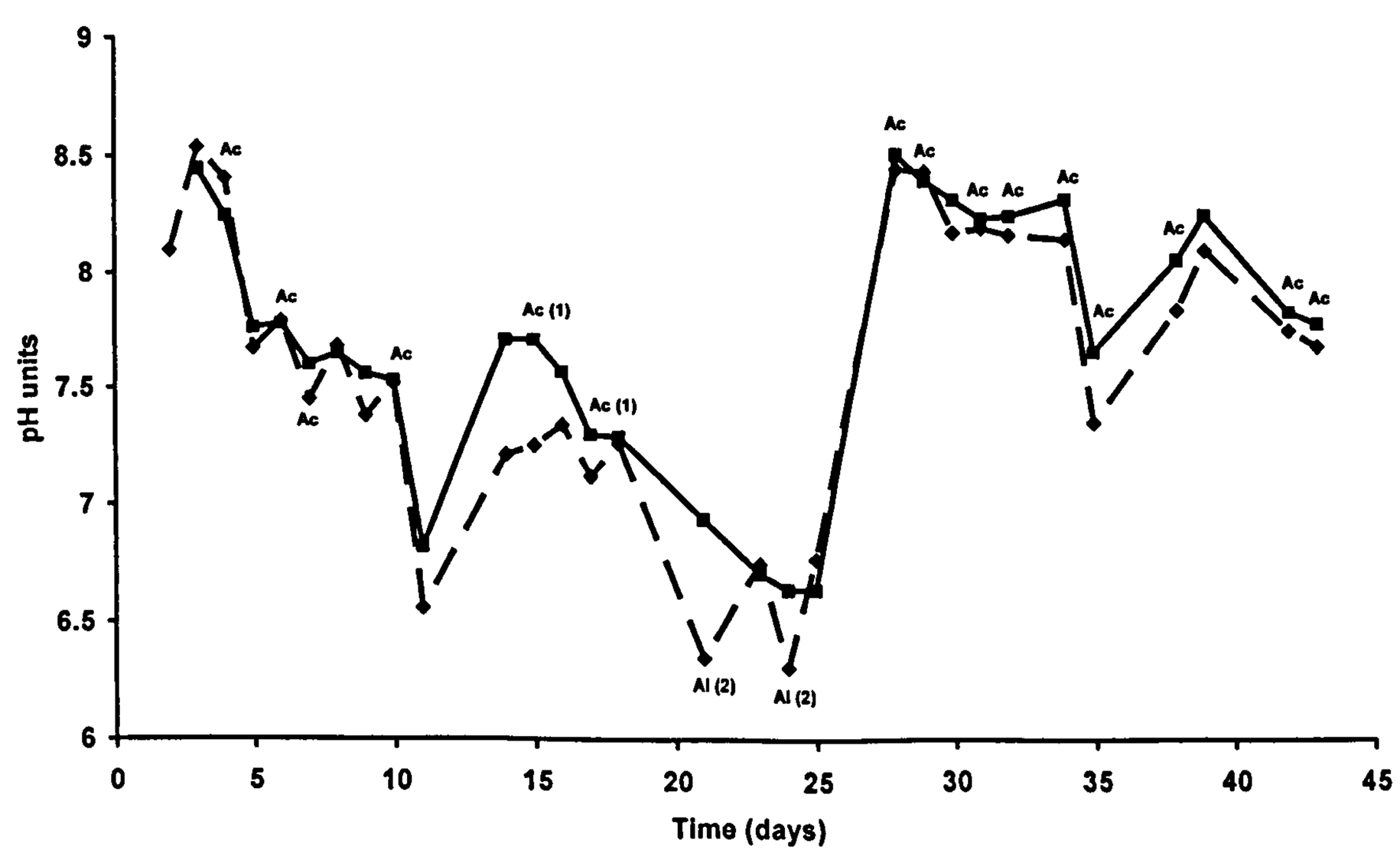


Figure 7.18 - pH monitoring during initial chemostat startup for reactors 1 (■) and 2 (♦), with instances of acid (Ac) and alkali (Al) addition indicated

B. Phase I bromate and glucose concentration trials

A total of 21 combinations of different bromate/glucose levels were trialled during phase I, encompassing three bromate levels (0.2, 1.4 and 80 mg L^{-1}) and seven glucose concentrations within the range 10 – 150 mg L^{-1}. At all three influent bromate concentrations biomass increased with increasing glucose concentration from 0.008 – 0.012 g dry wt L^{-1} with 10 mg L^{-1} glucose influent to 0.085 – 0.104 g dry wt L^{-1} at the 150 mg L^{-1} glucose level, although biomass concentrations were generally slightly higher at the lower bromate contamination levels for a given glucose concentration. The reason for this is not clear, although it is possible continuing biomass acclimation at this early stage may have led to a gradual alteration of the microbial community during phase I. The increase in biomass with increasing influent glucose concentration indicated glucose was limiting overall microbial biomass concentration under all conditions examined. Average influent biomass reading for phase I was 0.006 ± 0.004 g dry wt L^{-1}. Biomass data for all glucose and bromate concentrations are given in Figure 7.19. DO concentrations measured in steady-state supernatant samples were all below 2 mg L^{-1}, with influent levels ranging from 0.9 to 4.4 mg L^{-1}. Since some oxygen entrainment was likely during sampling due to the sampling procedure, this suggests reactor DO concentrations were maintained below 1 mg L^{-1}. Although it is possible bromate reduction in the current trial was slightly affected by fluctuating influent DO concentrations, any variations were essentially random and would not have systematically affected the bromate reduction data.

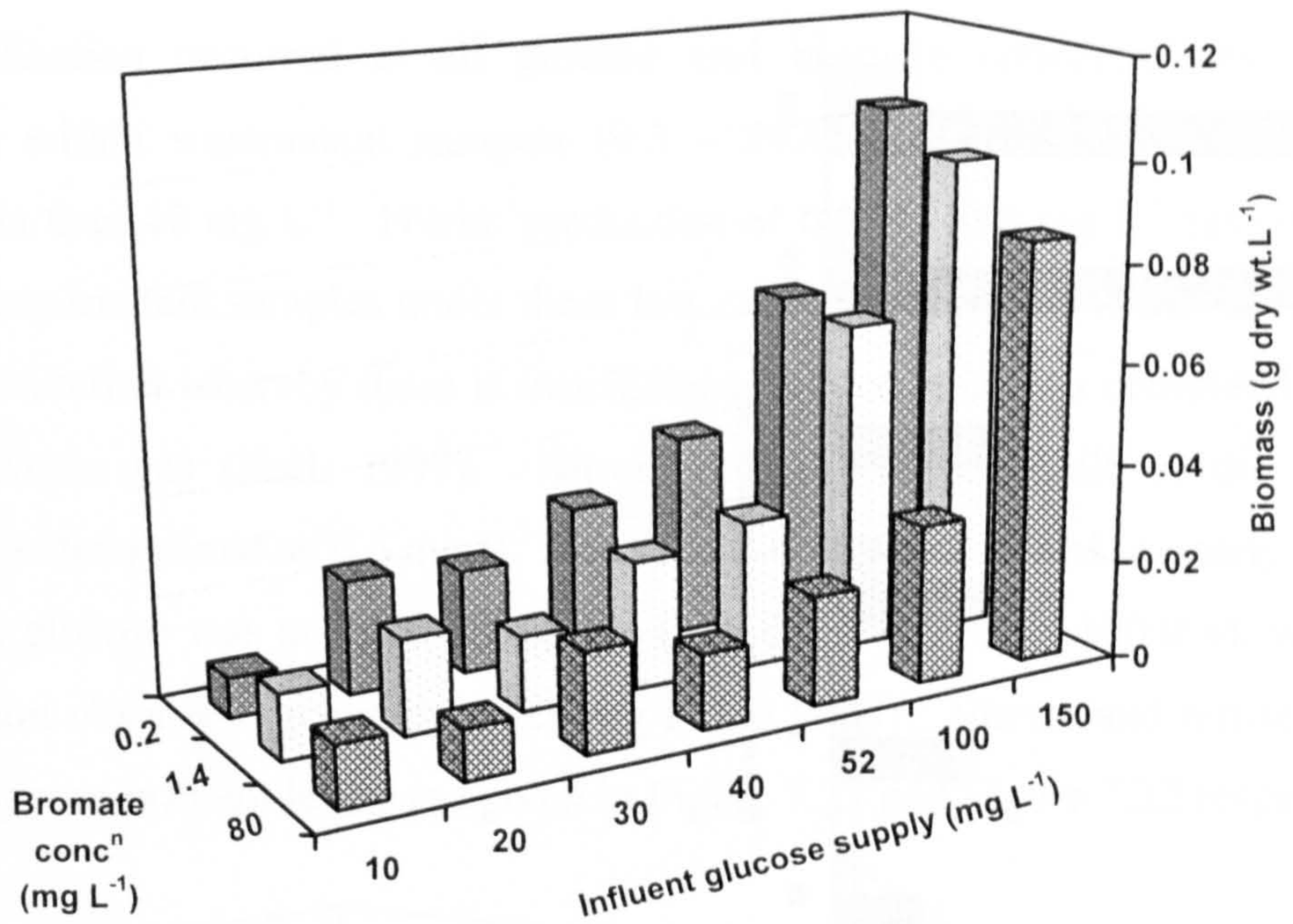
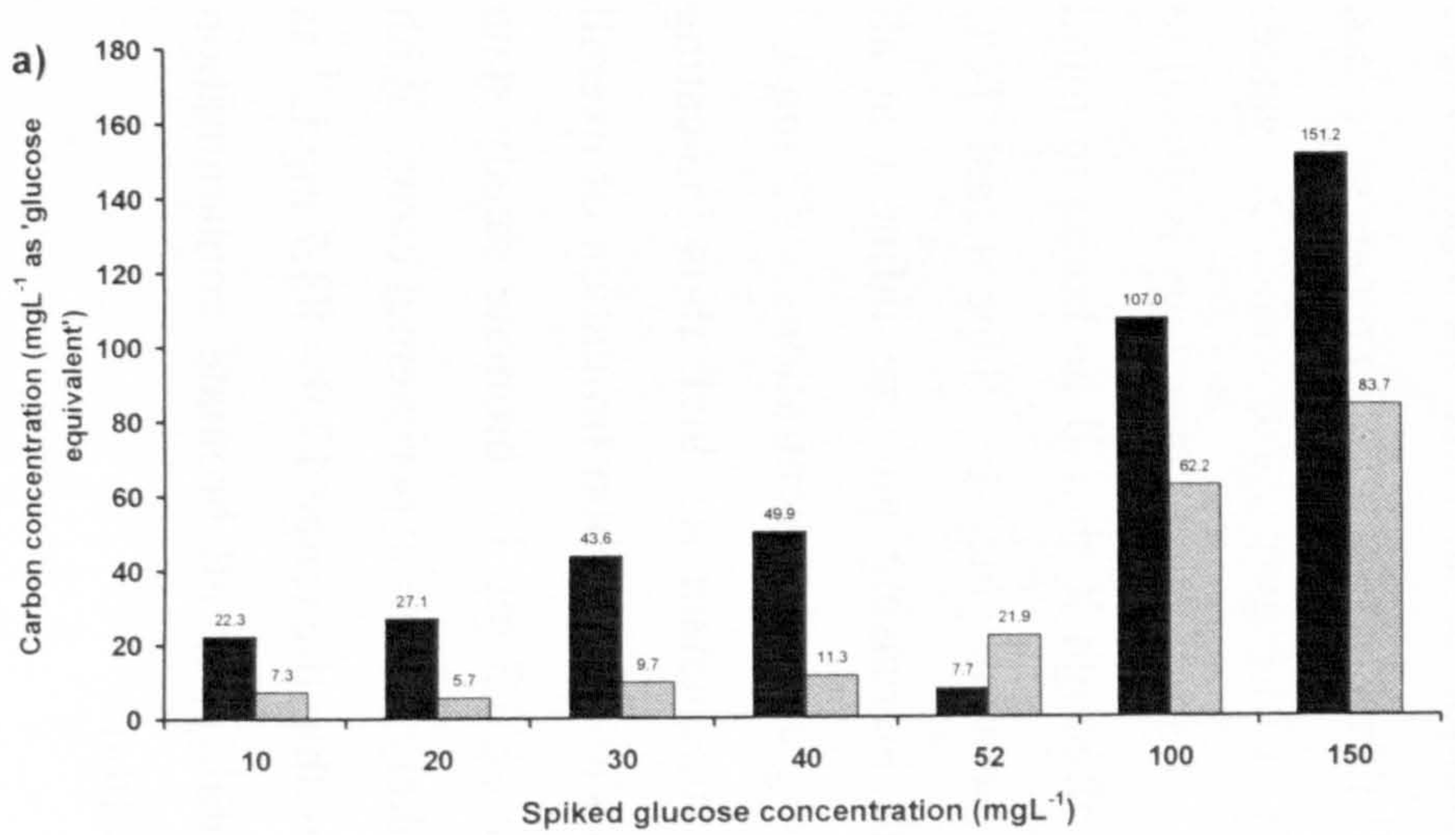
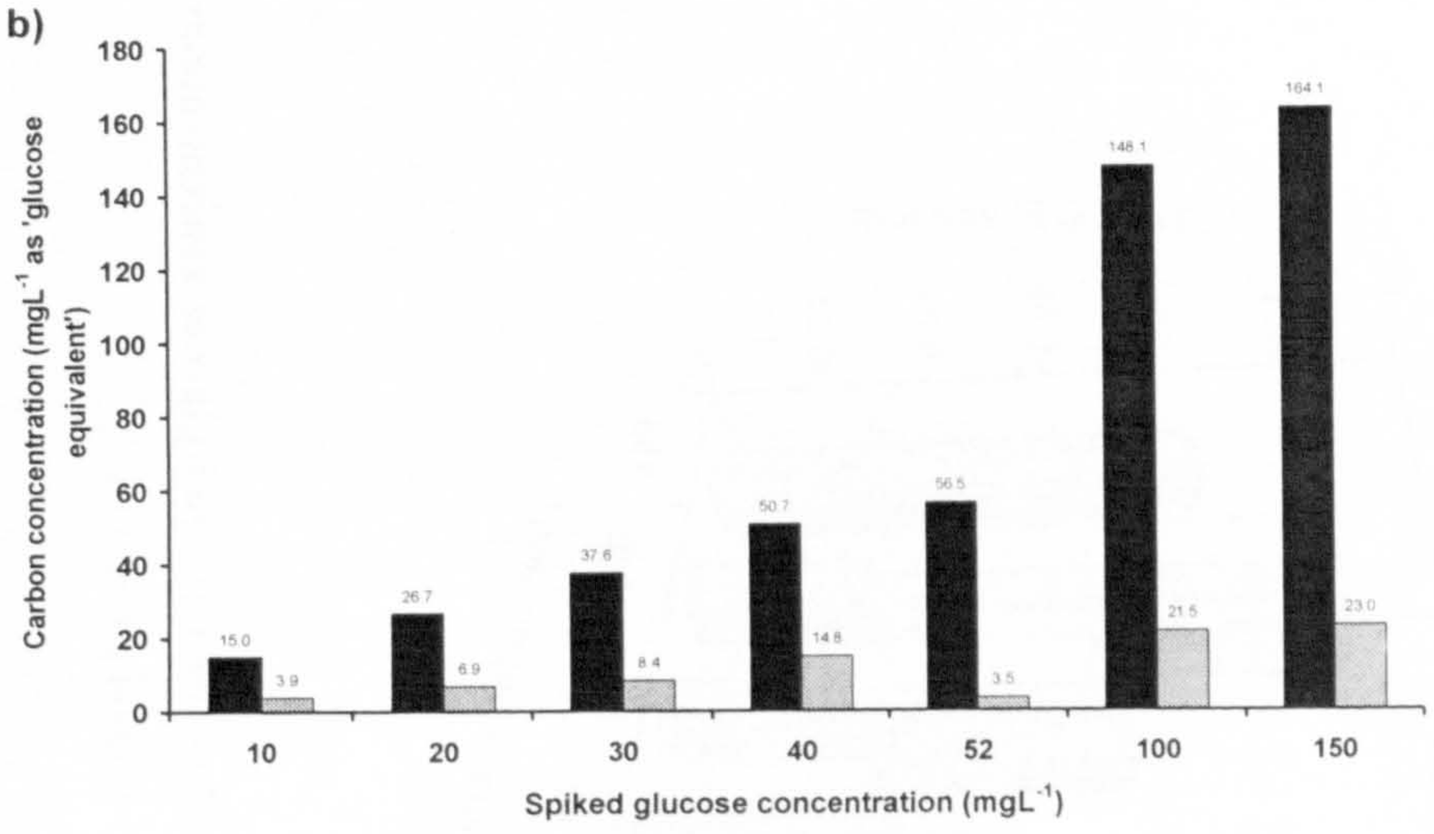


Figure 7.19 - Change in biomass concentration with varying bromate and glucose concentrations during phase I chemostat studies (Influent biomass = 0.006 ± 0.004 g dry wt L^{-1})

Soluble TOC results confirmed carbon utilisation at all three influent bromate concentrations, with similar trends observed for all three bromate contamination levels. Results for the three bromate concentrations are given in Figure 7.20, with TOC values converted to units of 'mg L^{-1} as glucose equivalent'. Use of this terminology is useful to confirm expected influent glucose concentrations, although it should be borne in mind that supernatant TOC may not all actually be present as glucose. Supernatant TOC concentrations generally showed an increase with increasing glucose influent at all bromate contamination levels. With spiked influent glucose concentrations < 52 mg L^{-1} there was up to 16.1 mg L^{-1} glucose equivalent supernatant residual, thus indicating incomplete TOC utilisation despite biomass results showing carbon limitation of overall biomass growth. The result at the 30 mg L^{-1} glucose/0.2 mg L^{-1} bromate steady state condition suggests an increase in TOC, but this is likely due to experimental error. With ≥ 52 mg L^{-1} glucose influent supernatant TOC was within the range 17.9 – 83.7 mg L^{-1} as glucose equivalent, indicating the potential availability, at all bromate contamination levels, of electron donor to bacteria capable of utilisation.





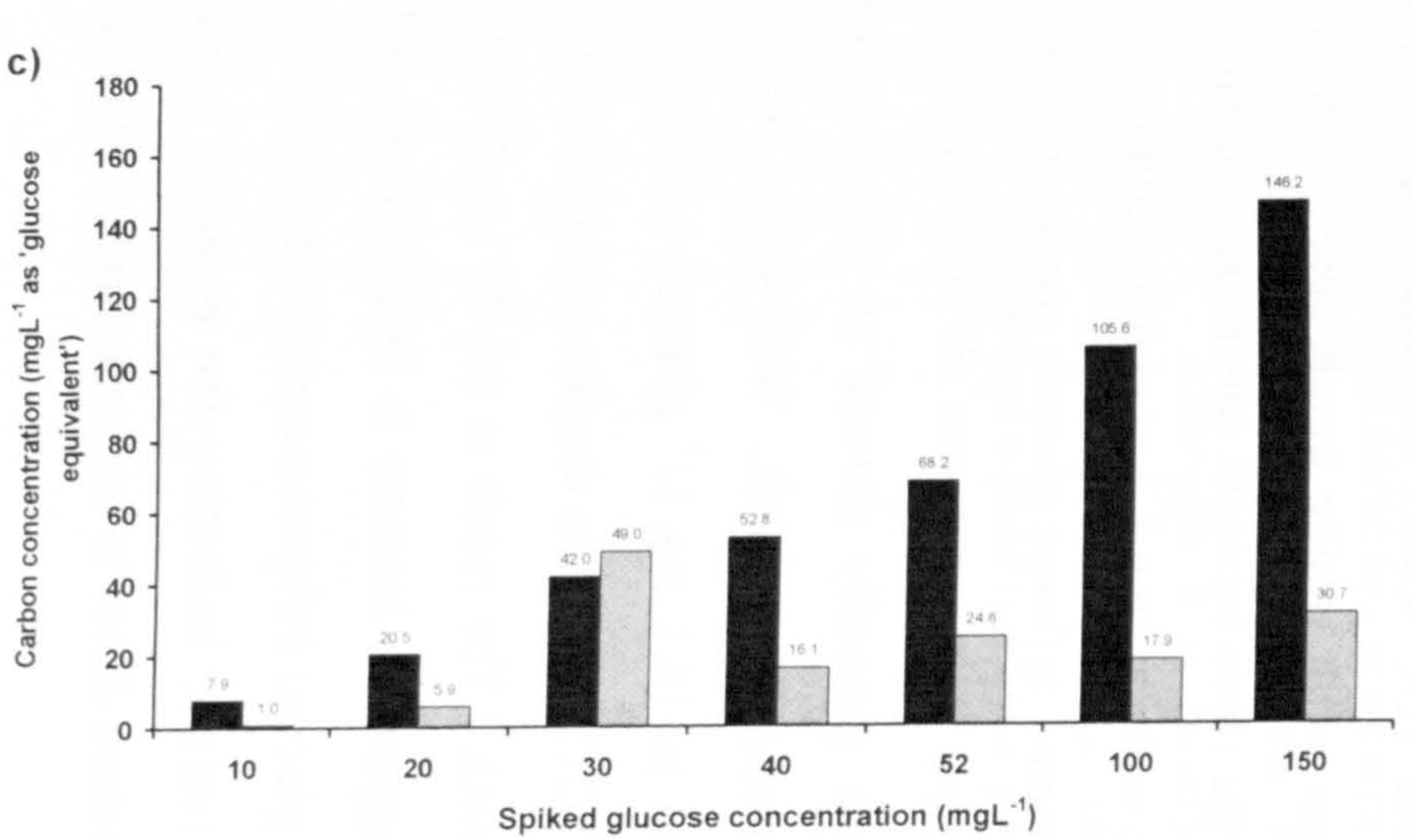


Figure 7.20 - Soluble TOC concentrations expressed as mg L^{-1} glucose with a) 80 mg L^{-1}, b) 1.4 mg L^{-1} and c) 0.2 mg L^{-1} bromate contamination during phase I chemostat studies. Influent concentrations given as black bars and supernatant concentrations as grey bars

Denitrification occurred at all glucose and bromate concentrations. Partial denitrification within supernatant samples (9.3 – 89.6%) was observed with glucose addition of less than 40 mg L^{-1}. Nitrite production of 0.03 – 10.0 mg L^{-1} (as NO_2^-) was also noted in supernatant samples under these low carbon conditions, which is consistent with carbon limitation whereby there is insufficient electron donor to completely reduce nitrate to nitrogen gas (Hall, 1997). Nitrate removal to the limit of the test kits (experimentally determined as 0.6 mg L^{-1} nitrate as NO_3^-) was attained in nearly all cases when influent glucose was at least 40 mg L^{-1} (reduction of 85.5 – 100.0%), with little nitrite formation observed (generally < 0.6 mg L^{-1} as NO_2^-). Nitrate and nitrite data for all bromate/glucose influent levels are given in Figure 7.21 and Figure 7.22 respectively.

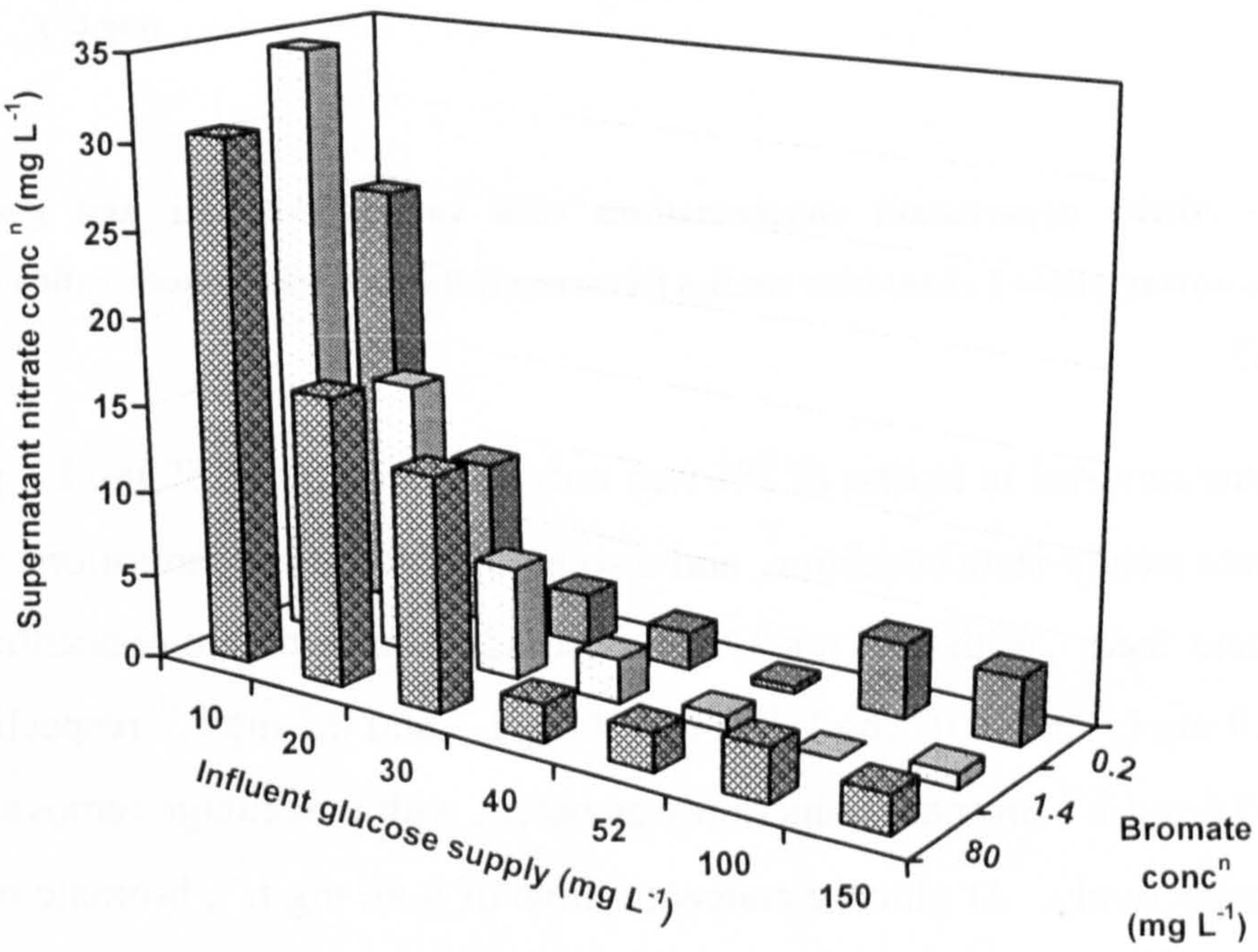


Figure 7.21 - Nitrate supernatant concentrations with varying bromate and glucose spiking concentrations during phase I chemostat studies (Influent nitrate concentrations of 42.7 ± 0.2, 38.0 ± 0.7 and 29.5 ± 0.9 mg L^{-1} as NO_3^- for 80, 1.4 and 0.2 mg L^{-1} bromate contamination levels respectively)

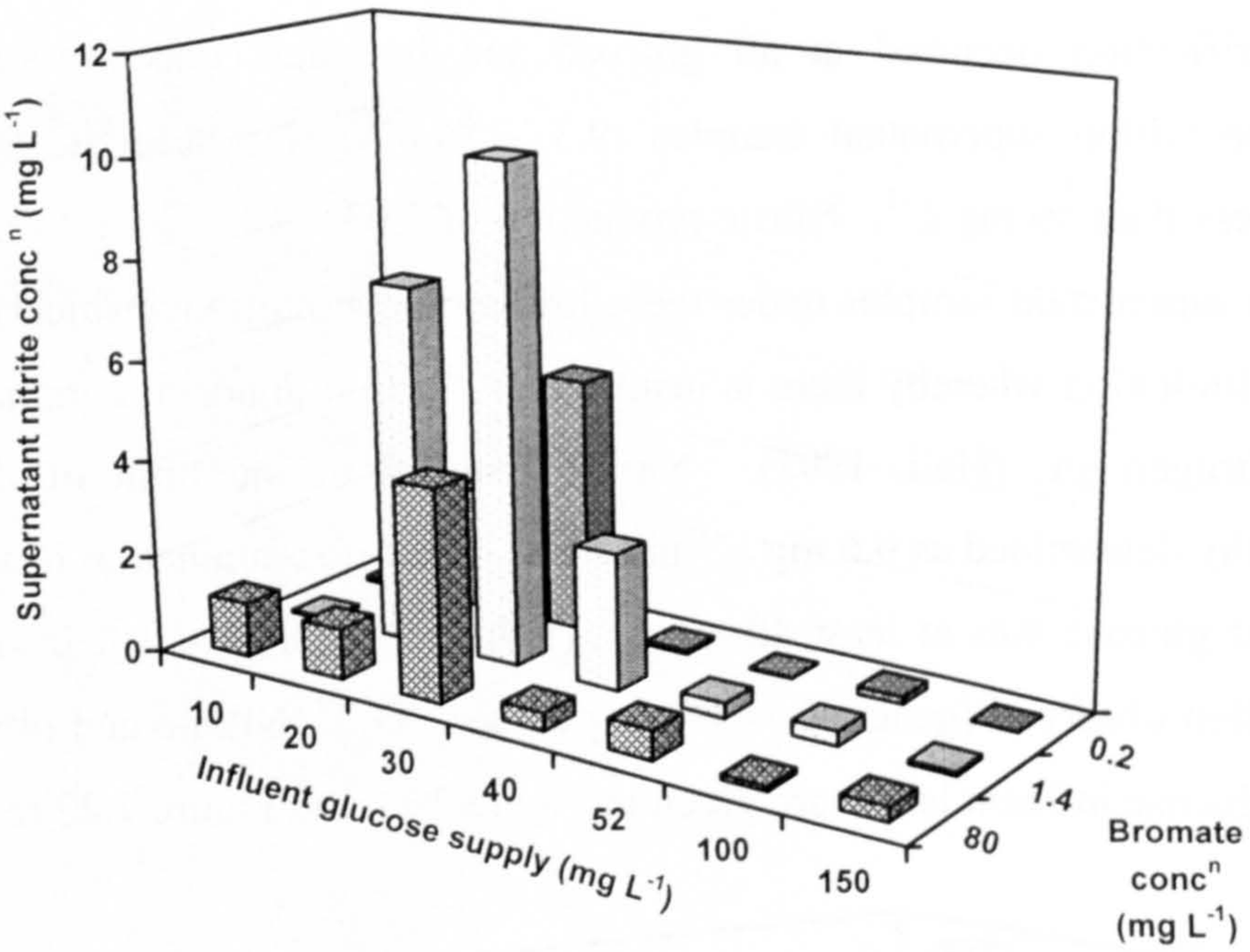


Figure 7.22 - Nitrite supernatant concentrations with varying bromate and glucose spiking concentrations during phase I chemostat studies (Average influent nitrite concentration of 0.04 ± 0.02 mg L^{-1} as NO_2^-)

Bromate removal in excess of 5% was only attained at the 150 mg L^{-1} glucose, 1.4 mg L^{-1} bromate steady-state condition, and also at all glucose concentrations with the 0.2 mg L^{-1} bromate feed. With 150 mg L^{-1} glucose addition, bromate concentrations were reduced to 0.9 mg L^{-1} and 0.02 mg L^{-1} from 1.4 mg L^{-1} and 0.2 mg L^{-1} respectively. Thus a maximum 0.6 mg L^{-1} bromate reduction was noted, with percentage removals of 39.3% and 91.2% respectively. At glucose concentrations of ≤ 40 mg L^{-1}, bromate reduction up to 0.05 mg L^{-1} was observed, but this only represented ≤ 25.5% of influent concentrations. Supernatant results for the 52 and 100 mg L^{-1} glucose concentrations at the 1.4 mg L^{-1} bromate contamination level were over 300x higher than the influent, an unlikely result. It is therefore thought these samples (which were both taken on the same day) were contaminated with bromate following collection and are therefore not valid results. At the highest bromate contamination level, a decrease in bromate concentration of 6.8 mg L^{-1} was noted at the 52 mg L^{-1} glucose concentration. Decreases of 0.3 mg L^{-1} and 0.03 mg L^{-1} were also recorded at the 100 mg L^{-1} and 40 mg L^{-1} glucose concentrations respectively. However, analysis of samples at this concentration was

using Method 2 (Chapter 5), which was subsequently proved to be a relatively crude method of analysis. It is thought accuracy of the technique during this analysis may have been limited, especially given that bromate increases in excess of 1 mg L^{-1} were apparently obtained for the three lowest glucose concentrations – an unlikely result given the data collected in subsequent studies. Therefore, results from this bromate concentration have not been included in any subsequent discussion. Bromate data for the three influent bromate contamination levels are given in Figure 7.23.

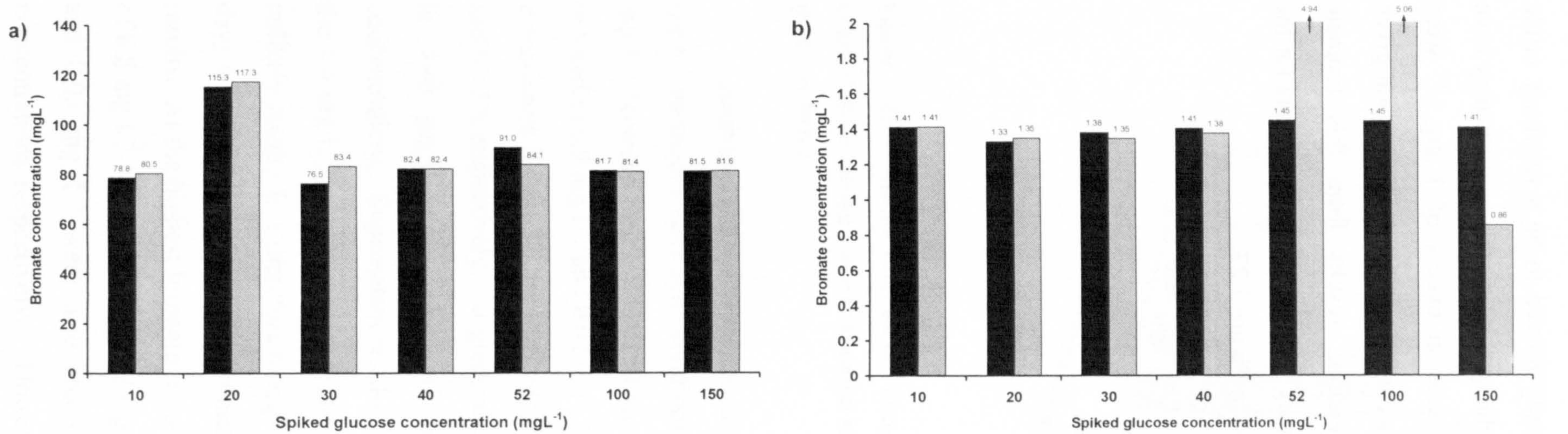


Figure 7.23 - Supernatant bromate concentrations with a) 80 mg L^{-1}, b) 1.4 mg L^{-1} and c) 0.2 mg L^{-1} influent bromate contamination during phase I chemostat studies. Influent concentrations given as black bars and supernatant concentrations as grey bars

C. Phase I reduction and specific reduction rates

With influent glucose concentrations in excess of 30 mg L^{-1} at both 1.4 mg L^{-1} and 0.2 mg L^{-1} bromate concentrations, nitrate reduction rate was maximal and relatively constant (1814.4 – 1858.7 and 1305.5 – 1460.4 µg L^{-1} NO_3 hr^{-1} respectively). Reduction rate only decreased under more carbon limiting conditions (≤ 30 mg L^{-1} glucose influent). Nitrate specific reduction rate was maximal (1689.3 and 1019.7 µmol N g dry wt^{-1} hr^{-1} respectively) with a 30 mg L^{-1} glucose input, decreasing at higher glucose concentrations due to high biomass content (minimum 205.9 µmol N g dry wt^{-1} hr^{-1}) and at lower glucose levels due to poor nitrate removal (minimum 219.6 µmol N g dry wt^{-1} hr^{-1}). Bromate reduction was poor with all glucose concentrations below the highest (150 mg L^{-1}), with a maximum reduction rate of only 5.1 µg L^{-1} BrO_3 hr^{-1} noted. At the 150 mg L^{-1} glucose input, reduction of 9.2 µg L^{-1} BrO_3 hr^{-1} was achieved with 0.2 mg L^{-1} bromate influent and 27.8 µg L^{-1} BrO_3 hr^{-1} at the 1.4 mg L^{-1} level, at best 1.5% of the nitrate reduction rate. Corresponding specific bromate reduction rates were also very low compared with those for nitrate, with a maximum of only 2.2 µmol Br g dry wt^{-1} hr^{-1}. At the highest glucose influent concentration, the relative rates on a molar basis (qN/qBr) for the 1.4 and 0.2 mg L^{-1} bromate contamination levels were 138.0 and 298.6 respectively, confirming that nitrate removal was favoured over bromate reduction during phase I trials. Bromate and nitrate reduction/specific reduction rates for chemostat phase I trials are given in Table 7.5.

Table 7.5 - Bromate and nitrate reduction/specific reduction rates during phase I chemostat trials (Temperature - 10°C; RT - 20 hours)

Bromate in feed (mg L^{-1})	Glucose addition (mg L^{-1})	Reduction rates		Specific reduction rates		qN/qBr
		Nitrate (µg L^{-1} NO_3 hr^{-1})	Bromate (µg L^{-1} BrO_3 hr^{-1})	qN (µmol N g dry wt^{-1} hr^{-1})	qBr (µmol Br g dry wt^{-1} hr^{-1})	
1.4	150	1858.7	27.8	309.1	2.2	138.0
	100	1836.5	-	448.8	-	-
	52	1836.5	-	987.4	-	-
	40	1814.4	1.5	1125.6	0.4	2563.7
	30	1571.0	1.7	1689.3	0.9	1920.5
	20	1128.5	-	958.0	-	-
	10	177.0	-	219.6	-	-
0.2	150	1327.6	9.2	205.9	0.7	298.6
	100	1305.5	0.9	314.3	0.1	3060.4
	52	1460.4	5.1	574.5	1.0	591.0
	40	1305.5	1.9	701.9	0.5	1432.5
	30	1327.6	2.6	1019.7	1.0	1037.4
	20	951.5	0.9	667.2	0.3	2282.3
	10	177.0	2.0	356.9	1.9	184.9

7.2.1.2 Phase II (Two-stage reduction and enrichment)

At the commencement of phase II, reactors were converted to series operation at an RT of 40 hours for each reactor, and groundwater source was altered to that from the House Lane borehole. Phase II was designed as a two-stage enrichment culture. Multi-reactor chemostat systems can be used with complex media to provide a series of different environments in the same stream (Pirt, 1975). In this case removal of the majority of influent nitrate and DO within reactor 1 with a denitrifying culture would potentially providing a low nitrate, high glucose influent to reactor 2 to encourage establishment of an enriched bromate reducing population. A continuous-flow enrichment culture was used by Malmqvist *et al.* (1994), for isolation of a chlorate reducing bacterium (*Ideonella dechloratans*). As with the current study, several months were required for attainment of a strong enrichment culture. Other enrichment trials including both batch (Wolterink *et al.*, 2002) and continuous flow systems (Bruce *et al.*, 1999) have also led to successful isolation of perchlorate reducing strains within the resultant enrichments.

Influent biomass concentration for phase II was generally < 0.005 g dry wt L^{-1}, with average influent pH and DO values of 7.9 ± 0.4 and 2.0 ± 0.9 mg L^{-1} respectively. Supernatant DO levels during the experimental phase were 1.0 ± 0.2 and 1.0 ± 0.4 mg L^{-1} for reactors 1 and 2 respectively, suggesting that anoxic conditions were maintained throughout. Despite the use of temperature control apparatus, influent temperature fluctuated within the range 11.5 – 22.4°C due to seasonal ambient temperature changes. However, the low influent flow rates meant that this did not affect temperature within the reactors, with reactors 1 and 2 having average supernatant temperatures of 20.3 ± 0.8°C and 20.2 ± 0.8°C respectively during phase II. Nominal influent glucose concentrations of 52, 75, 100 and 150 mg L^{-1} were utilised during this phase and the remainder of the trials (phase III), with analysis of averaged influent TOC samples (given as mg L^{-1} glucose) indicating these expected values were largely achieved with the exception of the 150 mg L^{-1} level, which was actually dosed at a higher concentration (219.2 mg L^{-1}). Influent TOC values at each concentration averaged over their period of use during phases II and III (expressed as mg L^{-1} glucose) are given in Table 7.6. Influent anion

concentrations averaged over the phase were also utilised for analysis of results, and are given in Table 7.7. Nitrite influent readings were too variable to be averaged.

Table 7.6 - Average influent TOC concentrations during phases II and III

Stated glucose addition (mg L^{-1} as glucose)	Average measured influent soluble TOC concentration (mg L^{-1} as glucose)	Number of samples (n)
52	57.9 ± 11.4	12
75	74.5	1
100	105.2 ± 30.7	8
150	219.2 ± 40.6	3

Table 7.7 - Average influent anion concentrations during phase II

Anion		Average measured influent concentration (mg L^{-1})	Number of samples (n)
Nitrate (phase IIa)		38.2 ± 4.8	7
Nitrate (phase IIb)		12.3 ± 2.7	10
Sulphate		50.5 ± 3.2	11
Bromate (Phase IIa - spiked)	1 mg L^{-1}	0.9	1
	2 mg L^{-1}	1.8	1
	4 mg L^{-1}	4.1	1
	8 mg L^{-1}	4.7	1
	16 mg L^{-1}	16.1	1
	32 mg L^{-1}	30.7	1
	64 mg L^{-1}	69.8	1
	128 mg L^{-1}	130.8	1
Bromate (Phase IIb – spiked)	32 mg L^{-1}	32.9 ± 1.9	6
	48 mg L^{-1}	50.9 ± 5.4	6
Bromide		10.8 ± 1.9	32

A. Phase IIa – Bromate concentration

A range of eight influent bromate concentrations were trialled during phase IIa, to ascertain a suitable influent concentration for subsequent enrichment trials (phase IIb). Concentrations trialled ranged from those commonly observed within the plume (1 – 2 mg L^{-1} as BrO_3^-) to the high groundwater contamination level of 128 mg L^{-1}. Biomass content within reactor 2 (0.029 – 0.080 g dry wt L^{-1}) was higher than that for reactor 1

(0.013 – 0.042 g dry wt L^{-1}) at all bromate concentrations, possibly reflecting the use of inoculated and acclimatised influent (reactor 1 effluent) to this vessel. A reduction in biomass was observed at the 128 mg L^{-1} bromate concentration, with a steady reactor 1 biomass concentration of 0.031 – 0.042 g dry wt L^{-1} reducing to only 0.013 g dry wt L^{-1}. Supernatant biomass concentrations within the two reactors are given in Figure 7.24. pH monitoring showed that, apart from an 8-day period at the start of the trial, pH was maintained within the range 6.5 – 8.0. pH readings for phase IIa are given in Figure 7.25.

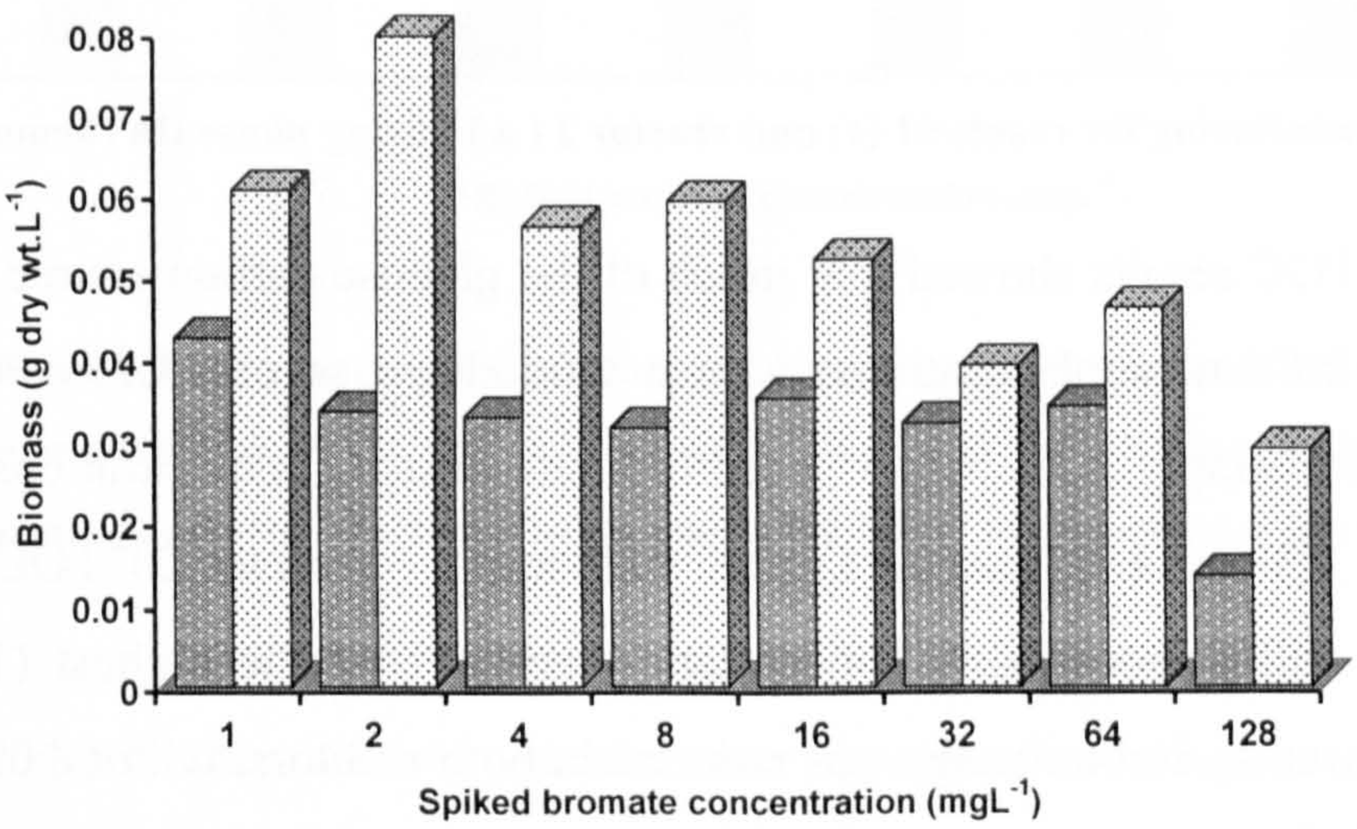


Figure 7.24 - Biomass concentrations at a range of bromate concentrations within reactor 1 (grey bars) and reactor 2 (white bars) during phase IIa chemostat trials

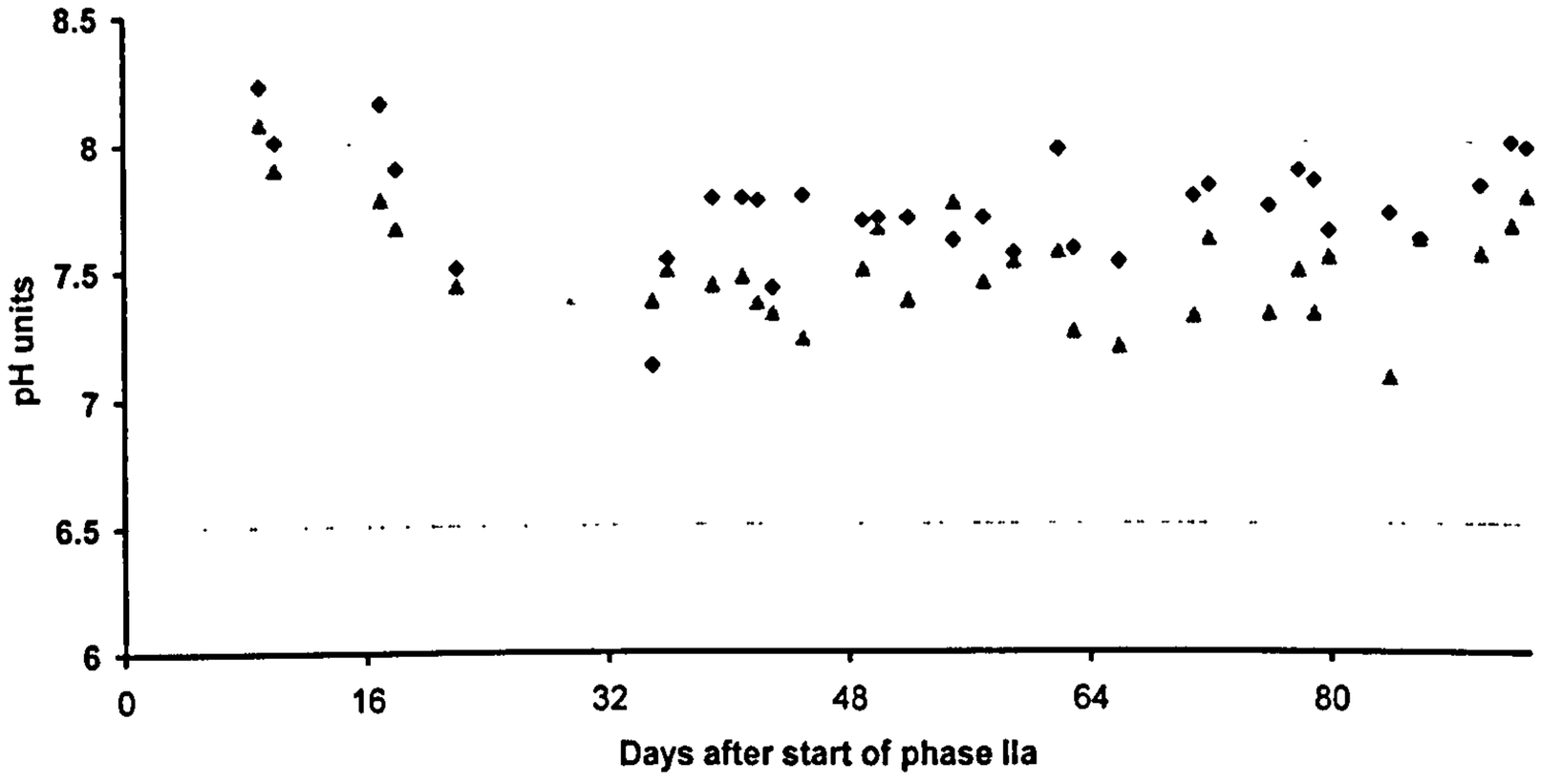


Figure 7.25 - pH monitoring for reactor 1 (♦) and reactor 2 (▲) during phase IIa chemostat trials

Soluble TOC results showed that much of the glucose carbon source was utilised at all bromate influent concentrations, with a total decrease of 78.7 – 90.7% of the combined influent supply at the lower seven bromate influent concentrations. However, a minimum of 8.3 mg L^{-1} (reactor 1) and 10.9 mg L^{-1} (reactor 2) TOC as glucose equivalent was still present in all supernatant samples. At the highest (128 mg L^{-1}) bromate concentration carbon usage was reduced in both reactors, with 68.0% utilisation within reactor 1 and only 54.1% in the combined system. Soluble TOC consumption (given as mg L^{-1} glucose) is shown in Figure 7.26.

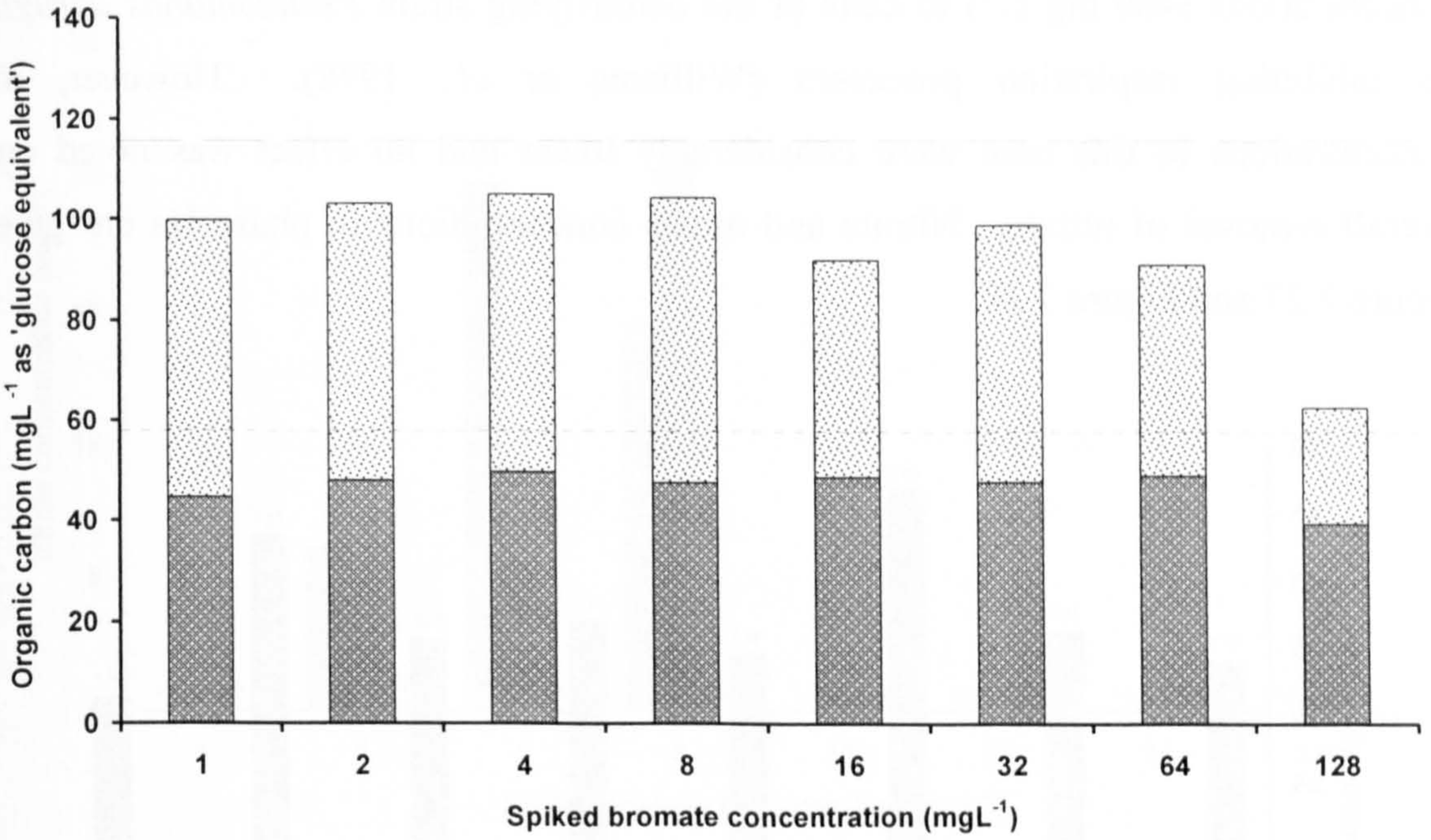


Figure 7.26 - Soluble TOC consumption in reactor 1 (grey bars) and reactor 2 (white bars) expressed as mg L^{-1} glucose during phase IIa. Total height of bar indicates carbon usage within the two-reactor system. Influent glucose to reactor 1 (----) and total input to two-reactor system (— — —) also indicated

Spiking of the influent groundwater produced a slightly variable nitrate concentration in the range 31.3 – 46.7 mg L^{-1}, with an average value of 38.2 ± 4.8 mg L^{-1}. Denitrification was observed in both reactors at all influent bromate concentrations, with the majority (72.6 – 98.7%) of nitrate reduced in reactor 1 within the first 40 hours and reactor 2 accounting for 0.5 – 24.4% of influent, leading to a total reduction of 90.6 – 98.9%. Nitrite concentrations within the supernatant of the second reactor were low at all times with concentrations ranging between < 0.06 mg L^{-1} and 0.3 mg L^{-1}, despite variable influent nitrite concentrations. Nitrite concentrations measured within the influent groundwater supply rose as high as 19.7 mg L^{-1} during phase IIa, which is thought to be due to the influent water 'ageing' whilst in storage and undergoing partial denitrification with nitrite as the end product during this time. Denitrification processes within the reactors were effective in removing this nitrite buildup within the 80-hour retention time, although nitrite production (6.8 mg L^{-1} as NO_2^-) was noted with an 8 mg L^{-1} bromate influent supply after 40 hours. Nitrite has been shown to exhibit direct toxicity at high

concentrations (460 mg L^{-1}) to cells of the denitrifying strain *Pseudomonas aeruginosa* by inhibiting respiration processes (Williams *et al.*, 1978). However, nitrite concentrations in this case were considerably lower and no effect was noted on the overall removal of nitrate. Nitrate and nitrite concentrations in phase IIa are given in Figure 7.27 and Figure 7.28.

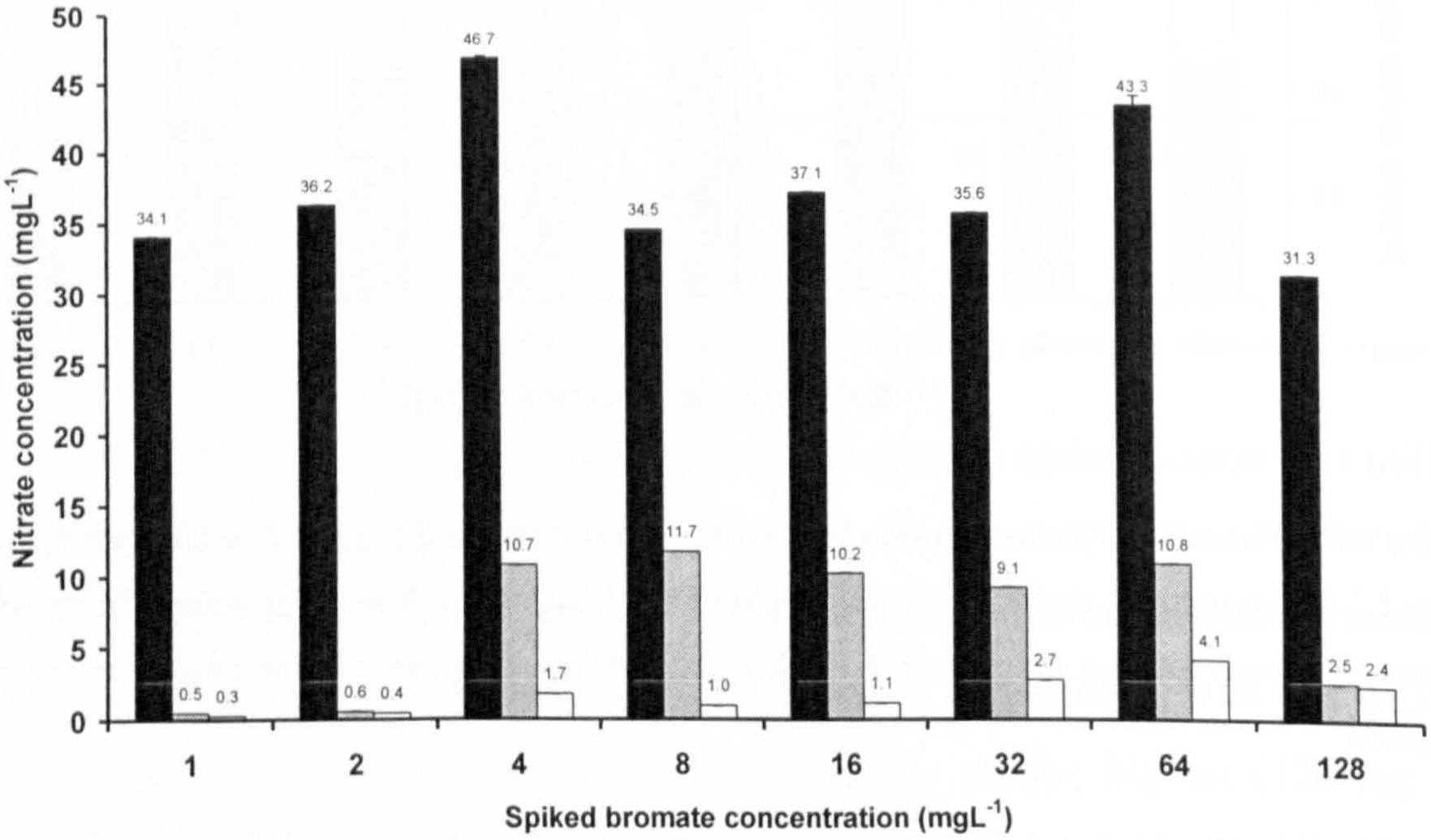


Figure 7.27 - Effect of bromate spiking on nitrate concentrations (in mg L^{-1}) during phase IIa chemostat trials. Influent concentrations given as black bars, reactor 1 supernatant concentrations (40-hour RT) as grey bars and reactor 2 supernatant (total 80-hour RT) as white bars.

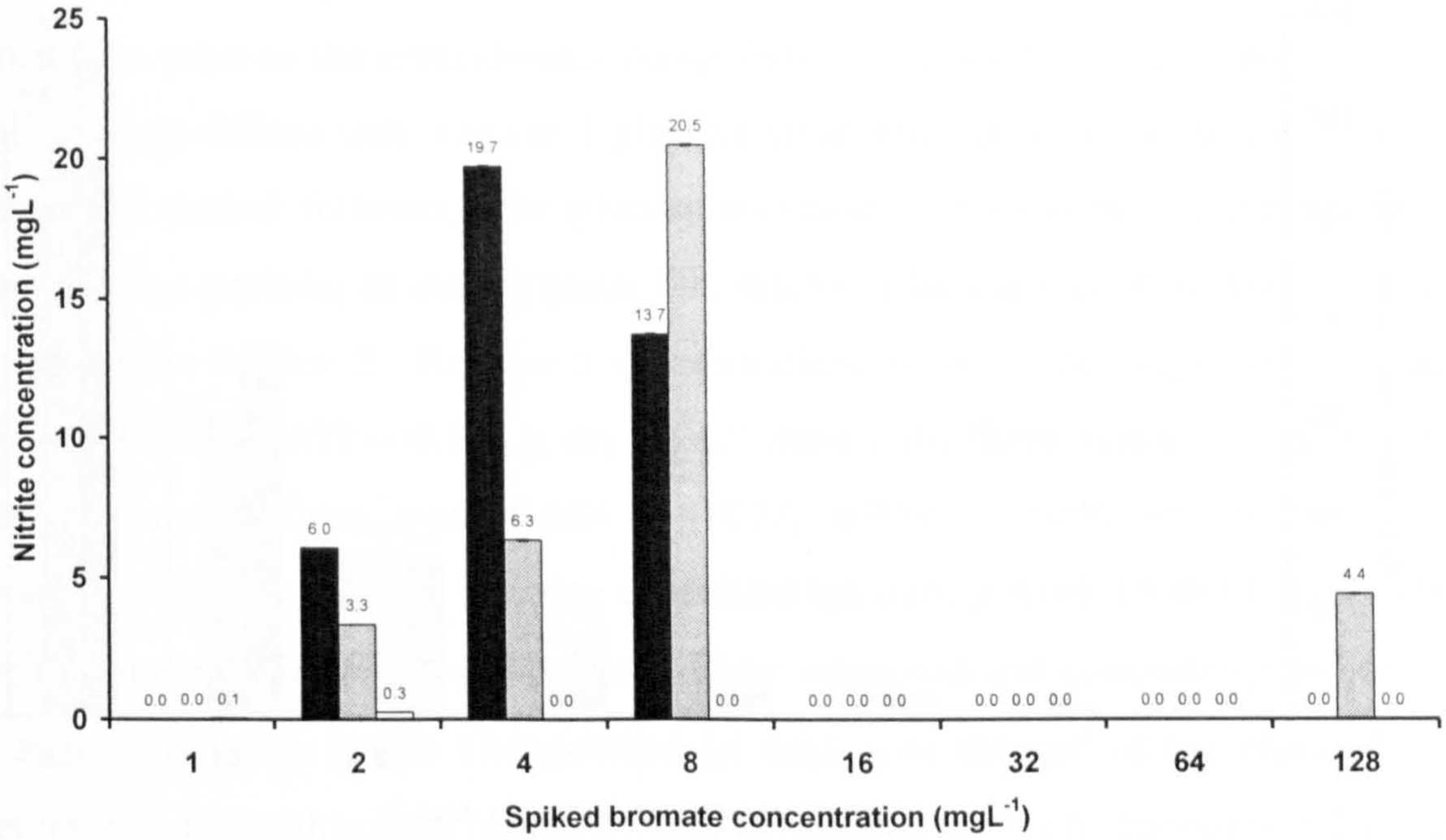


Figure 7.28 - Effect of bromate spiking on nitrite concentrations (in mg L^{-1}) during phase IIa chemostat trials. Influent concentrations given as black bars, reactor 1 supernatant concentrations (40-hour RT) as grey bars and reactor 2 supernatant (total 80-hour RT) as white bars.

Bromate reduction observed during phase IIa was in excess of that achieved during phase I. A maximum of 11.7 – 15.0 mg L^{-1} bromate was reduced with a nominal influent spiking concentration of 64 mg L^{-1} (measured value of 69.8 mg L^{-1}). There were slight increases in bromate concentration ($\leq$ 3.2 mg L^{-1}) within reactor 1 (40-hour RT) at the 4, 8 and 128 mg L^{-1} bromate concentrations, and reactor 2 at the 64 mg L^{-1} concentration. As no further bromate was added to either reactor, the reason for these discrepancies is not known. At the 1 mg L^{-1} and 2 mg L^{-1} bromate concentrations (measured values of 0.9 and 1.8 mg L^{-1} respectively) reduction to the limit of detection (0.01 mg L^{-1}) was observed with an 80-hour RT. Bromate influent spiking of 4 – 32 mg L^{-1} led to relatively constant bromate removal of 3.2 – 4.6 mg L^{-1} at the 80-hour RT. The 128 mg L^{-1} bromate spike induced lower bromate removal (10.1 mg L^{-1} with an 80-hour RT) than at the 64 mg L^{-1} level, with no reduction observed within reactor 1 (40-hour RT). Bromate concentrations in phase IIa are given in Figure 7.29.

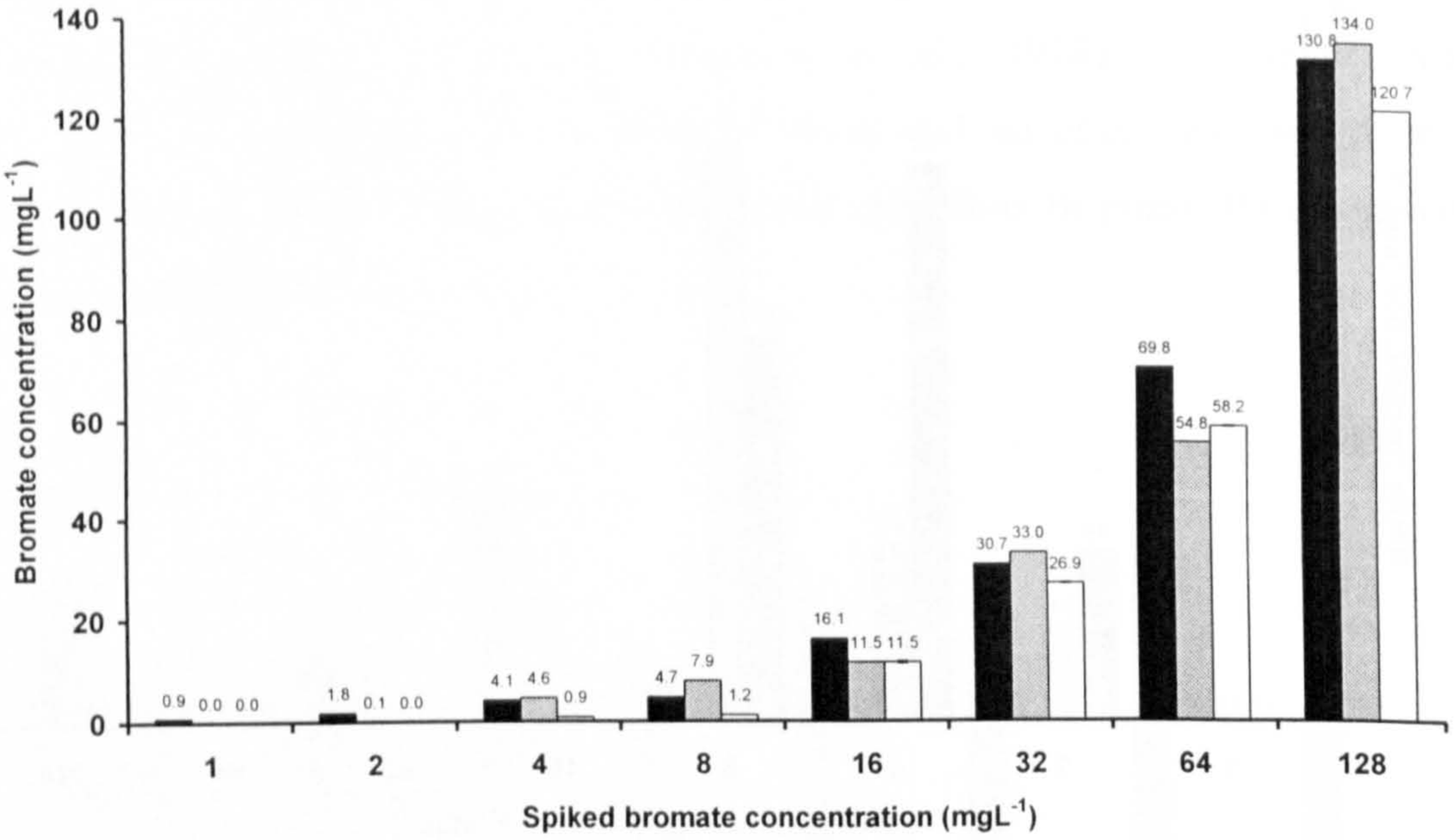


Figure 7.29 - Effect of influent bromate concentration on bromate reduction during phase IIa chemostat trials. Influent bromate concentrations given as black bars, reactor 1 supernatant concentration (40-hour RT) as grey bars and reactor 2 supernatant (total 80-hour RT) as white bars.

B. Phase IIb – Steady-state enrichment

Steady influent conditions (summarised in Table 6.4) were maintained for an extended (28-week) period during phase IIb, with the only alterations occurring being an increase in nominal bromate influent from 32 mg L^{-1} to 48 mg L^{-1} at week 15, and an increase in glucose influent to reactor 1 from 100 mg L^{-1} to 150 mg L^{-1} at week 23. These alterations were undertaken to attempt an increase in bromate reduction by providing excess electron acceptor and donor respectively. In addition there was a failure in the groundwater influent supply during week 7, which went unnoticed for a period of 6 days prior to rectification. Glucose influent supply was unaffected, leading to an inevitable increase in carbon availability within both reactors. Maximum soluble TOC values within the reactors during this period are unknown. However, analysis of supernatant samples collected at the end of the 6-day period showed that TOC concentrations of 89.6 and 27.8 mg L^{-1} were attained in reactors 1 and 2 respectively, compared with a maximum supernatant TOC value of 21.3 mg L^{-1} during phase IIa. Therefore, carbon was very much in excess during this 6-day period.

Biomass readings during the 28-week phase IIb showed three distinct periods. The first was prior to the groundwater pump failure (weeks 0 – 7), the second after the period of pump failure until reactor 1 glucose concentration was increased at week 23, and the final period following the glucose increase as also observed during phase I. Within all three periods, as during phase IIa, reactor 1 biomass concentration was lower than that within reactor 2. Reactor 1 concentrations were in the ranges 0.023 – 0.050, 0.025 – 0.065 and 0.031 – 0.106 g dry wt L^{-1} during the three periods. Corresponding reactor 2 concentrations were 0.034 – 0.078, 0.059 – 0.096 and 0.067 – 0.121 g dry wt L^{-1}. The increase in bromate concentration during week 15 did initially appear to elicit an increase in biomass, but this was not sustained and concentrations had fallen back to their previous levels after around 14 days. At the end of the phase, biomass concentrations fell within both reactors. This is likely to be due to operational difficulties leading to low glucose flows during this period. Biomass concentrations during phase IIb are given in Figure 7.30. pH monitoring showed that, as with phase IIa, levels in both reactors were within the range 6.5 – 8.0. Lower pH values of 5.7 (reactor 1) and 6.8 (reactor 2) were recorded immediately following the end of the groundwater pump failure period, but these had largely recovered within 6 days (approximately 3 retention times) of reinstatement of the groundwater supply. pH readings for phase IIb are given in Figure 7.31. A reduction in pH would occur during a period of fermentation, due to production of a range of acidic products (examples include acetic acid, lactic acid, formic acid and succinic acid). Fermentation can occur when a continual source of fermentable material is available (Pelczar *et al.*, 1993), which in this case was glucose. Following the pump failure, continued recording of elevated biomass readings indicated a sustained alteration in biomass composition, which also elicited increases in bromate reducing ability. The alterations observed during this key period of operation are outlined below, with implications further discussed in Chapter 8.

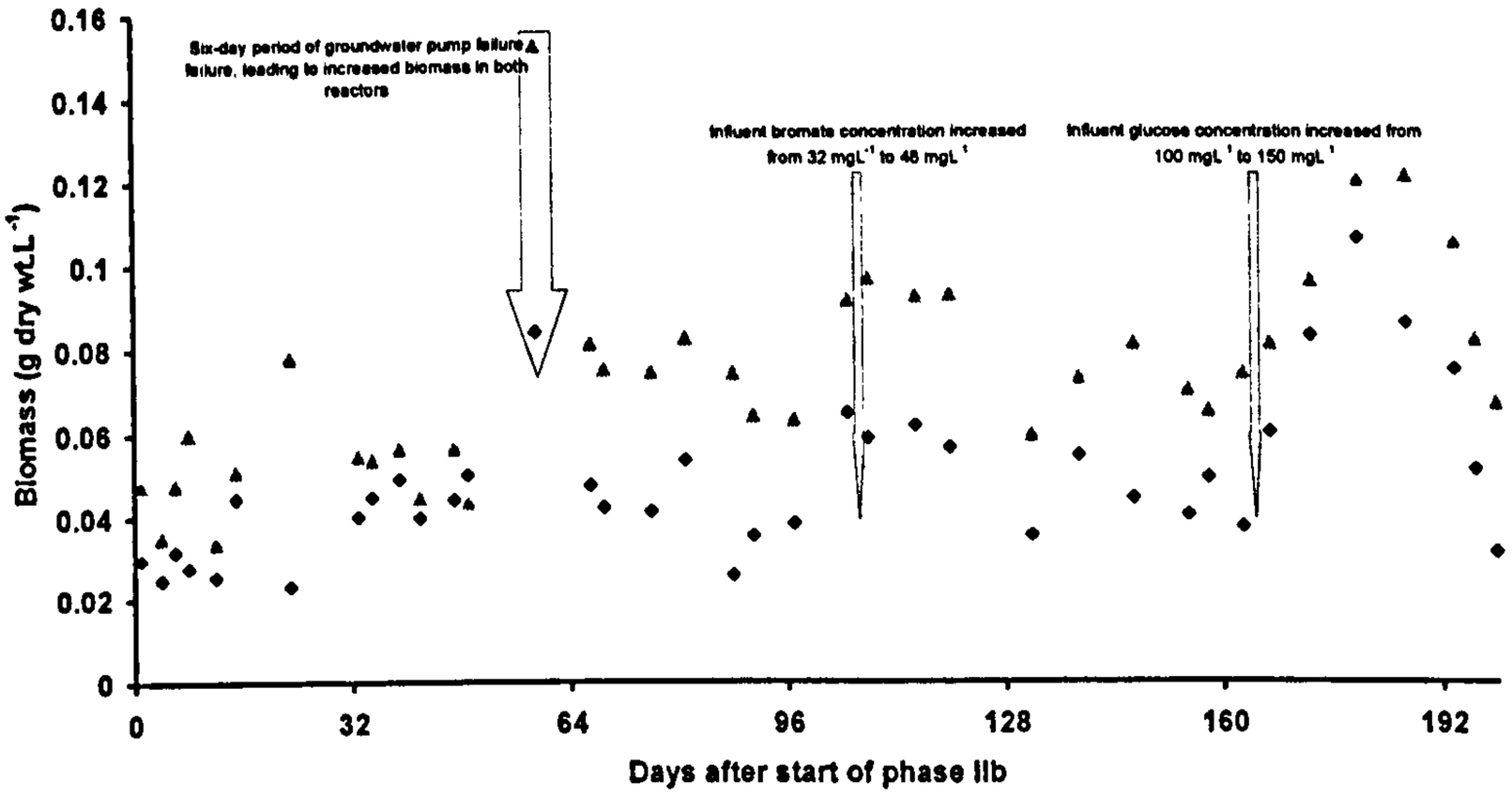


Figure 7.30 - Biomass monitoring for reactor 1 (♦) and reactor 2 (▲) during phase IIb chemostat trials

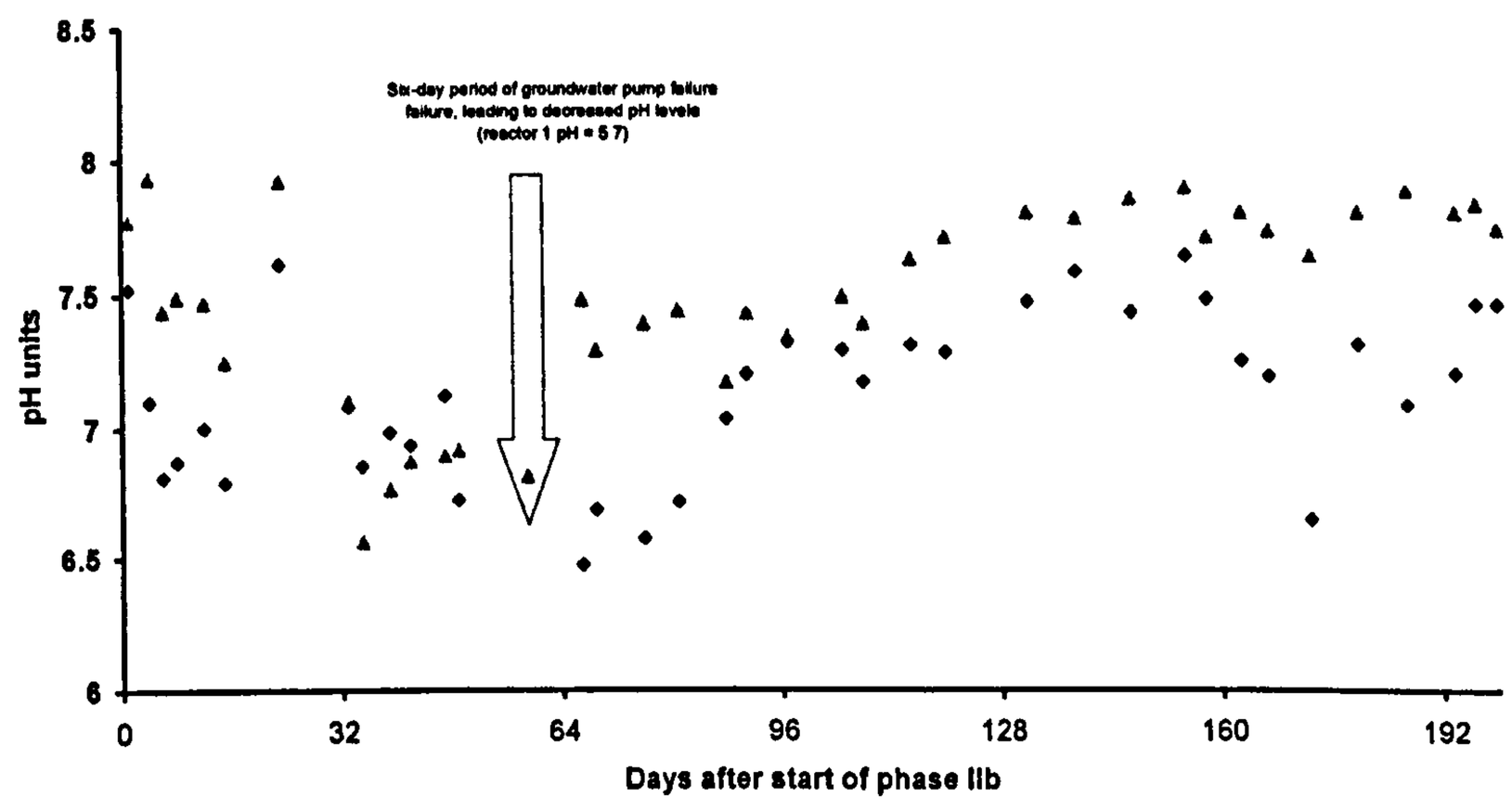


Figure 7.31 - pII monitoring for reactor 1 (♦) and reactor 2 (▲) during phase IIb chemostat trials

Soluble TOC was measured during phase IIb with the exception of weeks 1 – 5 and showed that, within both reactors, stable carbon consumption was maintained throughout the period. Following the increase in glucose influent to reactor 1 at week 23, carbon consumption did increase but overall percentage utilisation did not alter, suggesting a constant proportion of the carbon present was not available for use by the bacterial population despite the increase in biomass observed during this period. Percentage carbon utilisations during phase IIb were 50.3 ± 8.2% and 75.5 ± 5.1% for the

40-hour (reactor 1) and 80-hour (reactors 1 and 2) retention times respectively. Soluble TOC consumption during phase IIb (weeks 5 – 28) is shown in Figure 7.32.

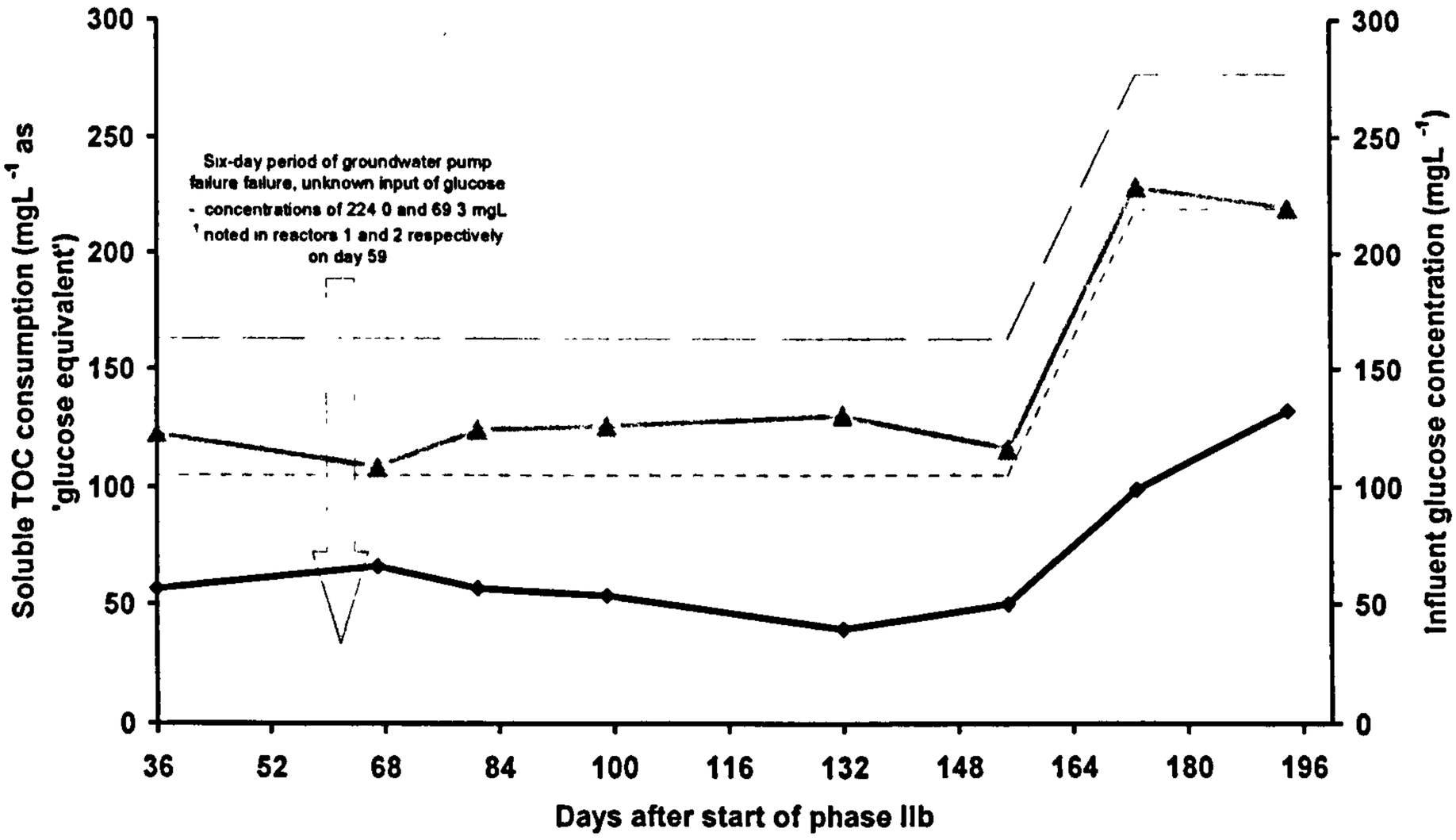


Figure 7.32 - Soluble TOC consumption within 40-hour (♦) and total 80-hour (▲) retention times expressed as mg L^{-1} glucose during phase IIb. Average influent glucose to reactor 1 (----) and total input to two-reactor system (———) also indicated

Denitrification within reactor 1 (40-hour RT) appeared to be variable throughout the phase, with results ranging from no observed reduction to almost total denitrification. The reason for this variation is not apparent. With the total 80-hour RT, denitrification was more stable, although a maximum nitrate supernatant concentration of 6.0 mg L^{-1} was still observed. Failure of the influent groundwater supply did not appear to affect denitrification, but results around the time of increased glucose addition are too variable to allow strong conclusions to be drawn. However, a temporary decrease in denitrification within both reactors was observed following increase of the bromate influent concentration, with the supernatant concentration at a 40-hour RT increasing from 0.7 mg L^{-1} to 6.3 mg L^{-1} over the following 18 days. Variable nitrite influent concentrations did not allow representative analysis of the supernatant nitrite concentrations, but a maximum of 0.8 mg L^{-1} was observed with an 80-hour RT, and all other results showed nitrite concentrations below 0.3 mg L^{-1}. Sulphate concentrations

indicated that during phase IIb, sulphate reduction was also occurring within the two reactors. Although the products of this reduction were not analysed, a strong hydrogen sulphide odour was noted upon occasion of sulphate reduction occurring. Previous to the groundwater pump failure, limited sulphate reduction was noted, with 47.3 mg L^{-1} still present after an 80-hour RT (50.5 mg L^{-1} average influent sulphate). However, immediately following the pump failure sulphate concentration within reactor 2 decreased to only 8.7 mg L^{-1}, showing occurrence of significant sulphate reduction under the high carbon conditions and suggesting the prior presence of a microbial population capable of sulphate reduction. Prior to the increase of bromate concentration from 32 mg L^{-1} to 48 mg L^{-1}, a supernatant sulphate concentration of only 13.5 mg L^{-1} was noted at the 80-hour RT. Following the bromate increase, the magnitude of sulphate reduction immediately decreased to give stable reactor 2 concentrations within the range 38.0 – 44.0 mg L^{-1}. Subsequent to the glucose increase, sulphate reduction with an 80-hour RT generally increased over the remaining experimental period, with 21.9 mg L^{-1} reduction observed by the end of the trial. The immediate decrease in sulphate reduction following bromate influent increase indicates that sulphate reduction does not occur in the presence of high bromate concentrations. Nitrate and sulphate concentrations during phase IIb are given in Figure 7.33 and Figure 7.34. No nitrate or sulphate analysis was available for the initial 5 weeks of phase IIb.

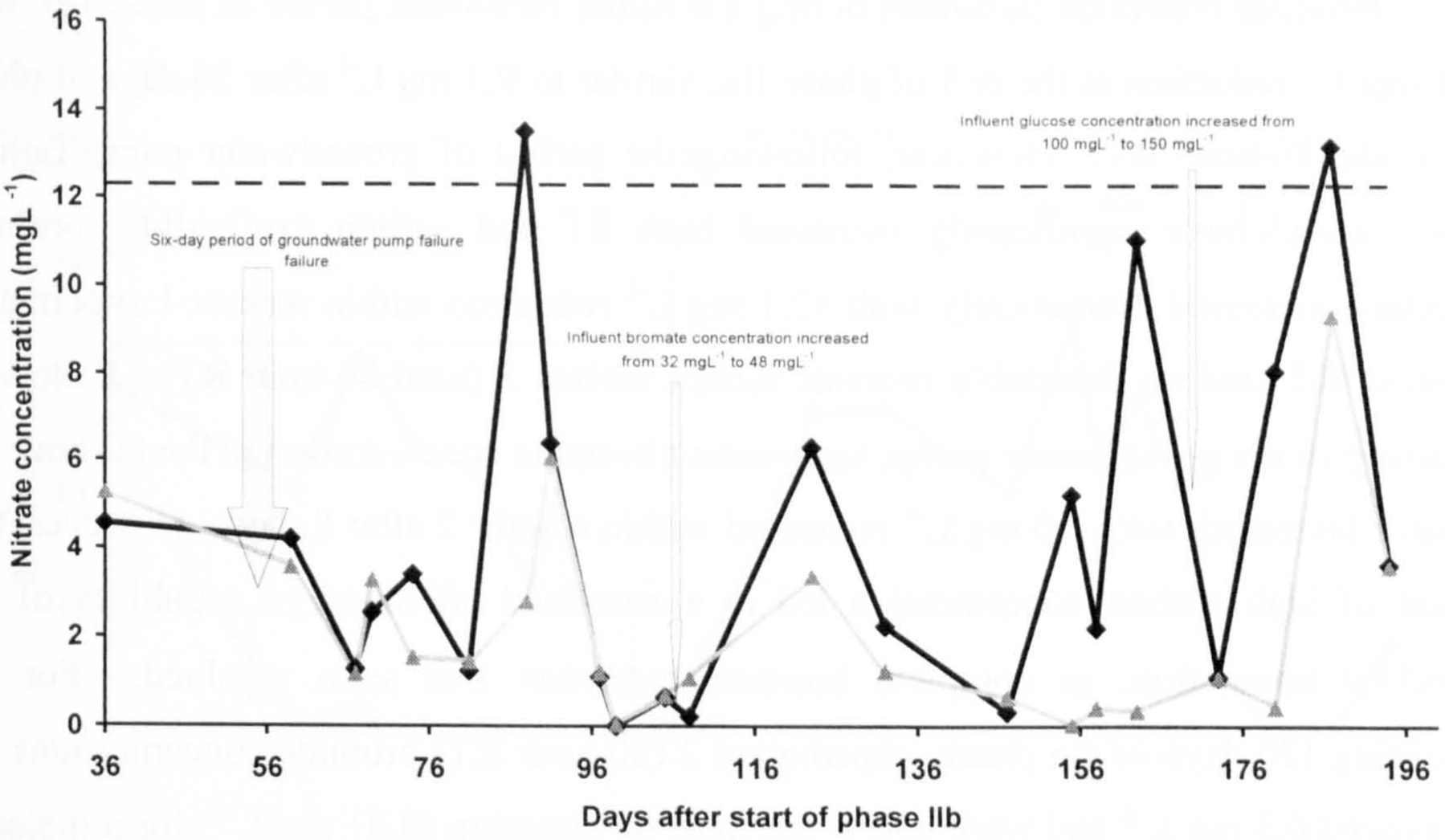


Figure 7.33 - Nitrate concentrations during the phase IIb (enrichment phase) for 40-hour (♦) and total 80-hour (▲) retention times. Average nitrate influent is given (— — —), with major perturbations to the steady influent supplies indicated

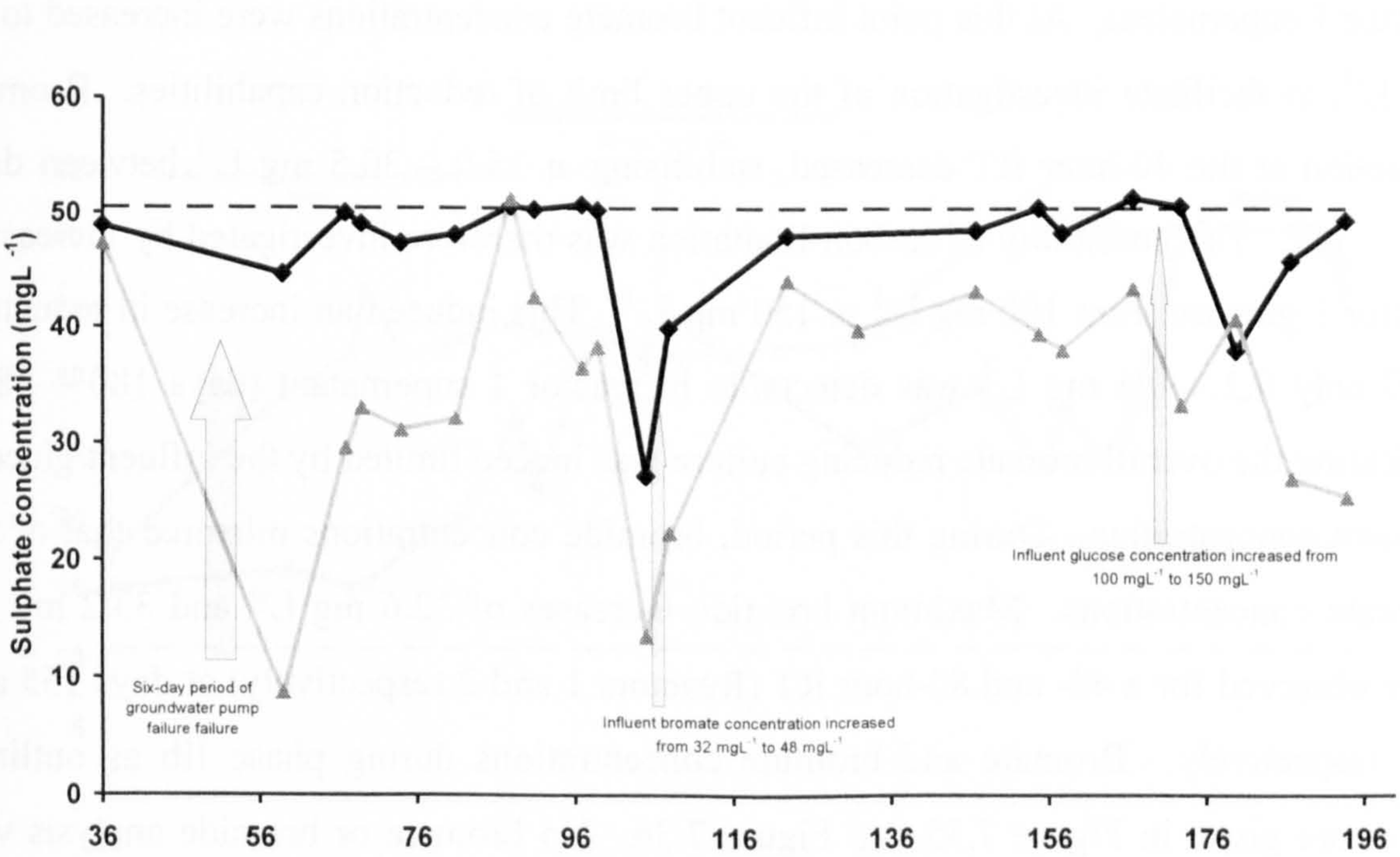


Figure 7.34 - Sulphate concentrations during the phase IIb (enrichment phase) for 40-hour (♦) and total 80-hour (▲) retention times. Average sulphate influent is given (— — —), with major perturbations to the steady influent supplies indicated

Bromate reduction stabilised during the initial five-week period of phase IIb, with 10.1 mg L^{-1} reduction at the end of phase IIa, similar to 9.1 mg L^{-1} after 36 days of phase IIb at the 80-hour RT. However, following the period of groundwater pump failure, which would have significantly increased both RT and carbon availability, bromate reduction increased dramatically with 12.1 mg L^{-1} reduction within reactor 1 supernatant (40-hour RT) and no detectable bromate within reactor 2 (total 80-hour RT). Following restarting of the groundwater pump, supernatant bromate concentration at the 80-hour RT initially increased, with 3.0 mg L^{-1} measured within reactor 2 after 8 days. However, this period of high carbon concentration led to a sustained alteration in capability of the microbial population, as complete bromate reduction was soon regained. For the remaining 120 days of the phase, supernatant 2 (80-hour RT) bromate concentrations did not exceed 0.3 mg L^{-1} and were below the limit of detection (0.01 mg L^{-1}) for a majority of readings taken, even following increase of influent level from 32 mg L^{-1} to 48 mg L^{-1}. At the 40-hour RT (reactor 1), bromate reduction initially fell following the restarting of groundwater pumps, but gradually increased again until only 0.3 mg L^{-1} was detected in reactor 1 supernatant. At this point influent bromate concentrations were increased to 48 mg L^{-1}, to facilitate investigation of the upper limit of reduction capabilities. Bromate reduction at the 40-hour RT decreased, stabilising at 15.0 – 30.5 mg L^{-1} between days 123 – 167. The possibility of carbon limitation was therefore investigated by increasing reactor 1 glucose from 100 mg L^{-1} to 150 mg L^{-1}, This induced an increase in reduction until only 0.2 – 0.4 mg L^{-1} was detectable in reactor 1 supernatant (days 180 – 187), indicating the overall bromate reducing culture was indeed limited by the influent glucose influent concentration. During this period, bromide concentrations mirrored that of the bromate concentrations. Maximum bromide increases of 32.6 mg L^{-1} and 33.2 mg L^{-1} were observed for a 40- and 80-hour RT (Reactors 1 and 2 respectively) at days 155 and 180 respectively. Bromate and bromide concentrations during phase IIb as outlined above are given in Figure 7.35 and Figure 7.36. No bromate or bromide analysis was available for the initial 5 weeks of phase IIb.

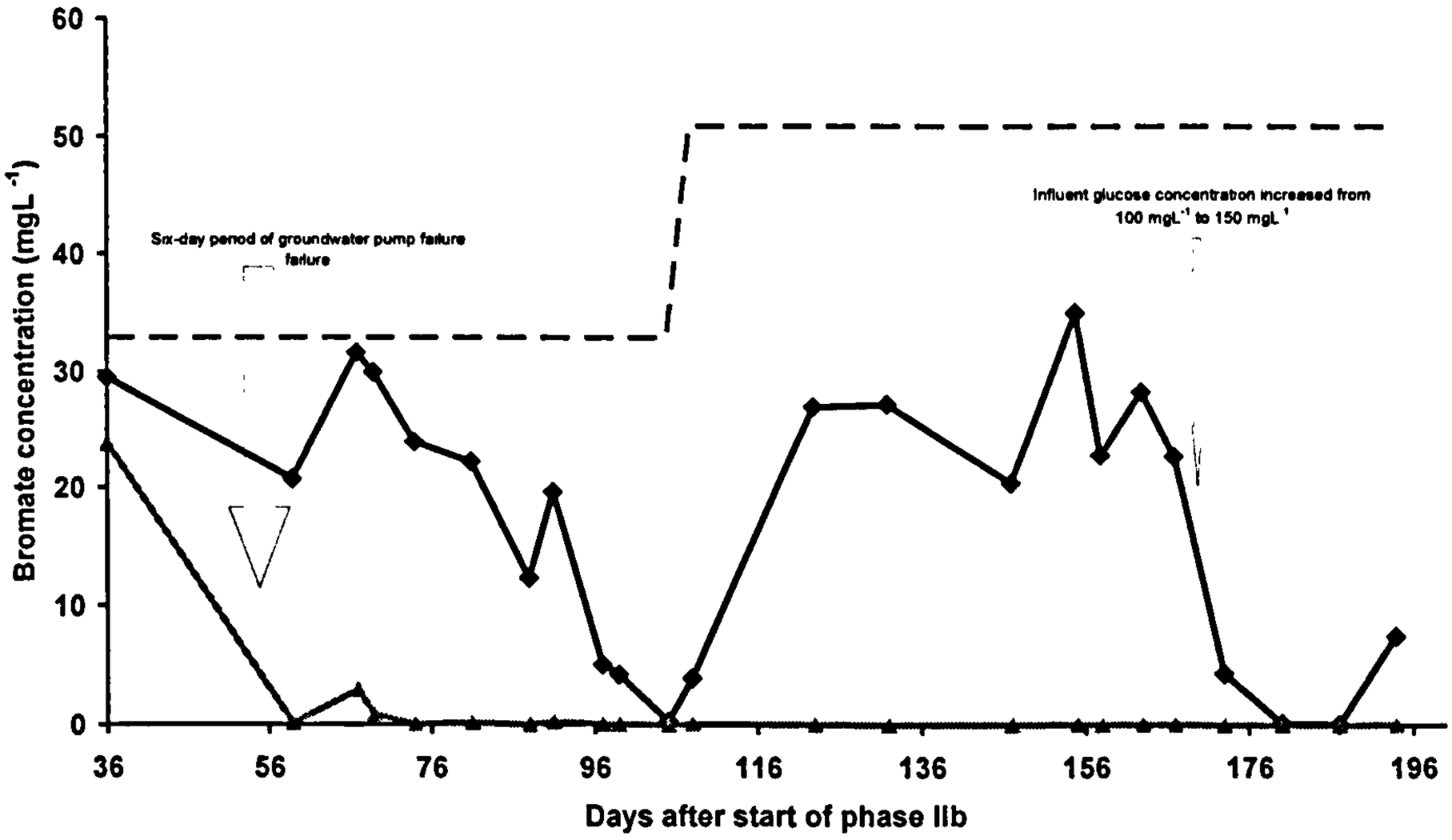


Figure 7.35 - Bromate concentrations during the phase IIb (enrichment phase) for 40-hour (♦) and total 80-hour (▲) retention times. Average bromate influent is given (— — —), with major perturbations to the steady influent supplies indicated

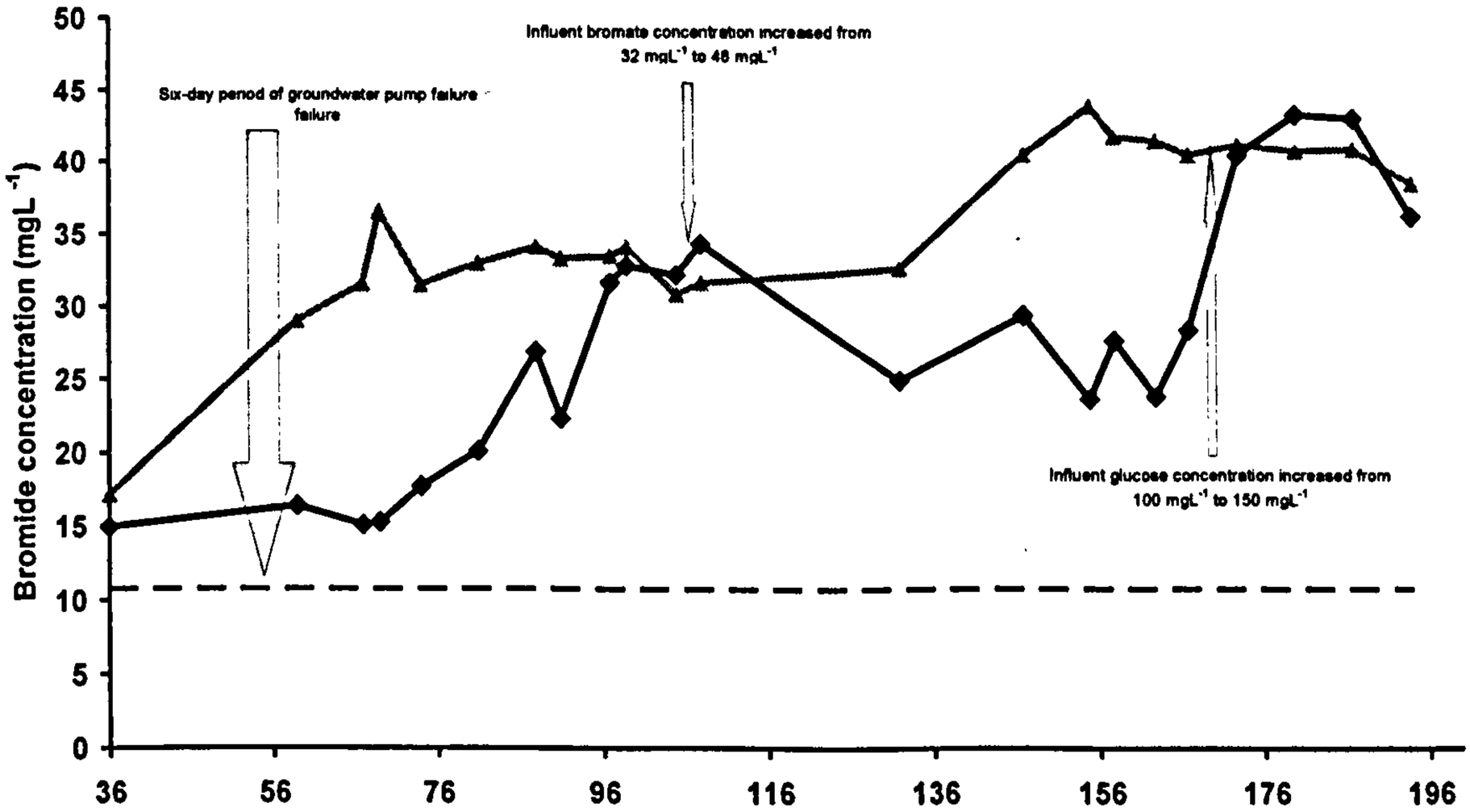


Figure 7.36 - Bromide concentrations during the phase IIb (enrichment phase) for 40-hour (♦) and total 80-hour (▲) retention times. Average bromide influent is given (— — —), with major perturbations to the steady influent supplies indicated

Overall percentage bromine recovery was calculated using an average of effluent values (80-hour RT) during the 28-week period of phase IIb. A bromine recovery of 100.0 ± 4.0% (n=6) was achieved over this extended period. This provided strong evidence for total conversion of bromate to bromide within the reactor system, with no formation of intermediates. Corresponding calculations for supernatant analyses gave comparable averages, although the error measured as standard deviations was greater in both cases. Bromine recovery for reactors 1 and 2 (n=10) gave percentage values of 99.8 ± 10.1% and 97.3 ± 10.7% respectively.

C. Phase II reduction and specific reduction rates

Bromate and nitrate reduction/specific reduction rates were calculated for both phase IIa and phase IIb trials, and are given in Table 7.8 and Table 7.9 respectively. During phase IIa nitrate reduction rates stayed within the ranges 662.4 – 943.7 μg L^{-1} NO_3 hr^{-1} (40-hour RT) and 439.0 – 474.5 μg L^{-1} NO_3 hr^{-1} (total 80-hour RT), with no overall trend discernible. Maximum nitrate reduction and specific reduction rates were lower than during phase I, partially due to the longer RT (40 hours versus 20 hours) with no additional influent nitrate. A maximum phase IIa specific reduction rate of 1105.9 μmol N g dry wt^{-1} hr^{-1} was observed at the highest bromate concentration with a 40-hour RT, due to the low biomass concentration of this sample. All other specific nitrate reduction rates measured during phase IIa were relatively constant, within the ranges 322.9 – 460.1 μmol N g dry wt^{-1} hr^{-1} at a 40-hour RT. Bromate reduction rates at the start of the phase were observed to be equivalent to the maximum rate achieved during phase I, with 22.1 μg L^{-1} BrO_3 hr^{-1} (40-hour RT) noted at a 1 mg L^{-1} bromate influent. Specific bromate reduction rates were also similar to the phase 1 maximum specific rate (2.2 μmol Br g dry wt^{-1} hr^{-1}), with 4.1 and 1.8 μmol Br g dry wt^{-1} hr^{-1} observed at 40- and 80-hour RTs respectively. During the course of phase IIa, bromate reduction at a 40-hour RT was inconsistent leading to no observed removal at some influent concentrations. However, after a total 80-hour RT consistent bromate reduction was achieved, thus allowing the calculation of bromate reduction and specific reduction rates. Both generally increased along with increase in bromate concentration, following a similar pattern. 80-hour RT reduction and specific reduction rates increased to maxima at the 64

mg L^{-1} bromate influent of 240.5 µg L^{-1} BrO_3 hr^{-1} and 30.3 µmol Br g dry wt^{-1} hr^{-1} respectively. Specific bromate reduction rate at the highest bromate influent concentration (128 mg L^{-1}) was 26.0 µmol Br g dry wt^{-1} hr^{-1} at an 80-hour RT. This is lower than may be expected were bromate reduction rate directly related to influent concentration, and reflects the low biomass concentration (Figure 7.30) and bromate reduction (Figure 7.35) observed at this high bromate contamination level. Relative rates (qN/qBr) decreased over phase IIa, largely due to increases in bromate specific reduction rates, from 87.9 to 3.8 at a 40-hour RT and from 85.5 to 3.9 at a total 80-hour RT (1 – 64 mg L^{-1} bromate influents).

Prior to the extended run during phase IIb nitrate spiking was discontinued. This is reflected in the lower phase IIb reduction rates, which stabilised and then stayed constant, increasing only marginally from 193.8 to 216.5 µg L^{-1} NO_3 hr^{-1} at a 40-hour RT. Specific reduction rates also decreased compared with phase IIa, with a continuing decreasing trend during phase IIb from 69.3 to 46.6 µmol N g dry wt^{-1} hr^{-1} (40-hour RT). By contrast, both bromate reduction and specific reduction rates increased significantly over the same period. A reduction rate of 84.9 µg L^{-1} BrO_3 hr^{-1} (40-hour RT) on day 36 had increased to 1086.5 µg L^{-1} BrO_3 hr^{-1} by the end of the phase on day 194. The corresponding increase in specific reduction rates during phase IIb was of almost an order of magnitude (14.7 µmol Br g dry wt^{-1} hr^{-1} (day 36) to 113.3 µmol Br g dry wt^{-1} hr^{-1} (day 194)), with a peak specific reduction rate of 160.5 µmol Br g dry wt^{-1} hr^{-1} on day 88 (40-hour RT). Relative molar ratios (qN/qBr) at a 40-hour RT within phase IIb fell from 4.7 (day 36) to 0.3 (day 194), supporting a continued improvement in bromate degradation ability relative to denitrification during phase IIb.

Table 7.8 - Bromate and nitrate reduction/specific reduction rates during phase IIa chemostat trials (Temperature - 20°C; Reactors in series with 40-hour (Reactor 1) and 80-hour (combined system) retention times; Influent glucose concentration – 52 mg L^{-1} to each reactor)

Bromate in feed (mg L^{-1})	Retention time (hours)	Reduction rates		Specific reduction rates		qN/qBr
		Nitrate (µg L^{-1} NO_3 hr^{-1})	Bromate (µg L^{-1} BrO_3 hr^{-1})	qN (µmol N g dry wt^{-1} hr^{-1})	qBr (µmol Br g dry wt^{-1} hr^{-1})	
1	40	943.7	22.1	362.4	4.1	87.9
	80	474.5	11.5	150.1	1.8	85.5
2	40	941.3	43.6	460.1	10.3	44.6
	80	474.0	22.5	88.9	2.0	43.5
4	40	686.7	-	335.6	-	-
	80	447.0	41.4	120.2	5.4	22.3
8	40	662.4	-	344.6	-	-
	80	437.6	42.5	91.7	4.3	21.3
16	40	700.8	114.9	322.9	25.7	12.6
	80	463.0	83.8	120.4	10.6	11.4
32	40	727.0	-	366.4	-	-
	80	450.7	52.3	142.5	8.0	17.8
64	40	684.4	374.9	324.7	86.2	3.8
	80	455.4	240.5	118.5	30.3	3.9
128	40	891.4	-	1105.9	-	-
	80	439.0	149.9	157.3	26.0	6.0

Table 7.9 - Bromate and nitrate reduction/specific reduction rates at commencement and conclusion of phase IIb chemostat trial (Temperature - 20°C; Reactors in series with 40-hour (Reactor 1) and 80-hour (combined system) retention times; Influent glucose concentration – 100 mg L^{-1} (Reactor 1) and 52 mg L^{-1} (Reactor 2)

Days after start of phase IIb	Retention time (hours)	Reduction rates		Specific reduction rates		qN/qBr
		Nitrate ($\mu g\ L^{-1}\ NO_3\ hr^{-1}$)	Bromate ($\mu g\ L^{-1}\ BrO_3\ hr^{-1}$)	qN ($\mu mol\ N\ g\ dry\ wt^{-1}\ hr^{-1}$)	qBr ($\mu mol\ Br\ g\ dry\ wt^{-1}\ hr^{-1}$)	
36	40	193.8	84.9	69.3	14.7	4.7
	80	174.4	117.0	20.4	13.3	1.5
194	40	216.5	1086.5	46.6	113.3	0.4
	80	209.9	636.2	14.2	41.8	0.3

7.2.1.3 Phase III (Parameter assessment)

With good bromate reduction observed at both 40 and 80-hour retention times, reactors were reconfigured back into separate units for Phase III, to allow examination of parameters affecting bromate removal within this high-rate reducing culture (temperature, influent glucose concentration and low nitrate/sulphate influent) on a test and control basis at a 40-hour RT. Over the entire period of phase III pH monitoring confirmed that pH control was again not required, with the majority of readings within the range 6.5 – 8.0. pH readings below 6.5 were noted to be concurrent with short periods of groundwater influent pipe blockage, but pH quickly rose on all occasions once flow recommenced. pH monitoring data for phase III are given in Figure 7.37. Average supernatant DO concentrations within reactors 1 and 2 were 1.4 ± 0.4 mg L^{-1} and 1.4 ± 0.3 mg L^{-1} respectively, again suggesting maintenance of anoxic conditions within the reactors. Supernatant temperatures were also kept constant, with average readings over the phase of 20.3 ± 0.8°C and 20.3 ± 0.7°C for reactors 1 and 2 respectively. Influent anion concentrations for House Lane groundwater averaged over the phase (Table 7.10) were utilised for analysis of results.

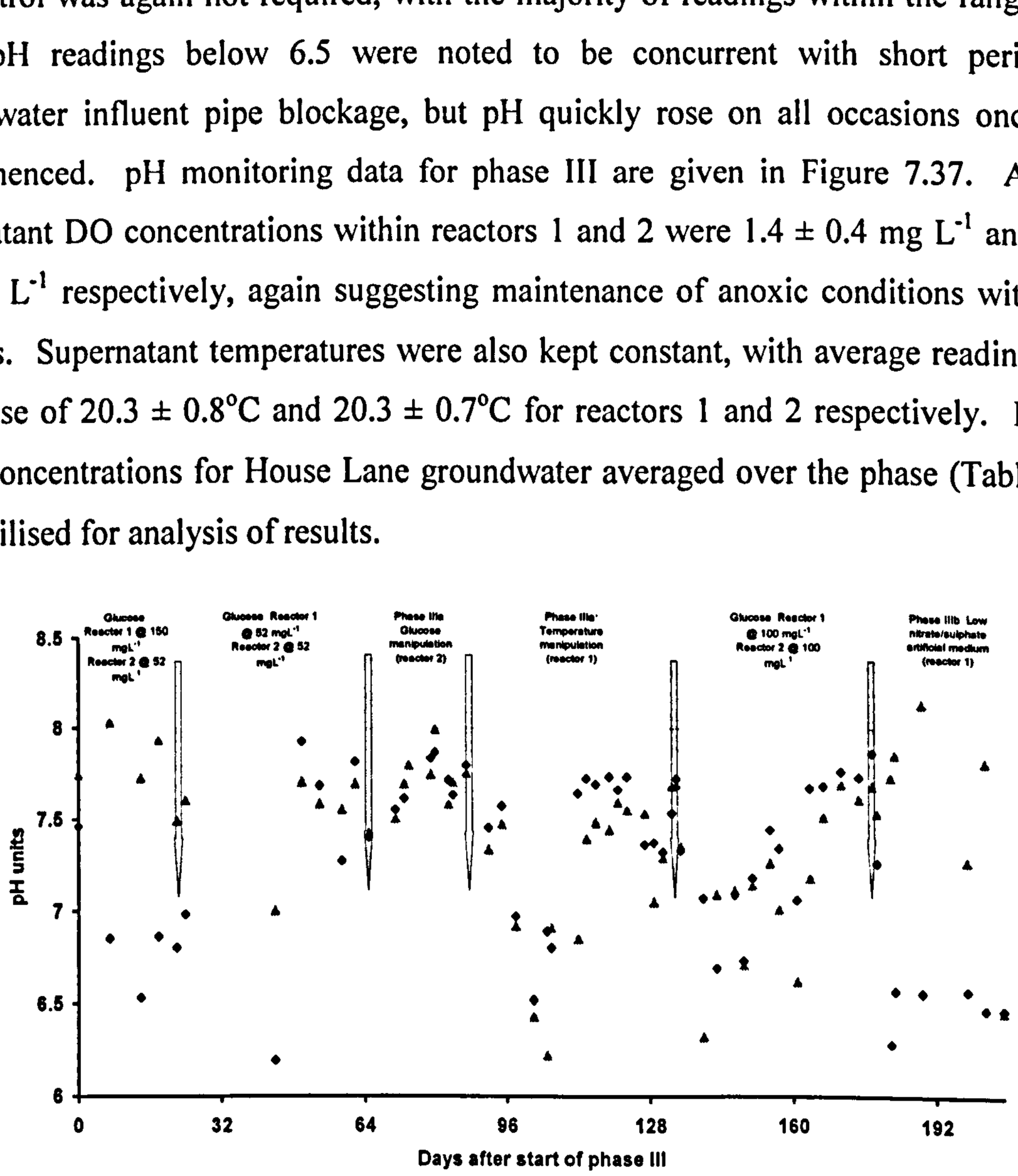


Figure 7.37 - pH monitoring for reactor 1 (♦) and reactor 2 (▲) during phase III chemostat trials

Table 7.10 - Average influent anion concentrations for House Lane groundwater supply during phase III chemostat trials

Anion	Average measured influent concentration (mg L^{-1})	Number of samples (n)
Nitrate	11.0 ± 2.5	12
Nitrite*	0.6 ± 1.6	15
Sulphate	54.3 ± 2.6	16
Bromate	25.9 ± 4.0	16
Bromide	14.3 ± 2.9	15

* 11 measurements were below the limit of detection (0.06 mg L^{-1})

Following separation of the two reactors into separate units upon commencement of phase III, bromate reduction within reactor 1 was observed to be inferior to that within reactor 2, with supernatant concentrations of 24.8 mg L^{-1} and 0.02 mg L^{-1} respectively. To enhance reduction within reactor 1, glucose influent concentrations were maintained at the phase II levels of 150 mg L^{-1} and 52 mg L^{-1} for reactors 1 and 2 respectively for a 3-week period. By the end of this period bromate reduction had increased within reactor 1, with comparable supernatant concentrations observed in both reactors 1 and 2 (0.7 mg L^{-1} and 0.8 mg L^{-1} respectively). Supernatant nitrate concentrations were within the range 0.02 – 1.4 mg L^{-1} in both reactors. Sulphate reduction within reactor 1 increased sharply within this period, with supernatant concentrations of 52.3 mg L^{-1} measured after two weeks decreasing to 1.7 mg L^{-1} one week later. This result, together with the bromate data, suggested that available carbon had been utilised to reduce all the bromate with excess carbon then stimulating sulphate reduction. Glucose influent concentration within reactor 1 was therefore dropped to 52 mg L^{-1}, creating identical conditions within the two systems. These conditions (32 mg L^{-1} bromate and 52 mg L^{-1} glucose influent concentrations in both reactors) were maintained for a further six-week acclimation period prior to commencement of phase IIIa glucose manipulations.

Despite maintenance of these steady conditions for a 6-week period, both reactors 1 and 2 showed a similar increase in bromate supernatant concentration over the first four weeks, rising from 0.7 mg L^{-1} and 0.8 mg L^{-1} to 10.8 mg L^{-1} and 11.9 mg L^{-1} respectively. Over the same period biomass readings dropped in reactor 1 from 0.130 to 0.039

g dry wt L^{-1} and in reactor 2 from 0.086 to 0.039 g dry wt L^{-1}. Figure 7.38 indicates this decrease, and also shows biomass concentrations during the entirety of phase III. Nitrate reduction remained stable with ≤ 3.9 mg L^{-1} supernatant concentrations in both reactors, but sulphate reduction decreased over the period with no observed reduction after four weeks. It is possible these alterations were due to use of an 'old' influent water which had been left un-refrigerated for a period of 35 days, leading to an increase in nitrite concentrations within the influent supply from 0.01 mg L^{-1} to 36.4 mg L^{-1}. The comparable influent nitrite increase noted during phase IIa (Section 7.2.1.2) did not appear to affect results in the same way, but reactors were not under steady conditions at the time so any effect would not be obvious. The phase III nitrite increase resulted in no effect on nitrate reduction whilst bromate reduction was decreased. This alteration in bromate reducing ability was sustained even following replacement of the influent supply, with reduction remaining stable over the following two-week period (10.2 – 16.3 mg L^{-1}). Given the similar response in both reactors, it is possible a slight perturbation in microbial composition occurred in response to the high-nitrite influent. This new balance of species may have subsequently stabilised thus causing the observed effects. However, for the purposes of this trial both reactors showed comparable operational characteristics after the 6-week period, which allowed use during phase IIIa as a test and control system.

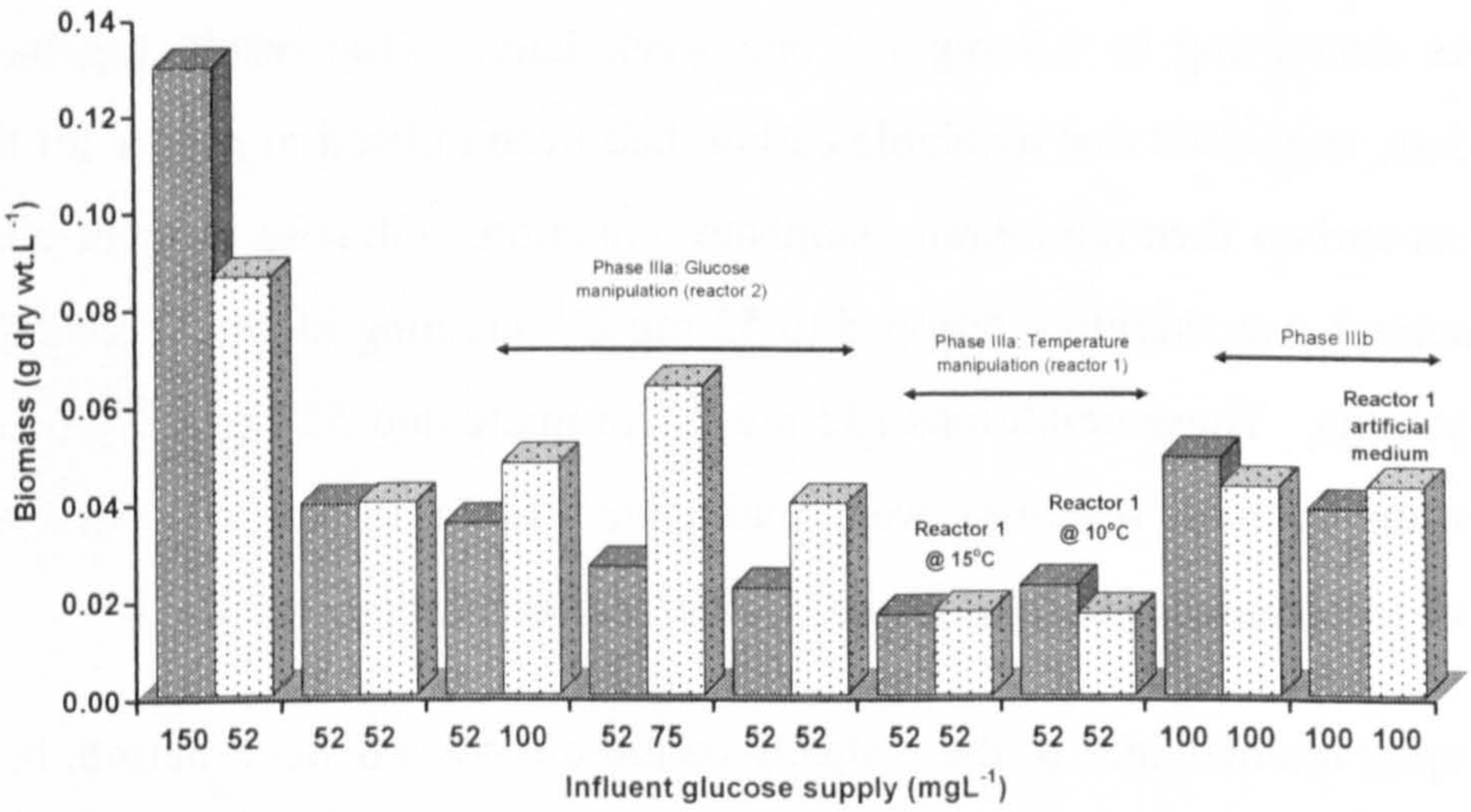


Figure 7.38 - Phase III biomass concentrations within reactor 1 (grey bars) and reactor 2 (white bars). Average influent biomass concentration of 0.002 g dry wt L^{-1}

A. Phase IIIa – Glucose and temperature manipulation

Steady state readings for influent glucose concentrations of 52, 75 and 100 mg L^{-1} were evaluated on a test and control basis. An increase in glucose supply from 52 to 100 mg L^{-1} precipitated a concurrent biomass increase in the test reactor from 0.039 to 0.047 g dry wt L^{-1}, although the subsequent glucose decrease from 100 to 75 mg L^{-1} was not reflected in biomass concentrations, with a further increase to 0.063 g dry wt L^{-1} noted (Figure 7.38). Following the subsequent drop back to 52 mg L^{-1} glucose influent, biomass concentrations did fall to the starting value of 0.039 g dry wt L^{-1}, confirming that carbon was a limiting factor to the overall microbial population at both 52 and 75 mg L^{-1} glucose influent supply. Over the same period, control reactor biomass fell slightly from 0.039 to 0.022 g dry wt L^{-1}. Soluble TOC utilisation was comparable in both control and test reactors with 52 mg L^{-1} glucose influent, with an average utilisation of 53.3 ± 4.8 %. With the higher (100 and 75 mg L^{-1}) glucose concentrations TOC usage was enhanced, with utilisation of 75.2% of the 100 mg L^{-1} influent and 76.0% of the 75 mg L^{-1} glucose supply. TOC concentrations (mg L^{-1} as glucose equivalent) are shown in Figure 7.39.

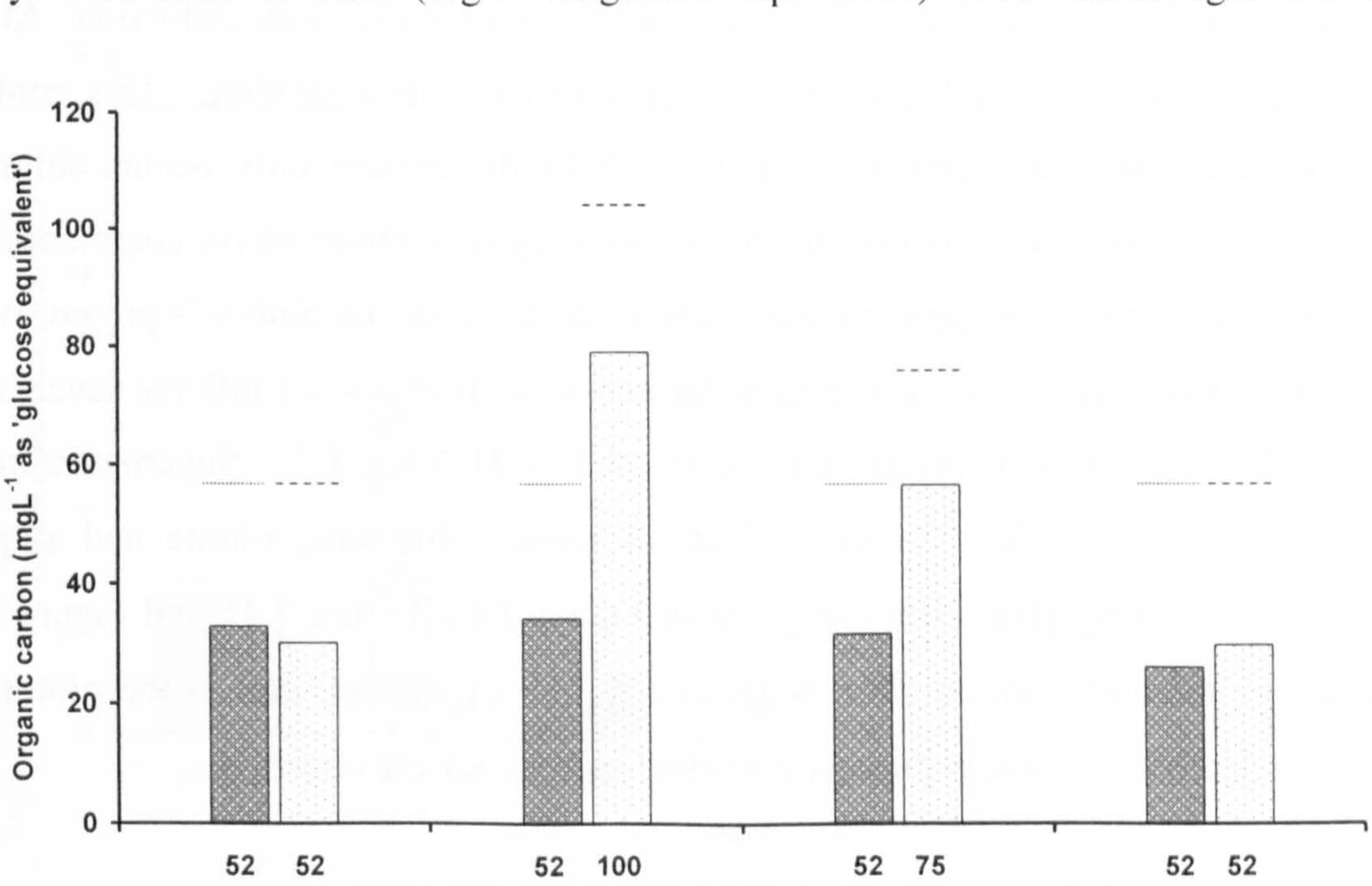


Figure 7.39 - Soluble TOC consumption in reactor 1 (grey bars) and reactor 2 (white bars) expressed as mg L^{-1} glucose with manipulation of influent glucose concentration in reactor 2 during phase IIIa. Influent glucose to reactor 1 (----) and reactor 2 (— — —) also indicated

Supernatant bromate concentrations within the control reactor were within the range 13.0 – 17.7 mg L^{-1}. Control nitrate concentrations fluctuated within the range 0.2 – 6.0 mg L^{-1}, with little sulphate reduction noted (supernatant concentrations of 51.0 – 55.4 mg L^{-1}). Within the test reactor, supernatant bromate concentrations were initially similar to the control (14.4 mg L^{-1}), but these fell with the glucose influent increase, with only 0.6 mg L^{-1} and 0.2 mg L^{-1} observed at the 100 mg L^{-1} and 75 mg L^{-1} glucose concentrations respectively. Further glucose decrease back to the 52 mg L^{-1} concentration led to an increase in supernatant bromate concentration, but reduction was still enhanced over that within the control reactor (supernatant concentration of 8.6 mg L^{-1} compared with 16.4 mg L^{-1}). Sulphate reduction was also enhanced with glucose increase. No reduction was noted at the 52 mg L^{-1} glucose concentration, but decreases in sulphate concentration to 32.5 mg L^{-1} and 45.6 mg L^{-1} were apparent with 100 mg L^{-1} and 75 mg L^{-1} glucose influents respectively. This trial clearly shows that bromate reduction was being limited by glucose influent (either in the form of glucose or glucose fermentation products) at the 52 mg L^{-1} influent concentration, but at both the higher glucose dosing levels sufficient carbon was available for total bromate reduction. At both these levels excess carbon led to the initiation of sulphate reduction. This confirms earlier observations that sulphate reduction within the system only occurs following almost total bromate reduction but, due to the short lag time observed for commencement of sulphate reduction, it is apparent that latent sulphate reduction ability is present within the culture. Bromide supernatant concentrations were in excess of influent levels at all times, with concentrations within the range 18.5 – 31.3 mg L^{-1}. Supernatant nitrite concentrations were below 0.4 mg L^{-1} at all times. Bromate, nitrate and sulphate concentrations during phase IIIa are given in Figure 7.40, Figure 7.41 and Figure 7.42. Increases in sulphate concentration suggested by the figures are due to the plotting of averaged influent data, and in practice no actual increases were observed.

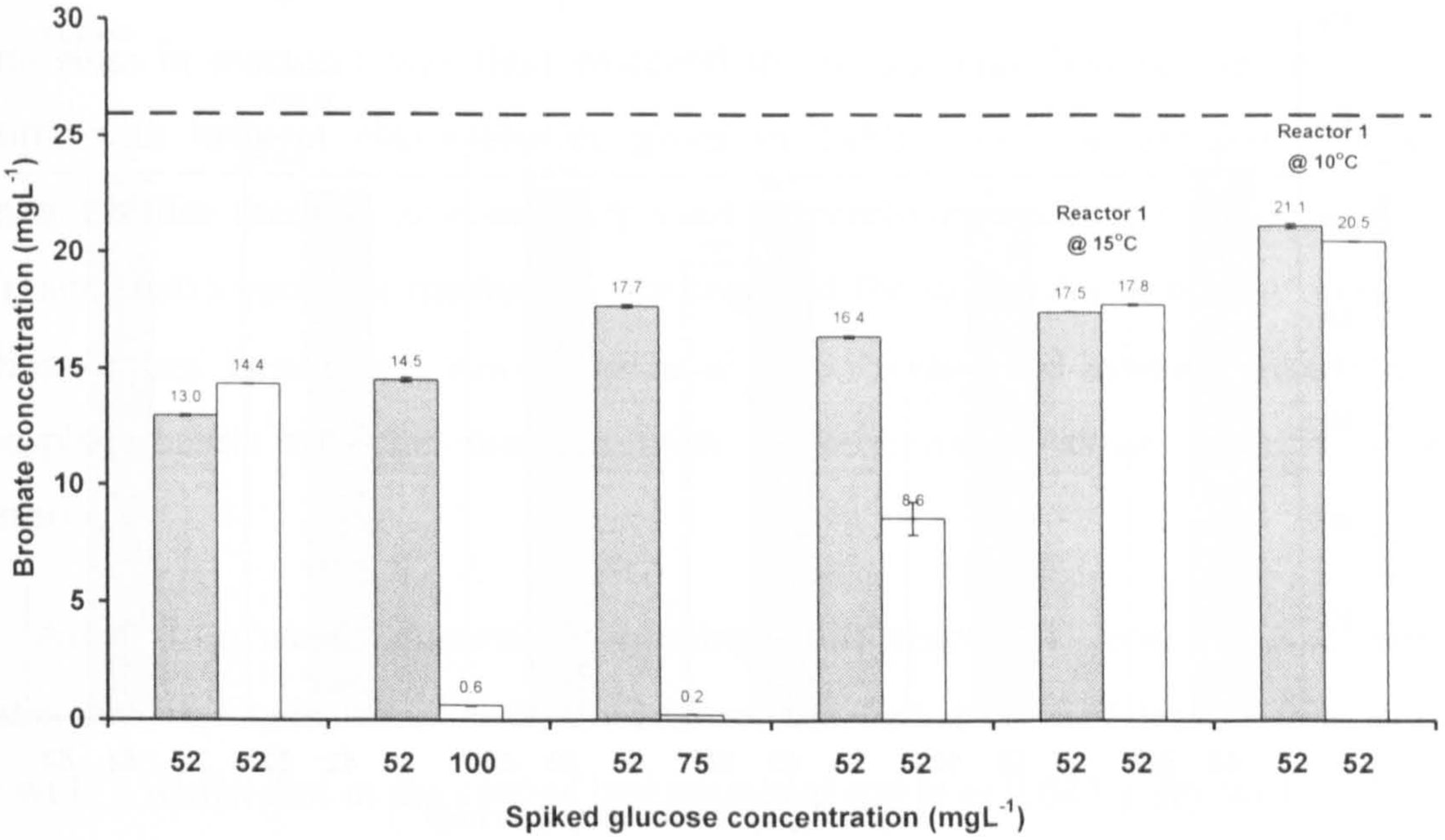


Figure 7.40 - Supernatant bromate concentrations in reactor 1 (grey bars) and reactor 2 (white bars) during phase IIIa chemostat studies. Average influent concentration also indicated (— — —)

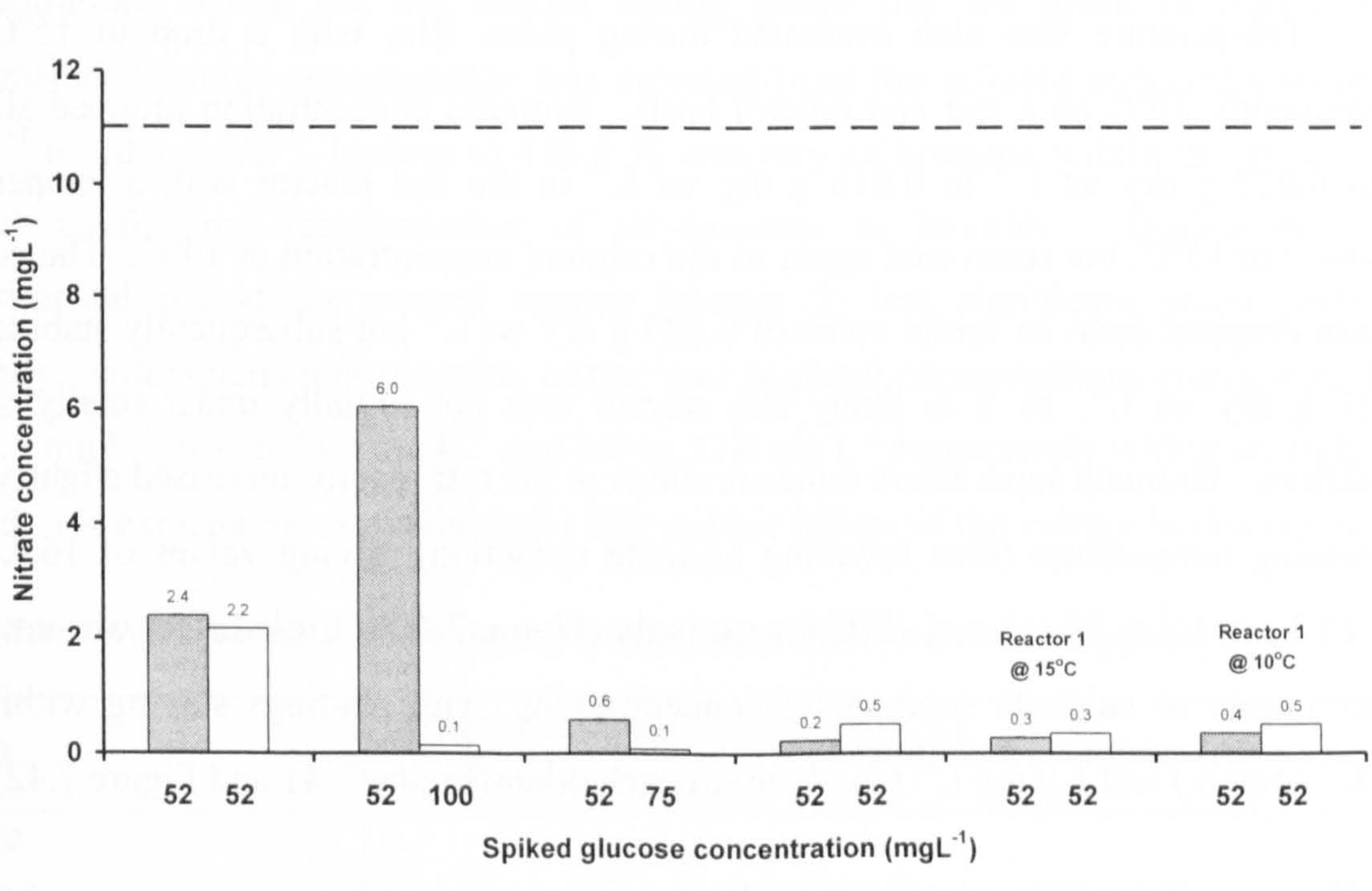


Figure 7.41 - Supernatant nitrate concentrations in reactor 1 (grey bars) and reactor 2 (white bars) during phase IIIa chemostat studies. Average influent concentration also indicated (— — —)

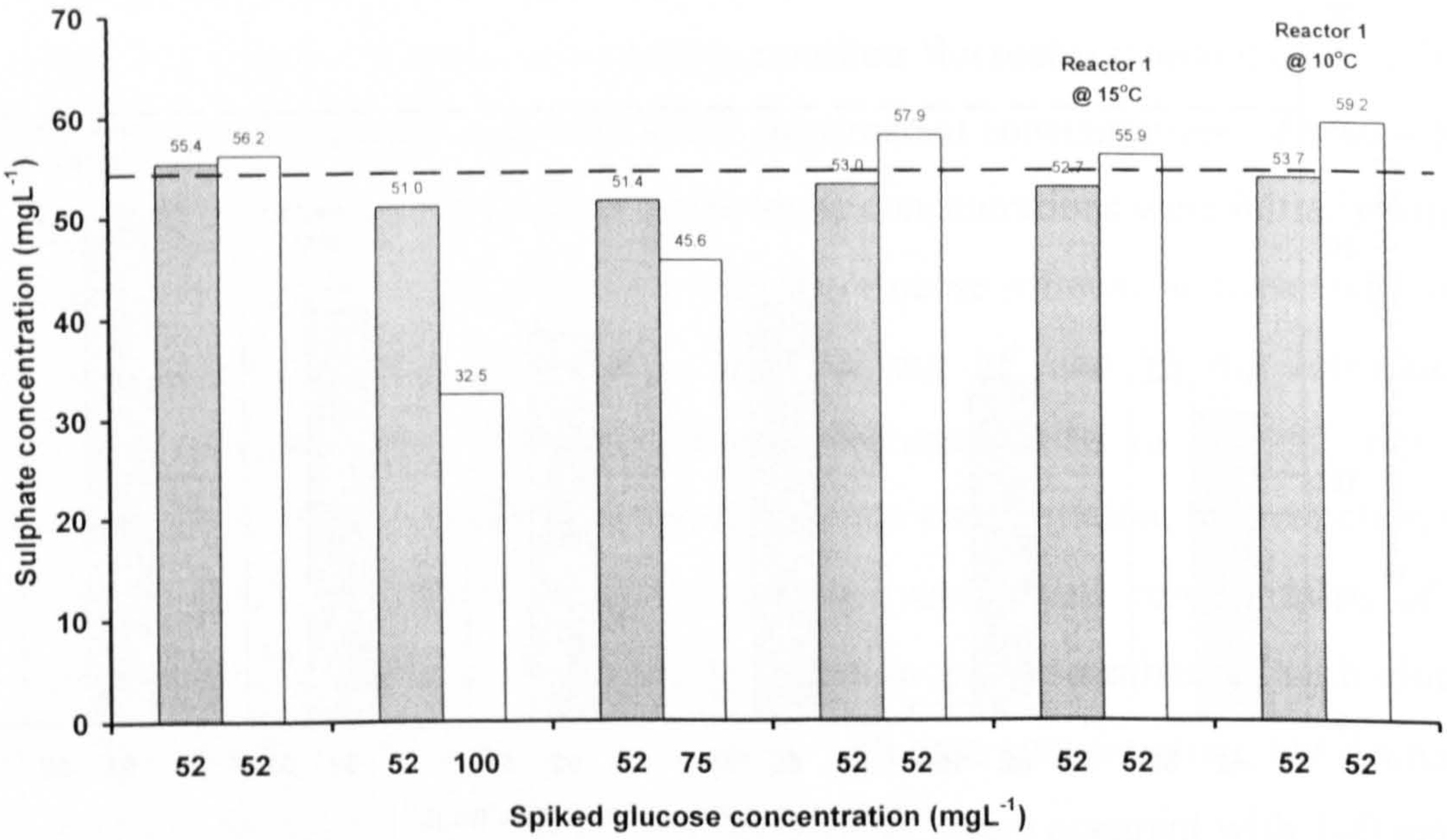


Figure 7.42 - Supernatant sulphate concentrations in reactor 1 (grey bars) and reactor 2 (white bars) during phase IIIa chemostat studies. Average influent concentration also indicated (– – –)

Temperature was also evaluated during phase IIIa, with a drop to 15°C and subsequently 10°C on a test and control basis. Biomass concentration dropped slightly from 0.022 g dry wt L^{-1} to 0.016 g dry wt L^{-1} in the test reactor with a temperature decrease to 15°C, but recovered again to the original concentration at 10°C. The control reactor dropped from an initial value of 0.039 g dry wt L^{-1} but subsequently stabilised at 0.017 g dry wt L^{-1}, so it is likely this reactor was not initially under totally steady conditions. Bromate supernatant concentrations in the test reactor increased slightly with decreasing temperature (thus lowering bromate reduction), giving values of 16.4, 17.5 and 21.1 mg L^{-1} at 20, 15 and 10°C respectively (Figure 7.40). Little effect was noted on either nitrate or sulphate supernatant concentrations, with readings staying within 0.2 mg L^{-1} (nitrate) and 1.0 mg L^{-1} (sulphate) of each other (Figure 7.41 and Figure 7.42).

B. Phase IIIb – Artificial medium

Following completion of phase IIIa trials, both reactors were operated at 20°C with 100 mg L^{-1} glucose for four weeks to enhance and stabilise bromate reduction prior to use of artificial medium. At the end of this period, both reactors showed good bromate

removal, with 0.3 mg L^{-1} and 0.7 mg L^{-1} remaining in supernatants 1 and 2 respectively. The medium in reactor 1 was then switched to the artificial (low nitrate and sulphate) medium, with influent characteristics given in Table 7.11. It is reported a simple phosphate buffer medium is commonly used in perchlorate reduction trials (Xu *et al.*, 2003), and that a complex medium is not required for successful culture of perchlorate reducing strains. In addition, van Ginkel *et al.* (2005b) observed bromate reduction using a phosphate-based artificial medium with no addition of other potential electron acceptors.

After four weeks continuous running with reactor 1 receiving the artificial medium, biomass concentration in the test reactor had decreased from 0.049 to 0.038 g dry wt L^{-1}, whilst that in the control had remained stable at 0.043 g dry wt L^{-1}. Bromate concentration in the test reactor gradually increased over the 4-week period, but only 6.7 mg L^{-1} still remained, showing that significant bromate reduction was occurring in the absence of spiked nitrate and sulphate within the artificial medium. Bromate concentrations within the test reactor during phase IIIb are given in Figure 7.43. Supernatant bromide concentration was elevated from the influent concentration of 1.5 mg L^{-1} to 20.5 mg L^{-1}, leading to 110.9 % recovery of bromine within the system and further confirming transformation of all bromate to bromide. During this final experimental period the control reactor (reactor 2) lost significant anion reduction capacity, with supernatant bromate, nitrate and sulphate concentrations rising from 0.01 to 23.9 mg L^{-1}, 0.1 to 23.4 mg L^{-1} and 1.9 to 52.6 mg L^{-1} respectively within an eight-day period. No explanation is available for this sudden failure of the culture in this reactor.

Table 7.11 - Average influent anion concentrations for artificial medium during phase IIIb

Anion	Average measured influent concentration (mg L^{-1})	Number of samples (n)
Nitrate	3.6	1
Nitrite*	0.06	3
Sulphate	3.1 ± 0.4	3
Bromate	33.2 ± 4.9	3
Bromide	1.5 ± 1.4	2

* All readings below the limit of detection (0.06 mg L^{-1})

stayed constant during this period (253.2 – 253.8 μmol N g dry wt^{-1} hr^{-1}). Bromate reduction rate and specific reduction rate reduced by 43% and 59% respectively (211.6 to 119.9 μg L^{-1} BrO_3 hr^{-1} and 101.5 to 42.0 μmol Br g dry wt^{-1} hr^{-1}), suggesting a temperature sensitivity to bromate reduction. However, the control reactor rates also decreased (33% and 32%) so it is possible some of this decrease can be explained by external parameters. Relative molar ratios were higher in both test and control reactors than during the glucose manipulations, with a qN/qBr value of 2.6 at 15°C and 4.6 at 20°C reflecting the declining bromate reduction rate. Phase IIIa reduction and specific reduction rates are summarised in Table 7.12.

During phase IIIb, nitrate reduction and specific reduction rates were low compared with control values (89.6 μg L^{-1} NO_3 hr^{-1} and 33.1 μmol N g dry wt^{-1} hr^{-1} compared with 247.0 μg L^{-1} NO_3 hr^{-1} and 53.8 μmol N g dry wt^{-1} hr^{-1}), but this was mainly due to the low-nitrate influent supply. Bromate reduction rate in both test and control reactors was comparable to that observed in phase IIIa at similar glucose concentrations (range 631.5 – 713.5 μg L^{-1} BrO_3 hr^{-1}), with no rate decrease following use of artificial medium. Specific rate increased within the test reactor from 105.0 to 120.9 μmol Br g dry wt^{-1} hr^{-1}, reflecting the decrease in biomass concentration. Phase IIIb reduction and specific reduction rates are summarised in Table 7.13.

Table 7.12 - Bromate and nitrate reduction/specific reduction rates during phase IIIa chemostat trials (Temperature - 20°C; RT - 40 hours). Values for control reactor given in brackets.

Glucose in feed ($mg\ L^{-1}$)	Reactor temperature (°C)	Reduction rates		Specific reduction rates		qN/qBr
		Nitrate ($\mu g\ L^{-1}\ NO_3\ hr^{-1}$)	Bromate ($\mu g\ L^{-1}\ BrO_3\ hr^{-1}$)	qN ($\mu mol\ N\ g\ dry\ wt^{-1}\ hr^{-1}$)	qBr $\mu mol\ Br\ g\ dry\ wt^{-1}\ hr^{-1}$)	
Glucose manipulation						
52 (52)	20	221.6 (215.8)	289.0 (323.3)	110.6 (99.5)	70.0 (72.2)	1.6 (1.2)
100	20	272.1	632.1	92.8	104.5	0.9
75 (52)	20	273.5 (260.8)	641.7 (205.3)	70.0 (161.8)	79.7 (61.7)	0.9 (2.6)
52 (52)	20	263.0 (270.1)	433.2 (237.2)	108.8 (200.8)	86.9 (85.5)	1.3 (2.4)
Temperature manipulation						
52	15 (20)	268.8 (266.9)	211.6 (203.6)	266.0 (253.2)	101.5 (115.6)	2.6 (2.7)
52	10 (20)	266.8 (262.8)	119.9 (135.8)	192.9 (253.8)	42.0 (63.6)	4.6 (4.0)

Table 7.13 - Bromate and nitrate reduction/specific reduction rates during phase IIIb chemostat trials (Temperature - 20°C; RT - 40 hours; Influent glucose concentration – 100 mg L^{-1}). Values for control reactor given in brackets.

Conditions	Reduction rates		Specific reduction rates		qN/qBr
	Nitrate ($\mu g\ L^{-1}\ NO_3\ hr^{-1}$)	Bromate ($\mu g\ L^{-1}\ BrO_3\ hr^{-1}$)	qN ($\mu mol\ N\ g\ dry\ wt^{-1}\ hr^{-1}$)	qBr ($\mu mol\ Br\ g\ dry\ wt^{-1}\ hr^{-1}$)	
Prior to artificial medium in test reactor	n/d	640.4 (631.5)	n/d	105.0 (115.6)	n/d
Artificial medium in test reactor	89.6 (247.0)	676.0 (713.5)	33.1 (53.8)	120.9 (56.2)	0.3 (1.0)

n/d – no data

7.2.2 Groundwater biomass isolation and maintenance

Initial plating was completed with M-R2A plates using either glucose or pyruvate as carbon source, and a presence (25 mg L^{-1} or 250 mg L^{-1}) or absence of bromate. It was anticipated that colonies from the bromate-containing plates which were not visible on those lacking bromate might be strains that had benefited from bromate addition. Growth was observed after 8 weeks of incubation on all M-R2A plates inoculated with supernatant collected directly from either the chemostat or pilot-scale bioreactors, with colonies visible both in the presence and absence of bromate. In practice it was difficult to discern differences in colony morphology by eye between the two types of plate at this stage, so a range of colonies derived from the bromate plates were subcultured onto both types of medium over the course of the project. The aim of this subculturing was to gradually remove interfering strains, thus facilitating identification of potential bromate reducing colonies. In this manner a number of colonies were identified, and were later subjected to examination for bromate reducing capabilities whilst in liquid batch culture (Section 7.2.3.5). Representative examples of colonies obtained are given in Figure 7.44. Names of colonies identified, their basic colony morphology and origin are summarised in Table 7.14. An attempt has been made at classifying colonies by size which, although a generally subjective measurement due to the range of colony sizes on a single plate, was guided by the size ranges outlined at the foot of the table.

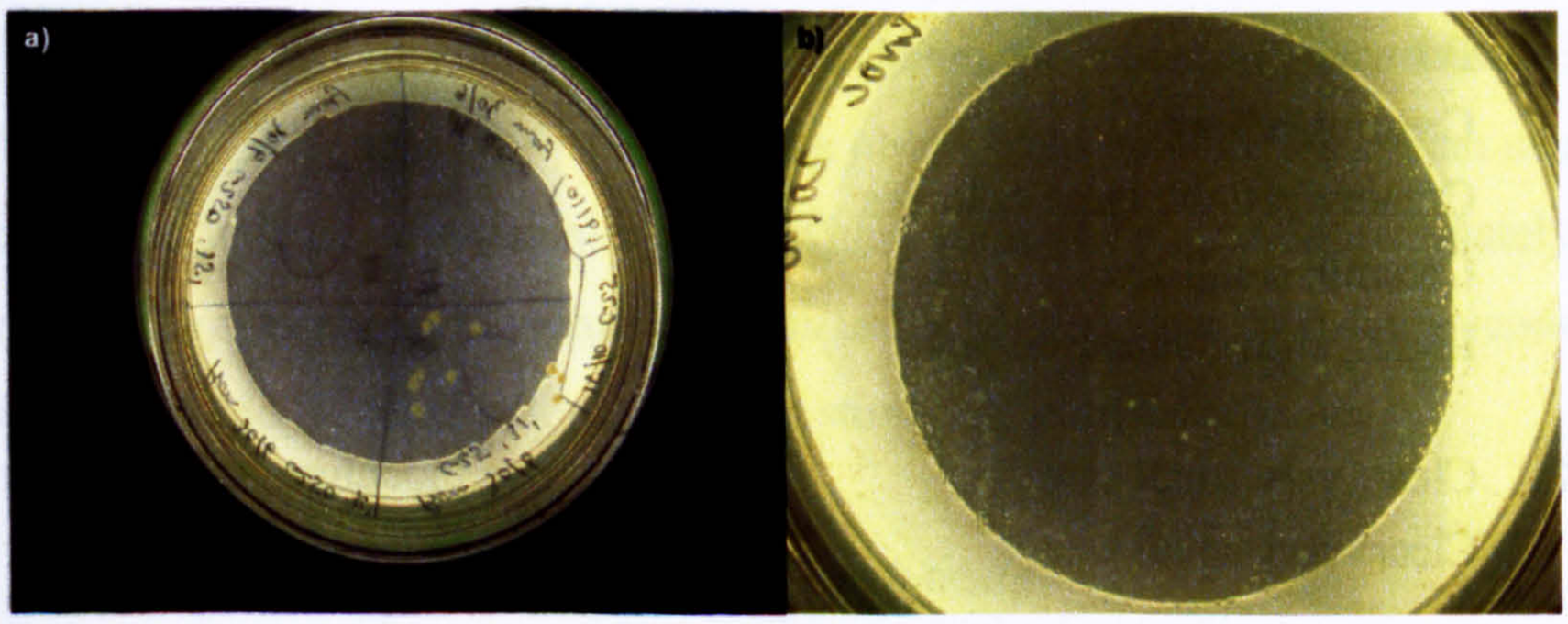


Figure 7.44 - Representative examples of a) large (lower right of plate) and b) small colonies on agar plates during isolation trials

Table 7.14 - Morphological summary of colonies isolated from chemostat liquid culture

Isolate name	Appearance on M-R2A agar (colony size* and colour)	Chemostat reactor origin	Notes
17	Clear; smear on plate	1	Subcultured from batch flasks
31	Creamy; small size	2	
32	Orange/red	1	
33	Creamy	2	
34	Bright yellow	1	
35	Off-white; small size	2	
36	Off-white; very small size	2	
37	Orange/red; large size	2	
38	White; very small size	2	
39	Light yellow; smear on plate	2	
40	White; large size	2	
41	Creamy; small size	1	
A – D	Off-white; small size	2	
E – G	Light yellow; small size	2	
H	Off-white; small size	2	
M – N	Brown	1	
O – P	Off-white; small size	2	
Q – U	Off-white; smear on plate	1	
V – W	Bright yellow	2	
X	Brown	1	
A1 – B1	White; small size	1	
C1	Brown	1	
D1	Creamy yellow	2	
E1	Creamy yellow; large colonies	2	
F1	Creamy; small size	2	
G1 – H1	Light yellow; smear on plate	2	
I1 – K1	Clear; very small size	1	Inoculum from dilution series (Section 6.4.3.5)
L1 – N1	Clear; very small size	2	
S1	Clear; very small size	1	Inoculum from batch incubation of colonies 32 (S1) and 35 (T1)
T1	Clear; very small size	2	

* very small = < 0.5 mm; small = 0.5 – 2 .0 mm; large = > 2 mm; smear = no distinct colonies visible within general growth

No consistent difference in colony morphology or growth success was observed between the use of glucose and pyruvate as carbon source, and both were utilised throughout the project period to maximise possibilities of isolating bromate reducers. No consistent difference between the two bromate concentrations was noted, but the higher (250 mg L^{-1}) concentration occasionally showed a tendency towards either no significant growth or growth of a small number of larger colonies, which were subcultured as bromate tolerance would be a suggestive feature of bromate reducing ability. On one occasion, light yellow colonies subcultured from plates containing the lower (25 mg L^{-1}) bromate concentration were observed to grow on equivalent plates but not on those containing 250 mg L^{-1}. On a separate occasion, an off-white colony subcultured from the high bromate concentration failed to grow on either control or 25 mg L^{-1} bromate plates, but did form colonies on a 250 mg L^{-1} plate. This promising colony was subcultured as colony 'K' but, along with colonies 'I', 'J' and 'L', was unfortunately lost due to contamination during subsequent incubation prior to investigation by batch culture. All other colonies were put into batch culture. Colonies X, 31, 35 and A1 – T1 were also plated onto ART+ and ART- plates to investigate growth in the absence of nitrate and sulphate. However growth on this medium was poor even in the presence of nitrate and sulphate (ART+) with little difference between the ART+ and ART- plates noted. It was concluded this medium was not able to support strains of interest without modification of the composition, and this line of investigation was not therefore further pursued.

Agar stabs were inoculated using direct chemostat inoculum and colonies 31, 33 and 35, on the M-R2A agar type. Growth was observed to be more rapid than using the plate culture technique, with colonies noted within some stabs after approximately three weeks. Growth was apparent within all stabs using M-R2A agar, and it was concluded this technique could be suitable for short-term storage of purified cultures.

7.2.3 Groundwater bioremediation batch trials

Batch trials were completed within sealed 100 mL conical flasks to allow rapid investigation of parameters and other factors potentially determining efficacy of bromate groundwater bioremediation.

7.2.3.1 Parameter assessment

Batch trial A was completed to investigate effects of initial bromate concentration on bromate reduction, and confirm results observed during chemostat phase I. After 35 days incubation, nitrate concentrations in all flasks had decreased to ≤ 0.2 mg L^{-1}, with nitrite results largely below the limit of detection (0.06 mg L^{-1}). Bromate concentration at the highest (100 mg L^{-1}) initial concentration showed no significant decrease (Mann-Whitney, $p = 1.000$) even after 35 days of incubation. A bromide increase of 4 mg L^{-1} was however observed, suggesting that, as with the chemostat system, high concentrations may lead to small decreases being masked during analysis. Decreases in bromate concentration were observed in both lower initial bromate concentrations even after 4 days incubation, with remaining concentrations after 35 days of 2.3 ± 2.6 mg L^{-1} and below the limit of detection (0.01 mg L^{-1}) for the 10 mg L^{-1} and 2 mg L^{-1} spikes respectively. Corresponding bromide increases of 5.8 and 4.4 mg L^{-1} respectively were noted. Calculated bromine recoveries over 35 days for the 100, 10 and 2 mg L^{-1} bromate spikes were 104.2%, 102.7% and 103.9% respectively. Sulphate reduction did not occur at the 100 mg L^{-1} bromate concentration, but at the lower bromate concentrations reduction did occur, with 17.5 mg L^{-1} removed at the 10 mg L^{-1} bromate concentration and almost the entire initial sulphate content of 41.0 ± 0.2 mg L^{-1} removed leaving only 0.7 ± 0.5 mg L^{-1} after 35 days with the 2 mg L^{-1} bromate spike. Anion values during trial A are given in Table 7.15.

Trial B compared three carbon sources for efficacy at stimulating bromate reduction. After 35 days, nitrate reduction was noted in all cases to ≤ 0.8 mg L^{-1}, with nitrite largely to the limit of detection. Bromide production was also noted in all cases, within the range 2.8 – 5.8 mg L^{-1}. Glucose was the most effective carbon source for bromate reduction, with 2.3 ± 2.6 mg L^{-1} bromate remaining after 35 days. Acetate and ethanol reduced the initial 10.6 – 10.7 mg L^{-1} bromate spike to 7.3 ± 0.2 mg L^{-1} and 5.4 ± 0.5 mg L^{-1} respectively. Having been acclimatised to glucose within the chemostat system, the culture may however be expected to utilise this carbon source more effectively. Calculated bromine recoveries over 35 days for glucose, acetate and ethanol treatments were 102.7%, 104.0% and 105.6% respectively. Only glucose stimulated

sulphate reduction (17.5 mg L^{-1}), although only in the presence of a low (2.3 mg L^{-1}) bromate concentration after 35 days incubation. A small apparent increase in sulphate concentration ($\leq$ 5.7 mg L^{-1}) was noted with the other two carbon sources. Anion values during trial B are given in Table 7.16.

Table 7.15 - Batch trial A: Effect of bromate concentration on bromate reduction

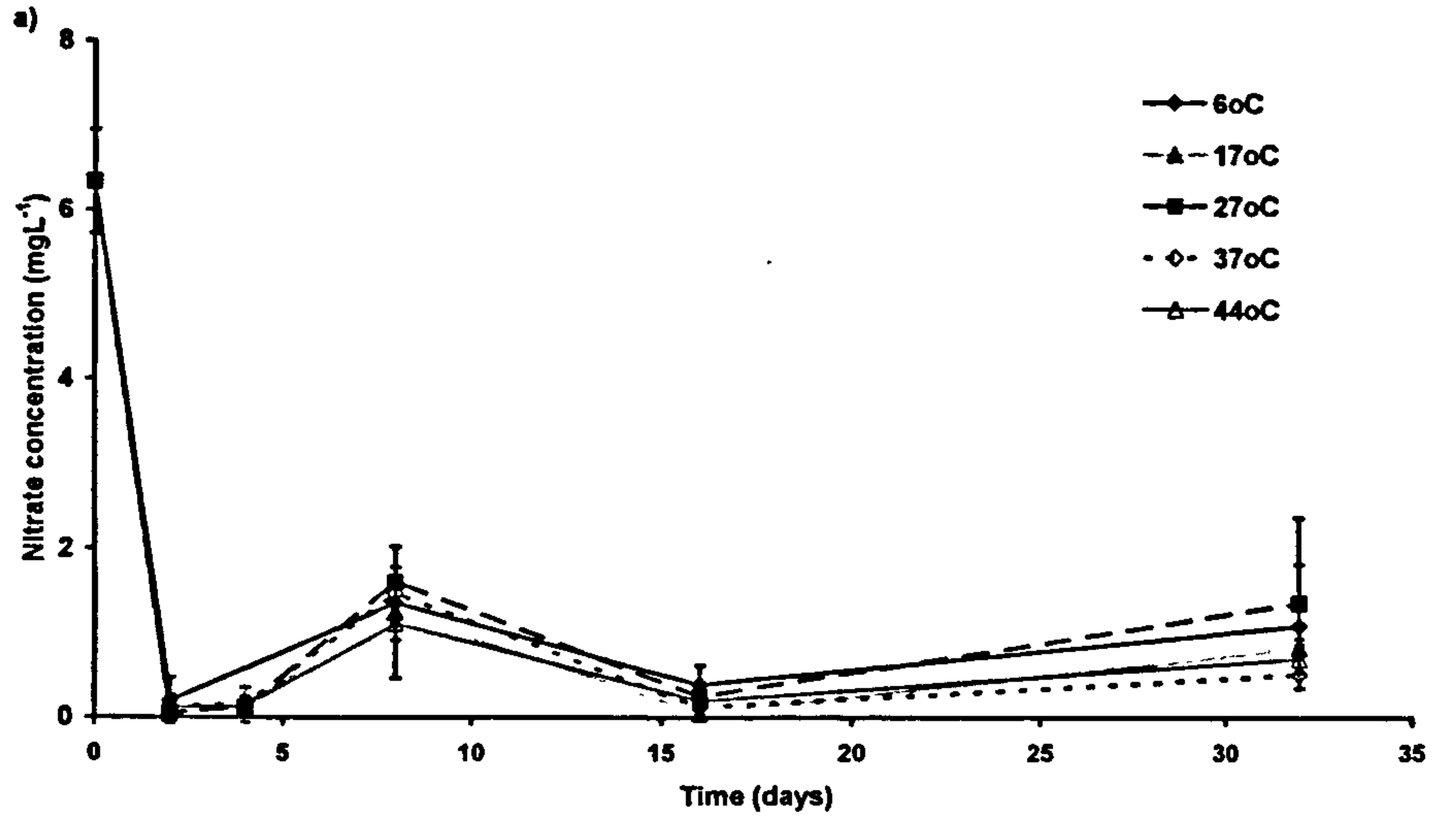
Nominal initial bromate (mg L^{-1})	Time (Days)	Bromate (mg L^{-1})	Bromide (mg L^{-1})	Nitrate (mg L^{-1})	Nitrite (mg L^{-1})	Sulphate (mg L^{-1})	Significant bromate reduction? (p-values in brackets)
100	0	99.6 ± 0.06	12.7 ± 0.04	8.2 ± 0.04	≤ 0.06	43.9 ± 0.06	
	4	97.0 ± 3.8	14.3 ± 0.06	11.4 ± 0.03	0.2 ± 0.4	44.6 ± 0.2	No (1.000)
	35	98.3 ± 3.0	16.7 ± 0.05	0.2 ± 0.2	≤ 0.06	46.5 ± 0.4	No (1.000)
10	0	10.7 ± 0.02	12.3 ± 0.006	7.0 ± 0.03	≤ 0.06	40.4 ± 0.04	
	4	8.9 ± 0.07	13.8 ± 0.09	5.9 ± 5.8	≤ 0.06	43.9 ± 0.06	Yes (0.0286)
	35	2.3 ± 2.6	18.1 ± 2.1	0.2 ± 0.2	1.2 ± 1.3	22.9 ± 23.0	Yes (0.0286)
2	0	3.3 ± 0.01	12.5 ± 0.01	7.5 ± 0.1	≤ 0.06	41.0 ± 0.2	
	4	1.3 ± 0.02	13.8 ± 0.1	5.3 ± 6.1	≤ 0.06	44.3 ± 2.7	Yes (0.0286)
	35	≤ 0.01	15.1 ± 0.01	0.06 ± 0.1	≤ 0.06	0.7 ± 0.5	Yes (0.0286)

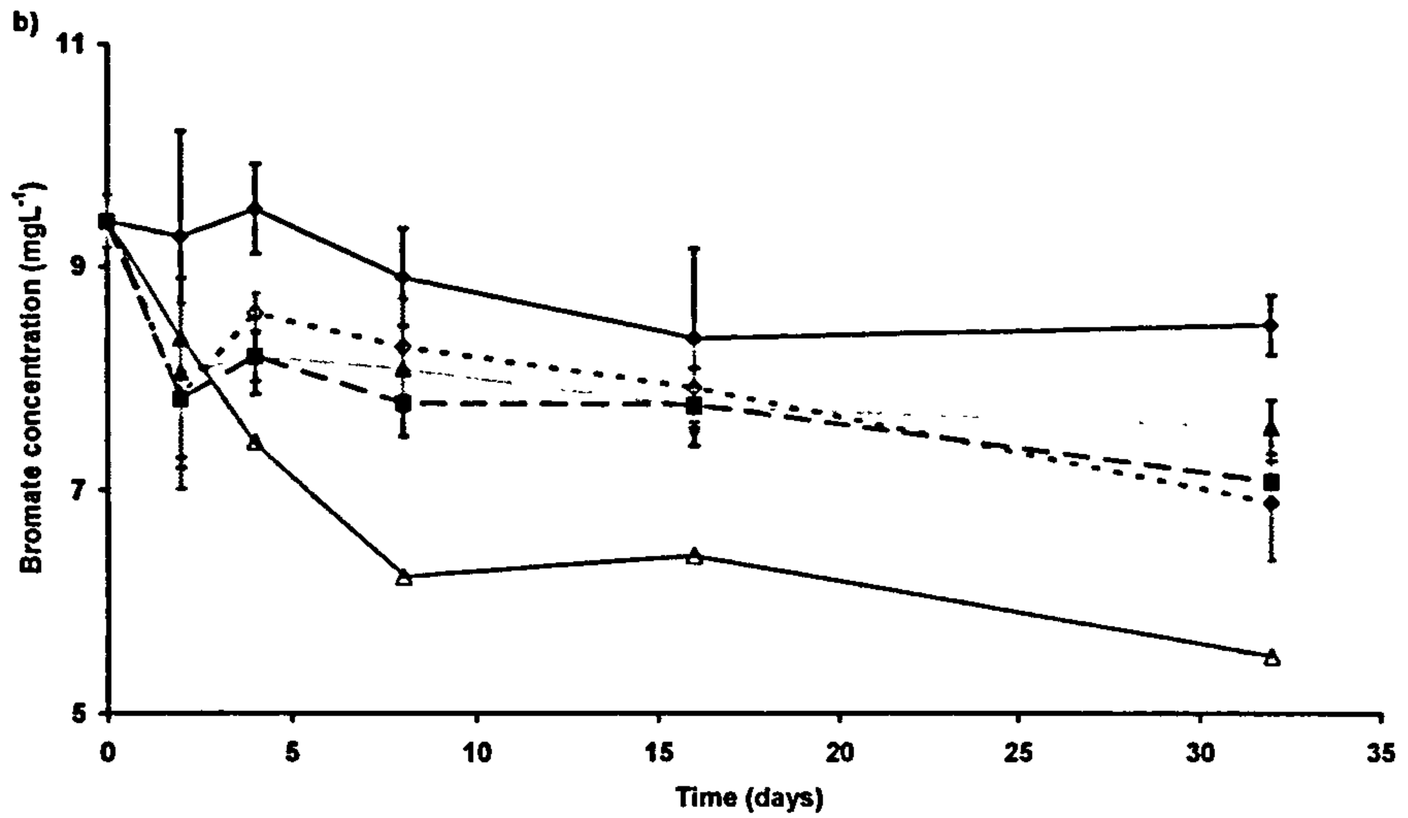
Table 7.16 - Batch trial B: Effect of carbon source on bromate reduction

Carbon source	Time (Days)	Bromate (mg L^{-1})	Bromide (mg L^{-1})	Nitrate (mg L^{-1})	Nitrite (mg L^{-1})	Sulphate (mg L^{-1})	Significant bromate reduction? (p-values in brackets)
Glucose	0	10.7 ± 0.02	12.3 ± 0.006	7.0 ± 0.03	≤ 0.06	40.4 ± 0.04	
	4	8.9 ± 0.07	13.8 ± 0.09	5.9 ± 5.8	≤ 0.06	43.9 ± 0.06	Yes (0.0286)
	35	2.3 ± 2.6	18.1 ± 2.1	0.2 ± 0.2	1.2 ± 1.3	22.9 ± 23.0	Yes (0.0286)
Acetate	0	10.6 ± 0.03	12.3 ± 0.04	7.0 ± 0.04	≤ 0.06	40.0 ± 0.09	
	4	8.6 ± 0.9	13.7 ± 0.04	2.1 ± 0.4	≤ 0.06	43.8 ± 1.2	Yes (0.0286)
	35	7.3 ± 0.2	15.1 ± 0.1	0.8 ± 0.2	≤ 0.06	45.7 ± 1.5	Yes (0.0286)
Ethanol	0	10.7 ± 0.008	12.3 ± 0.005	7.1 ± 0.05	≤ 0.06	40.1 ± 0.07	
	4	7.9 ± 0.07	14.4 ± 0.3	4.1 ± 2.0	0.1 ± 0.3	42.3 ± 0.5	Yes (0.0286)
	35	5.4 ± 0.5	16.7 ± 0.2	0.7 ± 0.05	≤ 0.06	43.2 ± 0.2	Yes (0.0286)

Trial C tracked anion concentrations over a 32-day timecourse at temperatures within the range 6 – 44°C. Nitrate removal was rapid at all temperatures, with an initial 6.3 mg L^{-1} concentration reduced to < 0.2 mg L^{-1} within the first two days at all temperatures (Figure 7.45a). After this period concentrations appeared to fluctuate and even increase slightly, with values in the range 0.5 – 1.4 mg L^{-1} after 32 days. The reason for this is not known, although Kirisits and Snoeyink (1999) observed nitrification activity in BAC filters with no external carbon addition. Ammonia was converted to nitrate under carbon limiting conditions in this trial, leading to a nitrate increase of 2.4 – 4.7 mg L^{-1} with a 20 minute RT. However, subsequent addition of a carbon source led to almost 100% reduction of 1.6 mg L^{-1} influent nitrate. Thus under the increasingly carbon limiting conditions within a batch flask it is possible a nitrifying population became more significant during latter stages of the trial, leading to the nitrate increases observed. However, ammonia concentrations were not measured so this hypothesis cannot be tested. Nitrite values were generally below the limit of detection, with a maximum of only 0.1 mg L^{-1} detected throughout the trial at all temperatures.

Bromate reduction was noted at all temperatures, but a trend was observed whereby higher temperatures resulted in more and faster bromate removal. The lowest bromate removal after 32 days was at 6°C incubation (0.9 mg L^{-1}), with the highest at 44°C (3.9 mg L^{-1}). Statistical analysis (Mann Whitney U-test) of the bromate data after 32 days showed a significant difference ($p < 0.05$) between overall bromate reduction at all pairs of temperatures apart from those of 27°C and 37°C ($p = 0.6991$). Increases in bromide concentration mirrored those for bromate reduction, with the lowest bromide increase (0.7 mg L^{-1}) at the 6°C temperature and the highest (2.3 mg L^{-1}) with incubation at 44°C. Calculated bromine recoveries over 32 days for the 6°C and 44°C temperatures were 100.4% and 99.5% respectively. Bromate concentrations over the timecourse of the trial are given in Figure 7.45b and bromide concentrations in Figure 7.45c. Sulphate concentrations did not decrease over the 32-day trial period, with the exception of one flask at the 17°C incubation temperature where analysis suggested that only < 0.1 mg L^{-1} sulphate remained.





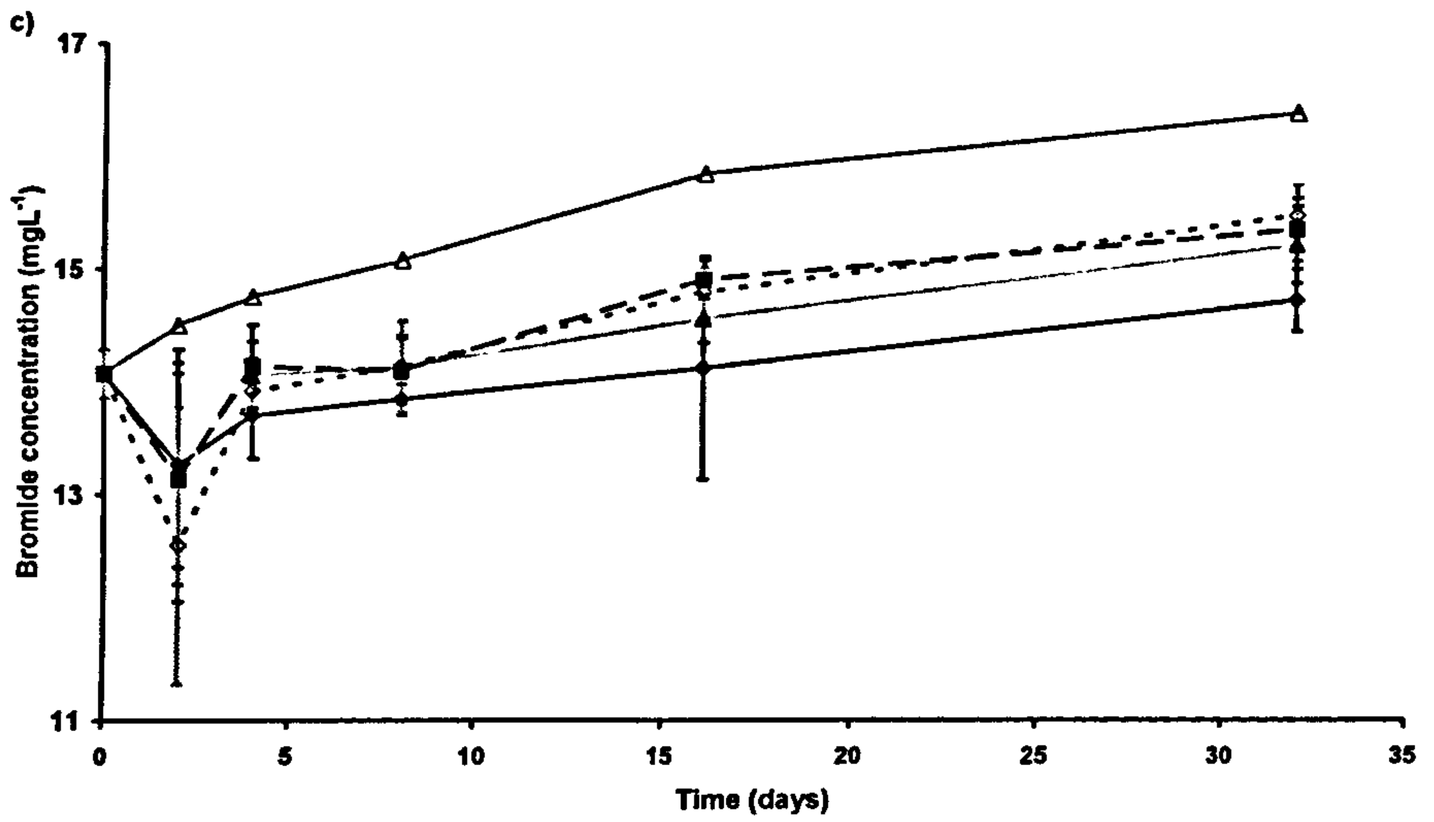


Figure 7.45 - Effect of temperature on a) nitrate, b) bromate and c) bromide concentrations during batch trial C. Error bars not shown for bromate and bromide concentrations at 44°C

7.2.3.2 Biotic versus abiotic reduction

Trial D aimed to confirm that the bromate reduction observed was biotic in nature, using filtration of influent groundwater by filters of two pore sizes (0.45 μm and 0.22 μm) in uninoculated samples to remove all bacteria from the liquid medium. Anion reduction in these flasks was then compared with unfiltered and also filtered but reinoculated samples. Removal of the majority of influent nitrate (5.8 – 6.8 mg L^{-1}) was observed within all flasks after 7 days, including those filtered and uninoculated, with a maximum concentration of only 0.7 mg L^{-1} noted after 36 days incubation. Nitrite was below the limit of detection in all cases after both 7 and 36 days incubation. Bromate reduction to the limit of detection (0.01 mg L^{-1}) was obtained both with filtered and reinoculated water and also unfiltered groundwater. Within filtered samples, small but significant (Mann-Whitney U-test; $p < 0.05$) reductions in bromate concentration (1.3 – 2.8 mg L^{-1}) were observed after 36 days. There was no significant difference in bromate reduction between the two filter pore sizes after both 7 and 36 days (Mann-Whitney; $p = 0.8182$). The lack of significant difference confirmed that use of the 0.45 μm filter in batch trials as opposed to the smaller pore size was justified. Bromide concentration did not increase with the 0.45 μm pore size, although a slight increase was noted with the 0.22 μm-filtered samples. Bromide increases of 6.2 mg L^{-1} and 4.7 mg L^{-1} were obtained with reinoculated filtered water and unfiltered groundwater respectively, indicating that bromate reduction to bromide had occurred in these samples containing groundwater bacteria. Reduction in sulphate concentration was slight ($\leq$ 1.8 mg L^{-1}) within uninoculated filtered samples, but high with both the inoculated and also unfiltered flasks (up to 56 mg L^{-1} after 36 days). Anion values during trial D are given in Table 7.17.

Batch trial D suggested that removal of microbes from House Lane groundwater almost totally halted bromate reduction, whilst reinoculation of filtered water with enrichment culture supernatant restored reduction ability. This result showed bromate reduction within groundwater was biotic in nature, with bacteria both from contaminated aquifer groundwater and the enrichment culture able to carry out these reactions. However, slight decreases in bromate concentration and also total nitrate reduction were observed during batch trial D, even in the absence of a microbial inoculum. It was speculated this could be due to use of unsterile glassware in all batch trials. Sterile techniques were not used in the current study as the major

aim was only to quantify variations in bromate reduction between different culture conditions, and not ascertain absolute reduction rates. Therefore it was possible that naturally-occurring facultative denitrifiers could be present on the glassware and be responsible for both the total nitrate and very limited bromate reduction observed.

Trial E investigated this possibility by comparing uninoculated sterile and unsterile batch flasks, all containing 0.45 μm-filtered groundwater. During the trial, analytical problems led to no reliable nitrate data for 0 and 8-day timepoints. However, almost total nitrate reduction had still occurred by day 36 in both sterile (0.2 mg L^{-1}) and unsterile (0.5 mg L^{-1}) flasks. Initial nitrate concentrations may be estimated at approximately 6 mg L^{-1} by comparison to batch trial D. Nitrite concentrations were below limits of detection in all cases. No bromate reduction was observed in the sterile flasks after 8 days, although a small (0.3 mg L^{-1}) decrease was apparent at the end of the trial. A decrease in bromate concentration of 1.2 mg L^{-1} occurred in the unsterile samples after 36 days. No bromide increase was noted in either sterile or unsterile flasks. Anion values during trial E are given in Table 7.18.

The observation of a decrease in bromate concentration even under apparently sterile conditions in batch trial E could be interpreted to suggest some form of abiotic reduction was occurring. However, a more likely explanation of this small decrease (0.3 mg L^{-1} after 36 days) may simply be that changes of this magnitude were due to the between-run analytical error of the ion chromatography methodology. Alternatively, it could suggest that microbial contamination occurred during trial setup within the sterile flasks. With recorded decreases in bromate concentration over 36 days higher under unsterile conditions (1.2 mg L^{-1}) than following sterilisation, a small amount of biotic bromate reduction may be occurring through use of unsterile equipment. Examination of other batch trials within the current study reveals a similar pattern. However, the small magnitude of this decrease is such that results of the other batch trials were not greatly affected, with any effect being equal for each treatment investigated. The occurrence of nitrate reduction within sterile flasks could also be interpreted as due to contamination by opportunistic denitrifiers within the environment although, with almost total nitrate removal occurring, this may suggest an abiotic nitrate removal mechanism. Therefore, whilst it is not thought an abiotic bromate mechanism was operating within batch flasks, further trials would be required to investigate the possibility of abiotic nitrate reduction.

Table 7.17 - Batch trial D: Effect of groundwater filtering on bromate reduction

Filter pore size	Inoculum type	Time (Days)	Bromate (mg L^{-1})	Bromide (mg L^{-1})	Nitrate (mg L^{-1})	Nitrite (mg L^{-1})	Sulphate (mg L^{-1})	Significant bromate reduction?
0.45 µm	RO water	0	9.7 ± 0.07	11.2 ± 0.2	6.0 ± 0.06	≤ 0.06	55.1 ± 0.9	
		7	9.6 ± 0.09	11.5 ± 0.1	0.01 ± 0.01	≤ 0.06	55.3 ± 0.3	No (0.1320)
		36	8.4 ± 0.5	11.0 ± 0.3	0.04 ± 0.05	≤ 0.06	53.3 ± 0.2	Yes (0.0022)
0.22 µm	RO water	0	9.2 ± 0.02	11.1 ± 0.09	5.8 ± 0.05	≤ 0.06	54.1 ± 0.2	
		7	9.2 ± 0.08	11.3 ± 0.1	0.01 ± 0.01	≤ 0.06	53.6 ± 0.4	Yes (0.0260)
		36	6.4 ± 3.0	12.2 ± 1.9	0.2 ± 0.3	≤ 0.06	53.6 ± 0.8	Yes (0.0022)
0.45 µm	Chemostat	0	11.4 ± 0.3	11.6 ± 0.1	6.8 ± 0.9	0.2 ± 0.01	57.7 ± 0.5	
		7	10.5 ± 0.3	13.0 ± 0.1	0.2 ± 0.3	≤ 0.06	57.0 ± 1.7	Yes (0.0022)
		36	≤ 0.01	17.8 ± 0.2	0.6 ± 0.3	≤ 0.06	1.7 ± 0.9	Yes (0.0022)
None	RO water	0	9.5 ± 0.08	11.2 ± 0.1	5.9 ± 0.08	≤ 0.06	54.1 ± 0.4	
		7	8.2 ± 0.2	12.1 ± 0.1	0.01 ± 0.01	≤ 0.06	53.9 ± 0.4	Yes (0.0022)
		36	≤ 0.01	15.9 ± 0.08	0.7 ± 0.9	≤ 0.06	4.1 ± 2.3	Yes (0.0022)

Table 7.18 - Batch trial E: Comparison of bromate reduction in uninoculated sterile and unsterile batch flasks containing 0.45 µm-filtered groundwater

Treatment	Time (Days)	Bromate (mg L^{-1})	Bromide (mg L^{-1})	Nitrate mg L^{-1})	Nitrite (mg L^{-1})
Sterile	0	9.8 ± 0.07	11.1 ± 0.1	n/d	≤ 0.06
	8	9.8 ± 0.3	11.3 ± 0.1	n/d	≤ 0.06
	30	9.4 ± 0.1	10.8 ± 0.04	0.2 ± 0.3	≤ 0.06
Unsterile	0	10.0 ± 0.05	11.1 ± 0.08	n/d	≤ 0.06
	8	9.5 ± 0.06	11.2 ± 0.1	n/d	≤ 0.06
	30	8.8 ± 0.06	11.0 ± 0.3	0.5 ± 0.4	≤ 0.06

7.2.3.3 Electron acceptor and donor usage

Trial F aimed to confirm order of usage for the potential electron acceptors bromate, nitrate and sulphate over a timecourse of 32 days. Figure 7.46 and Figure 7.47 show plots for flasks inoculated with inoculum from the chemostat system and the isolated strain 35 respectively. Both cultures exhibited similar bromate and nitrate reduction trends. In both cases nitrate removal to < 1 mg L^{-1} occurred within the first two days, although the chemostat culture was more rapid over the first 24 hours with 5.6 mg L^{-1} reduction. Culture 35 exhibited an initial increase in nitrate to 9.3 mg L^{-1} (Day 0 concentration of 7.6 mg L^{-1}), which may suggest the occurrence of nitrification along with slower initiation of denitrification. Total denitrification in both cases was indicated by the absence of nitrite which was consistently < 1 mg L^{-1}, apart from on day 2 with strain 35 inoculum. Bromate reduction commenced after 24 hours whilst nitrate was still present (0.4 mg L^{-1} (chemostat inoculum) and 9.3 mg L^{-1} (culture 35 inoculum)), with no lag time noted and reduction subsequently occurring in a linear fashion in both cases. Sulphate reduction was not observed in the flasks containing isolate 35 despite the reduction of bromate to the detection limit by day 32. A mixed sulphate response was noted with the chemostat inoculum, leading to a large error on day 32. One flask reduced bromate to the detection limit, with substantial sulphate reduction (49.3 mg L^{-1}) also exhibited. The other two flasks showed little sulphate reduction (0.6 – 2.1 mg L^{-1}), with 1.3 – 1.8 mg L^{-1} bromate also remaining after 32 days. This discrepancy again suggests that sulphate reduction can only occur following the removal of bromate to low levels, in this case < 1.3 mg L^{-1}. Reduction rates showed that nitrate was reduced faster than bromate. A nitrate reduction rate of 156.1 and 141.0 µg L^{-1} NO_3^- hr^{-1} for chemostat and isolate 35 respectively over the first two days was observed. Equivalent rates for bromate reduction (over the first 16 days) were 12.1 and 15.2 µg L^{-1} BrO_3^- hr^{-1}. The observation of faster bromate reduction and slower nitrate reduction rates by isolate 35 may be indicative of a culture more efficient at bromate reduction relative to denitrification, although these data are not conclusive.

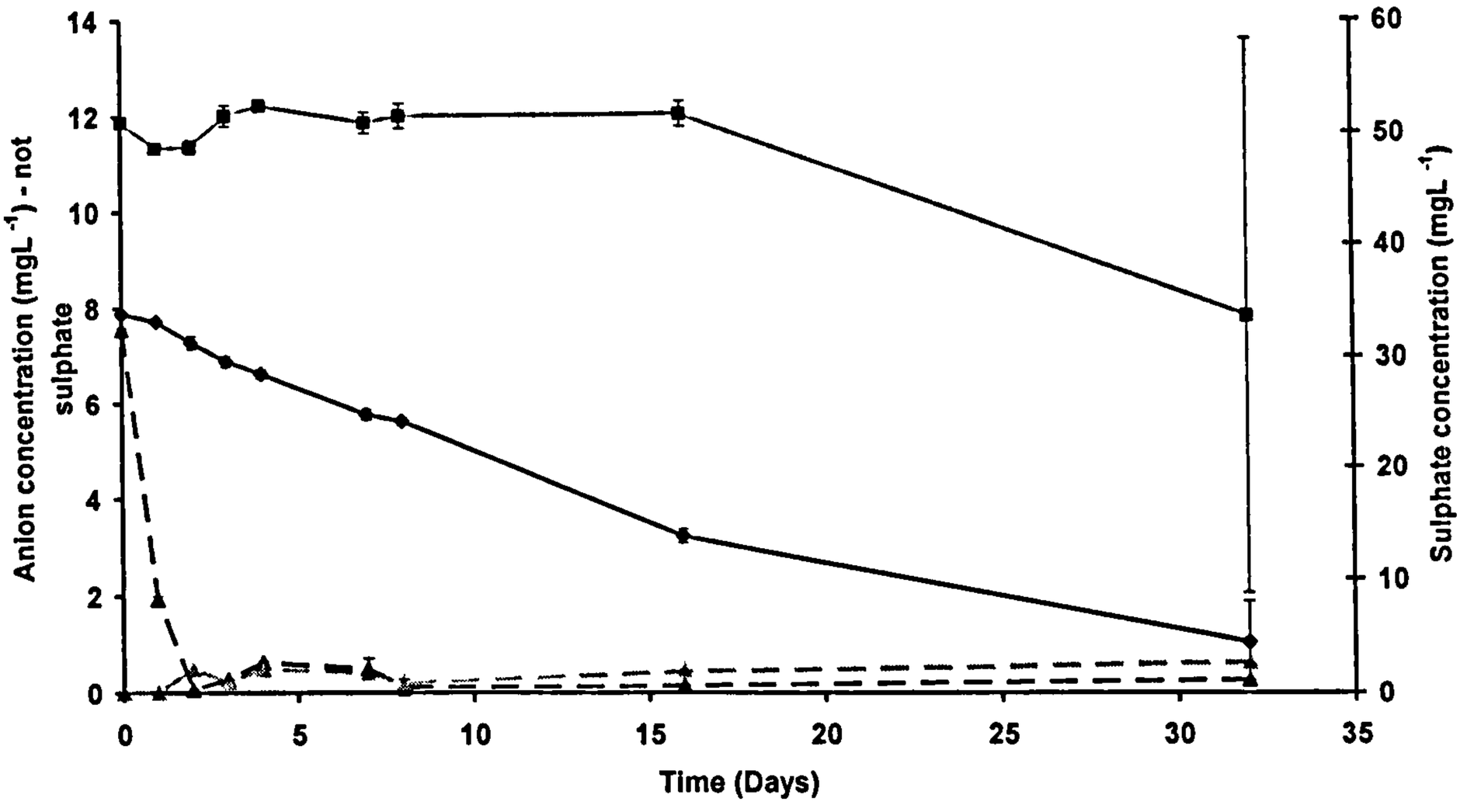


Figure 7.46 - Bromate (♦), nitrate (▲), nitrite (▲) and sulphate (■) concentrations over time during batch trial F with chemostat inoculum

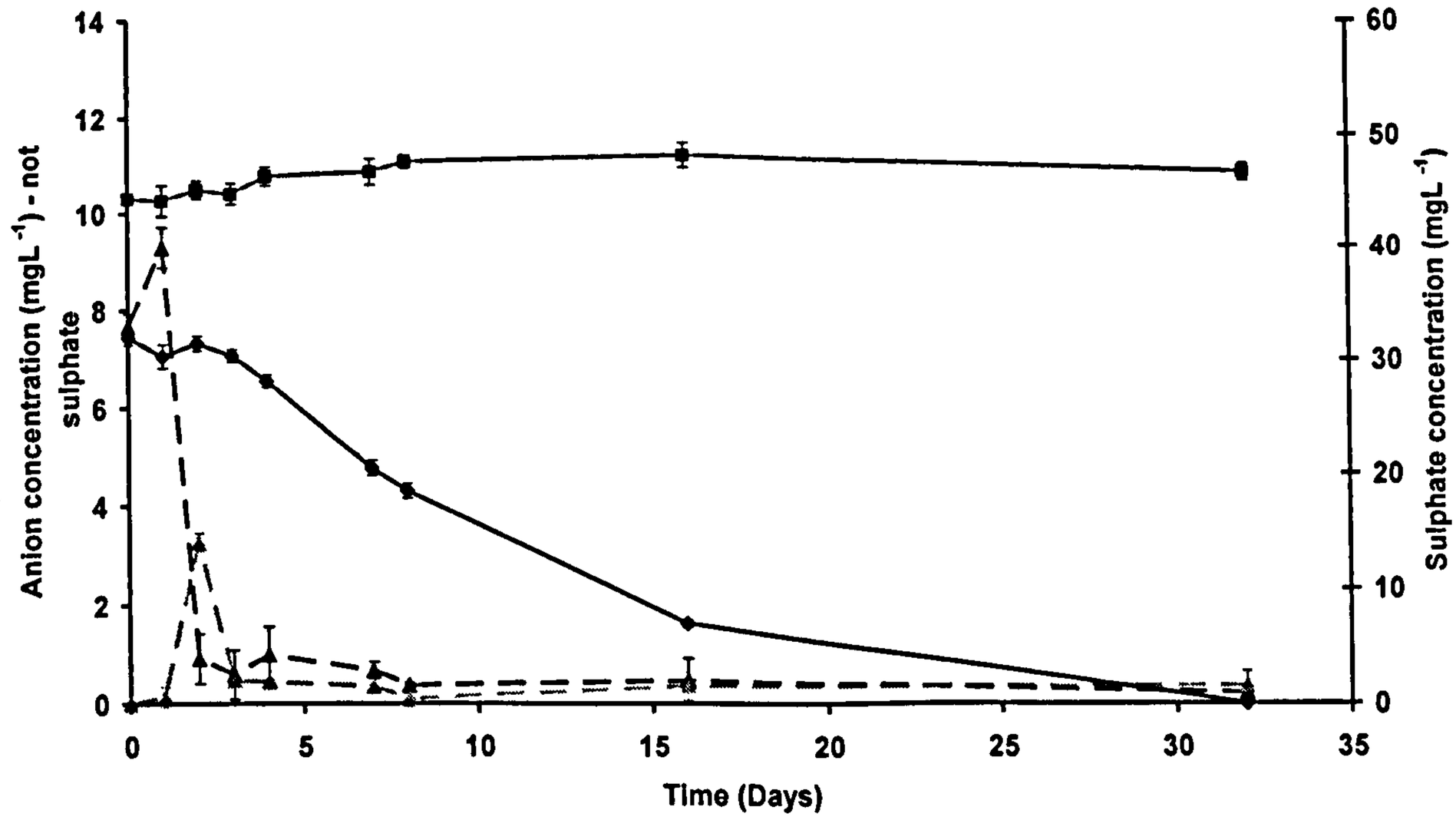


Figure 7.47 - Bromate (♦), nitrate (▲), nitrite (▲) and sulphate (■) concentrations over time during batch trial F with culture 35 inoculum

Analysis for the fermentation products lactate, ethanol and acetate was undertaken in batch trial G, and these were correlated with glucose and anion concentrations over a timecourse of 46 days. More frequent sampling than during previous batch trials showed that reduction of influent nitrate (3.9 mg L^{-1}) was largely completed (0.03 mg L^{-1}) within the first 4-hour period, leading to a reduction rate of 976 µg L^{-1} NO_3^- hr^{-1}. This again suggested the presence of abiotic nitrate reduction, as biomass only increased by 0.001 g dry wt L^{-1} during this time, indicating that sufficient biomass for denitrification had not accumulated in this short time. Nitrite was not detected at any time during the course of the trial. Bromate concentration again decreased in a linear fashion (Linear regression over the first 19 days; R-squared value of 0.962), but was slower than during trial F with a reduction rate of 7.3 µg L^{-1} BrO_3^- hr^{-1}. Control bromate concentrations decreased by only 1.2 mg L^{-1} over the 46 day period. The slower reduction rate may have been due to a less-active chemostat inoculum, which varied in efficacy over the course of the project. Calculated bromine recovery over the experimental period was 96.5%. Sulphate reduction was not observed. Anion concentrations in test and control flasks during trial G are given in Figure 7.48.

Glucose analysis revealed that all initial glucose was rapidly consumed in the test flasks, with no detectable glucose remaining after 48 hours. Biomass increased rapidly over the initial 3-day period, rising from an initial value of 0.028 g dry wt L^{-1} to 0.097 g dry wt L^{-1}. Within this period acetate and ethanol concentrations also increased rapidly, with acetate levels rising from < 30 mg L^{-1} (limit of detection) to 55.1 mg L^{-1} and ethanol from 11.5 mg L^{-1} up to 34.0 mg L^{-1}. No rise in lactate concentration was observed. Over the same time periods, minimal glucose decrease (5 mg L^{-1}) was observed in the control flask, with a detectable but small increase in biomass (0.008 g dry wt L^{-1} compared with 0.068 g dry wt L^{-1} for the test system).

Over the remaining period, acetate and ethanol concentrations decreased slightly following peaks at 19 (acetate) and 29 (ethanol) days of 56.3 ± 1.5 mg L^{-1} and 39.5 ± 0.7 mg L^{-1} respectively. After 46 days acetate and ethanol concentrations had reduced to 49.0 ± 0.5 mg L^{-1} and 31.0 ± 1.4 mg L^{-1} respectively, with lactate levels rising slightly from 2.6 ± 0.2 mg L^{-1} to 3.4 ± 0.1 mg L^{-1}. Concentrations of ethanol in the control flask

did not rise over the first few days, but instead gradually decreased from 17.0 mg L^{-1} to 10.0 mg L^{-1}. Control lactate concentrations increased from 2.4 mg L^{-1} to 3.9 mg L^{-1} over the first 19 days then decreased back to 1.9 mg L^{-1}. Control acetate concentrations did not rise above the 30 mg L^{-1} limit of detection. Average carbon and biomass concentrations in the test flasks during trial G are given in Figure 7.49. Calculated recoveries of bromine and carbon are given in Table 7.19. Following completion of the trial, bromine recoveries of 96.4% and 97.7% were obtained for test and control treatments, suggesting almost total reduction of bromate to bromide. Carbon recovery (glucose, acetate, ethanol and lactate) for test and control treatments was much lower, only 44.7% and 77.5% respectively.

This trial shows strong evidence of glucose fermentation prior to the majority of bromate reduction, which suggests that bromate metabolism does not occur in the presence of glucose but instead one or more of its potential fermentation products. Usage of carbon within batch and continuous flow systems is further explored in Chapter 8.

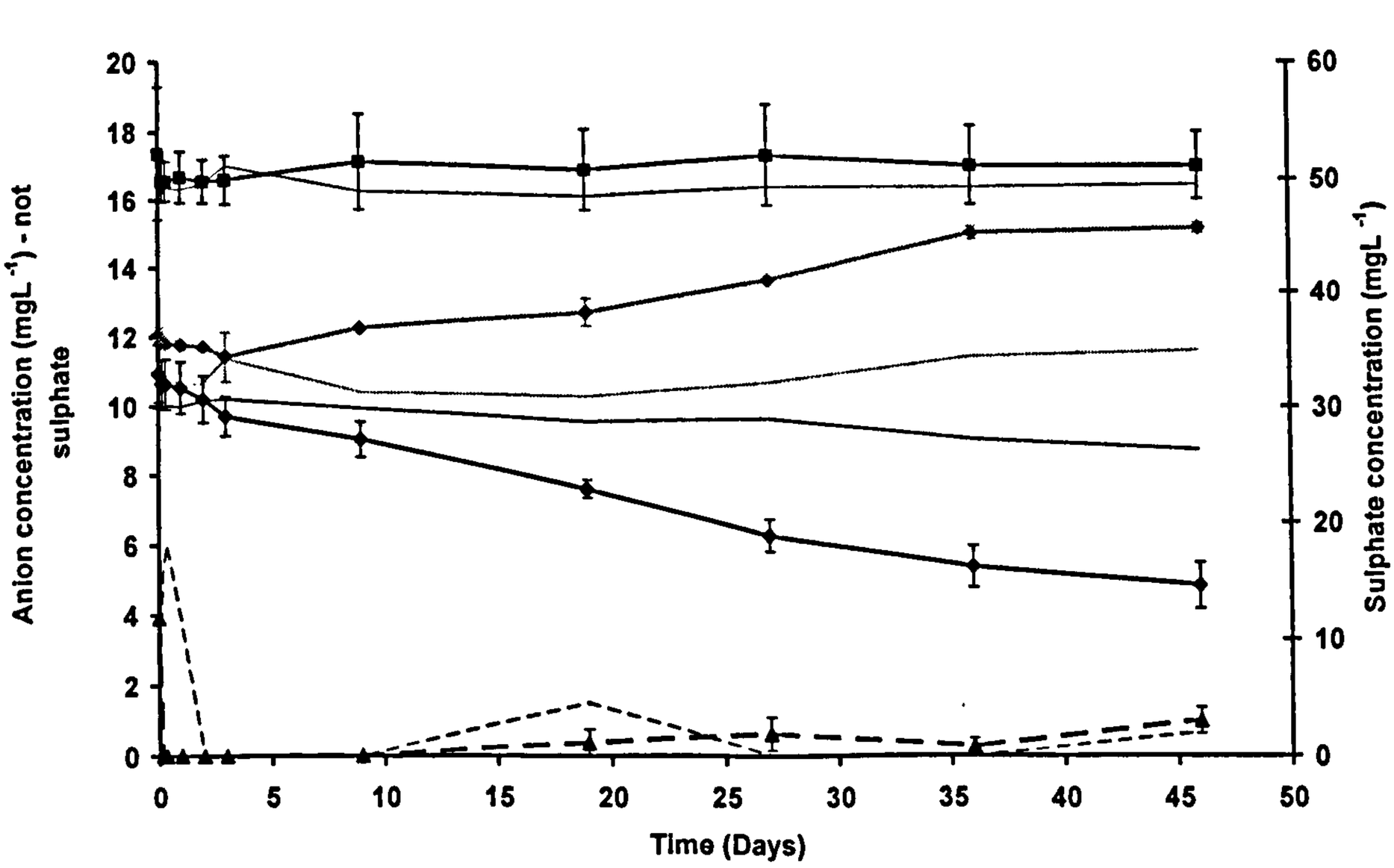


Figure 7.48 - Bromate (♦), bromide (♦), nitrate (▲) and sulphate (■) concentrations against time during batch trial G (n=4). Control values shown as corresponding narrow lines

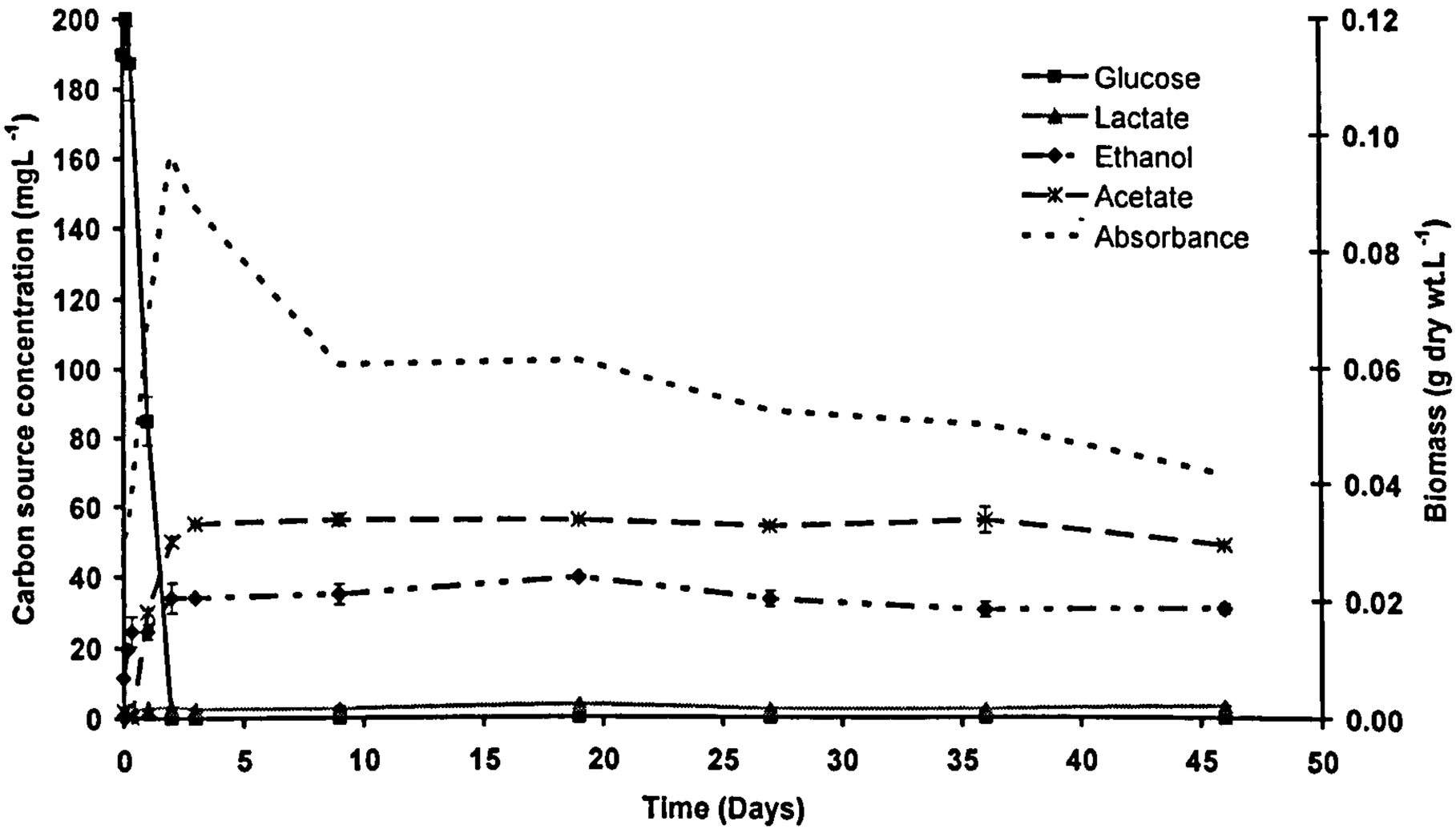


Figure 7.49 – Carbon source and biomass concentrations against time during batch trial G (n=2)

Table 7.19 - Bromine and carbon recovery during batch trial G

Time (Days)	Bromine recovery (%)		Carbon recovery (%)	
	Test	Control	Test	Control
4 hours	97.8	98.4	109.2	101.5
8 hours	97.4	98.1	106.1	102.5
1	97.0	96.3	71.6	92.7
2	95.6	97.1	46.9	95.0
3	92.4	101.4	49.1	94.2
9	94.7	94.9	50.3	93.6
19	92.3	92.8	53.4	89.1
27	93.0	95.6	48.2	78.8
36	97.4	97.7	47.3	80.5
46	96.4	97.7	44.7	77.5

7.2.3.4 Dilution-to-extinction

Dilution-to-extinction (Trial H) was trialled in tandem with agar plating trials as a

potential isolation method. Microorganisms that grow well in a mixed culture medium but are present in relatively low numbers will be selected for at lower dilutions as, at these dilutions, they are better able to outcompete those strains less well-suited to the medium but numerically abundant in the undiluted inoculum (Kirisits, 2000). Within the two dilution series attempted using chemostat inoculum, bromate reduction to ≤ 0.2 mg L^{-1} from an initial nominal 2 mg L^{-1} concentration was apparent at all dilutions after 30 days with reactor 2 inoculum (Flasks 10 – 16). However, using reactor 1 inoculum (Flasks 2 – 8) it was observed that, whilst bromate reduction was apparent at the highest 5 dilutions, at the lower two dilutions no bromate reduction over the uninoculated control occurred. It is unknown why an apparent bromate increase was seen at the lowest dilution, the most likely explanation being sample contamination. Bromate concentrations during trial H are given in Figure 7.50. Total nitrate removal to the limit of detection occurred with all dilutions.

To further examine the potentially partially-purified strains, the lowest dilutions exhibiting bromate reduction were subcultured into a further set of batch flasks (Trial I), this time containing a 10 mg L^{-1} bromate spike. In this trial, bromate reduction in excess of the uninoculated control was observed by all isolates, but reduction was superior with chemostat inoculum. However, bromate was still detectable both in flasks containing dilution series isolates (4.6 – 6.4 mg L^{-1}) and chemostat inoculum (2.8 ± 0.4 mg L^{-1}) at the end of the 36 day trial. Nitrate concentrations in all flasks at the end of the trial were < 0.5 mg L^{-1}. Bromide increases were observed in all flasks, with calculated bromine recoveries for the four isolates within the range 98.4 – 100.5%. Bromate and bromide concentrations during trial I are given in Figure 7.51.

The method of dilution-to-extinction is commonly used as a pre-treatment to further purification, possibly by agar plating. A comparable method was used by Kirisits (2000) using culture from BAC columns, although less dilutions were attempted and a long (3.5 month) incubation time was utilised. In this study nitrate was reduced to < 0.07 mg L^{-1} in all cases (initial concentration of 7.25 mg L^{-1}), and with one of the two inocula trialled all dilutions (1:1 – 1:10,000) reduced bromate to < 2.6 mg L^{-1} (initial concentration of 26.8 mg L^{-1}). The other inoculum showed partial bromate reduction

(> 2.5 mg L^{-1} bromate remaining) in all flasks apart from the undiluted sample. No plating was attempted from this trial. Plating of the four promising dilutions (Isolates 5, 6, 15 and 16) was attempted in the current study, but results in this case were inconclusive with little growth noted on any plate.

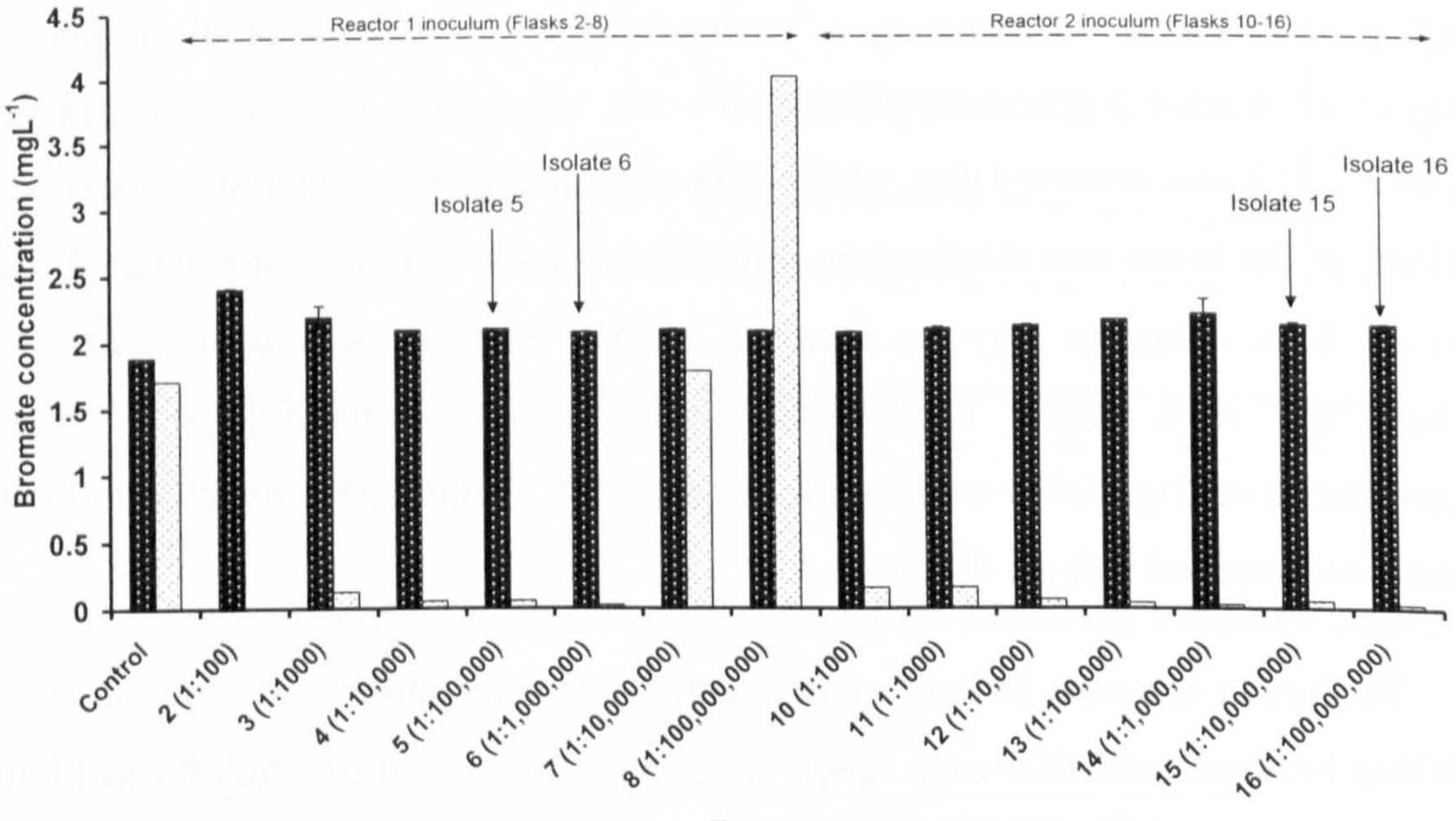


Figure 7.50 - Batch trial H: Bromate reduction with a batch dilution series. Initial (day 0) values given as black bars and final (day 30) values as light bars

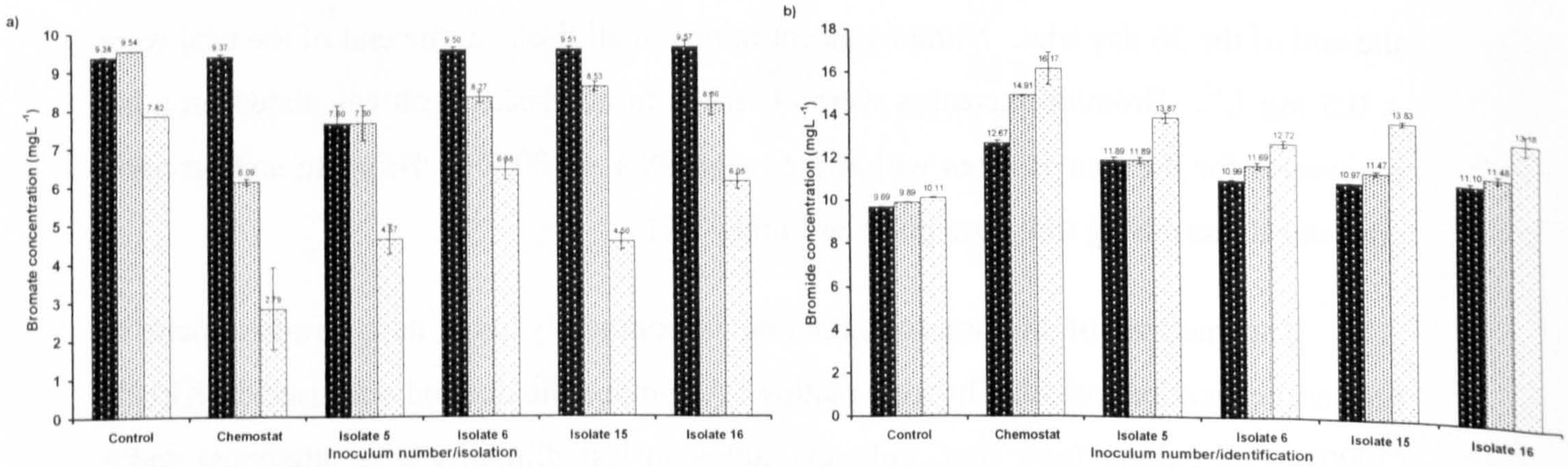


Figure 7.51 – Reduction of bromate (a) and increase in bromide (b) concentrations of dilution series isolates and controls during batch trial I. Initial (day 0) values given as black bars, day 8 as grey bars and final (day 36) values as light bars

7.2.3.5 Bromate removal by isolates

Batch trial J investigated bromate reducing ability of 10 strains (isolates 32 – 41) purified by plate culture techniques (Section 7.2.2), compared with an inoculum derived directly from chemostat culture, using a low (2 mg L^{-1}) bromate spike. Total nitrate removal to below the detection limit was obtained in all flasks after 8 days incubation, with no detectable nitrite observed at the end of the trial. Bromate reduction in excess of uninoculated control values was obtained in all cases, indicating bromate reducing ability within all the strains, with six isolates (32, 33, 35 – 38) plus the chemostat-derived inoculum (17) exhibiting reduction to the limit of detection. Corresponding bromide concentrations gave calculated percentage bromine recoveries within the range 101.0 – 104.9%. Bromate concentrations in trial J are given in Figure 7.52.

To further elucidate bromate reducing capabilities of the best-performing strains in trial J, five isolates (32, 33, 35, 36 and 39) were selected for subculture into a further set of batch flasks with a higher (10 mg L^{-1}) bromate spike (Trial K). These isolates were selected as they exhibited the fastest bromate reduction, given by the lowest remaining concentration after 8 days. In addition, culture 17 and a similar chemostat-derived inoculum (31) were also trialled. Nitrate removal to the limit of detection was observed in all flasks, with detectable nitrite levels (0.3 mg L^{-1}) apparent in only one case (inoculum 31). Bromate reduction in excess of uninoculated control values was observed in all cases, with a decrease in bromate concentration within the range 0.08 – 3.3 mg L^{-1} noted after 8 days. Three isolates (31, 33, 35) removed bromate to the limit of detection by the end of the trial, which was more than that observed for a direct chemostat inoculum where 2.8 ± 1.1 mg L^{-1} bromate remained. However, the fastest initial rate of reduction (over 8 days) was recorded for the chemostat inoculum at 17.1 μg L^{-1} hr^{-1}. Bromide concentrations increased in all cases, giving calculated percentage bromine recoveries within the range 97.2 – 100.8%. Sulphate reduction to a final value of < 3 mg L^{-1} was observed with flasks containing isolates 31, 33 and 35, with only slight decreases in initial sulphate concentration (< 3 mg L^{-1}) apparent in flasks where bromate still remained upon completion of the trial, again providing evidence for sequential reduction of bromate prior to sulphate. Bromate and bromide concentrations in trial K

are given in Figure 7.53. Isolates 33 and 35, plus the semi-purified cultures 17 and 31 were considered the most promising cultures from the results of trial K due to the exhibition of more complete bromate reduction than that observed with a direct chemostat inoculum. These isolates were therefore preserved by regular sub-culture in liquid batch flasks, and also frozen within the proprietary 'Protect' bacterial preservation system (Section 6.4.2) for later analysis in batch trial N.

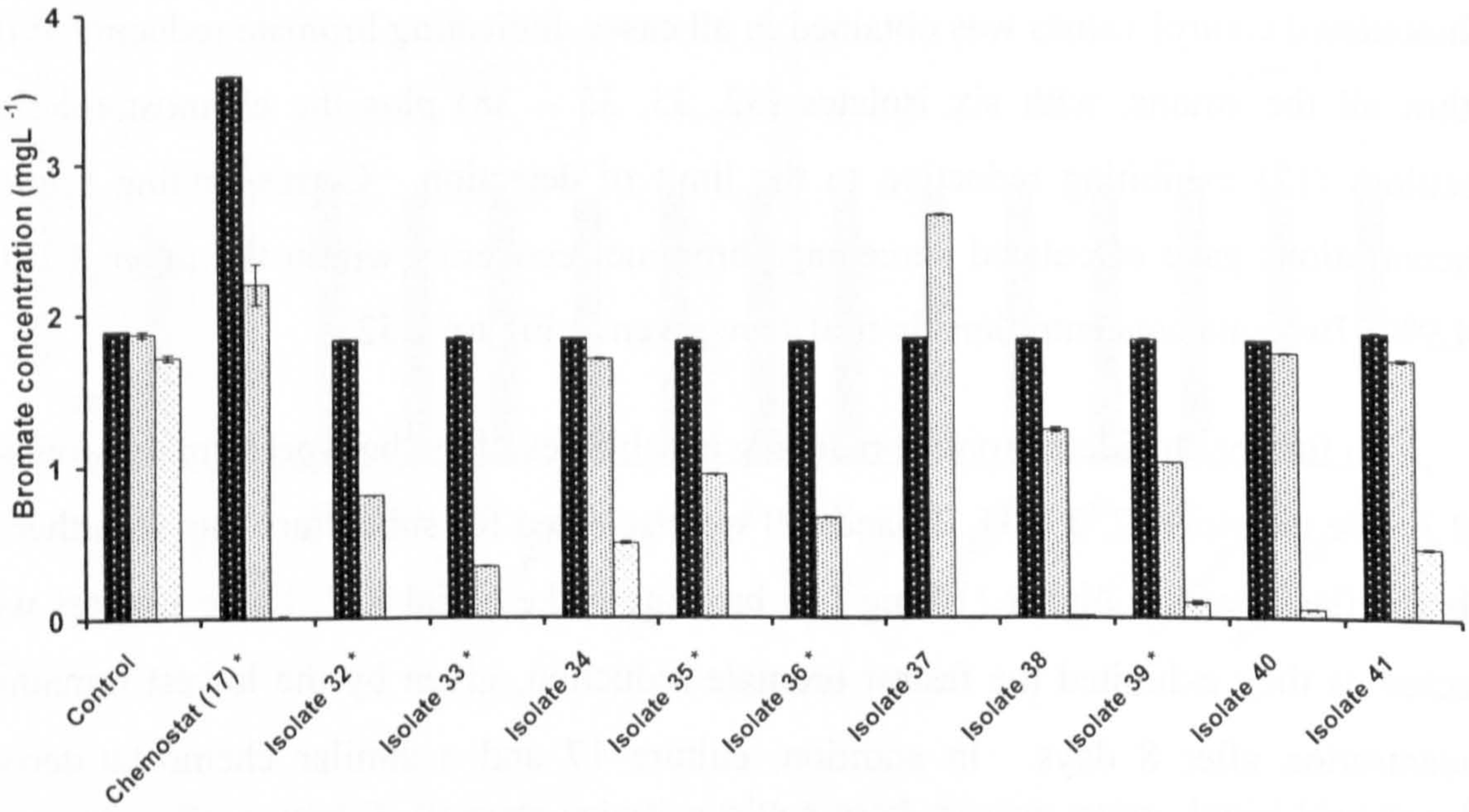


Figure 7.52 - Batch trial J: Bromate reduction of chemostat inoculum and isolated strains. Initial (day 0) values given as black bars, day 8 as grey bars and final (day 30) values as light bars

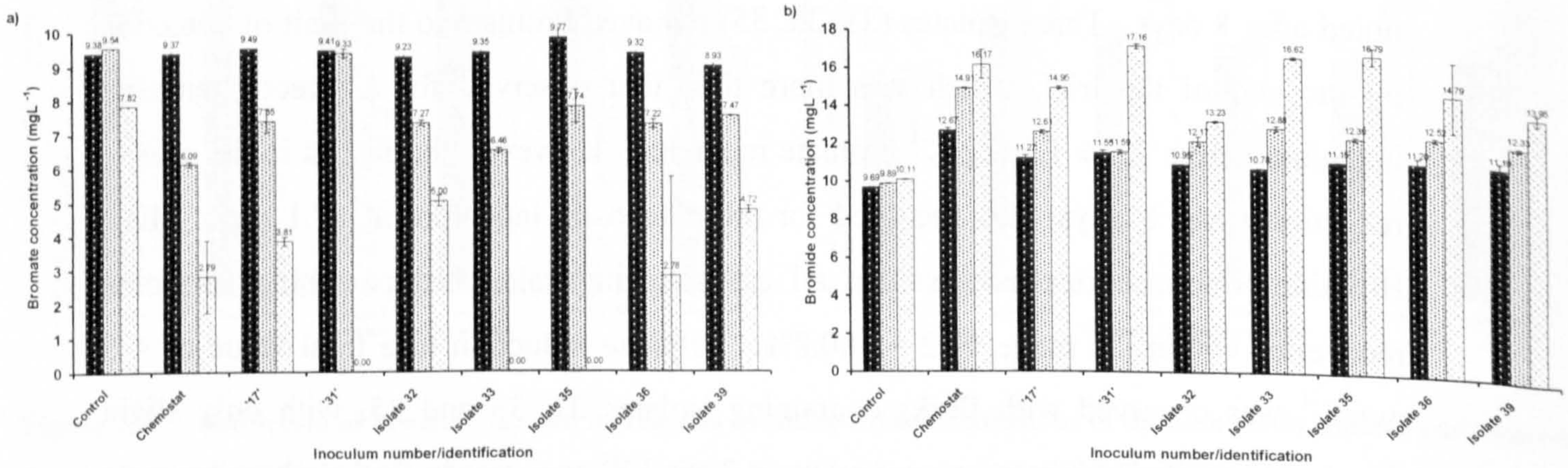


Figure 7.53 – Batch trial K: Reduction of bromate (a) and increase in bromide (b) concentrations of selected isolated str maintained from batch trial J. Initial values given as black bars, day 8 as grey bars and final values as light bars

TEXT BOUND INTO THE SPINE

Trial L examined efficacy of the artificial medium utilised in chemostat phase IIIb (Section 7.2.1.3) for culture of isolated strains and direct chemostat inoculum. Initial nitrate and sulphate levels were low but detectable, with concentrations of 0.2 mg L^{-1} and 4.2 mg L^{-1} respectively. After 30 days incubation, nitrate concentrations were below the detection limit in all cases, with sulphate levels slightly reduced by a maximum of 1.6 mg L^{-1}. Nitrite concentrations were below the limit of detection in all cases. Bromate reduction in excess of the uninoculated control was noted in all flasks. Chemostat inoculum exhibited the poorest reduction, with 2.3 mg L^{-1} bromate reduced after 30 days incubation. The highest reduction was noted for isolate 33, with a total reduction of 4.4 mg L^{-1} equating to a reduction rate of 6.0 µg L^{-1} hr^{-1}. Although bromate reduction rate was low in this trial compared with those previously (ie trial J maximum rate of 17.1 µg L^{-1} hr^{-1}), reduction rates of isolates cultured simultaneously within standard groundwater medium were similar (maximum rate of 7.5 µg L^{-1} hr^{-1}) suggesting overall culture conditions and not the medium used were responsible for the lower rates observed in this trial. Bromide concentrations increased in concert with bromate reduction, giving calculated percentage bromine recoveries within the range 94.5 – 117.2%. The highest percentage recovery was obtained for inoculum 33, with the others not exceeding 106%. Bromate and bromide concentrations in trial L are given in Figure 7.54. Bromate reduction within a low nitrate and sulphate medium and implications for possible reduction mechanisms are discussed in Chapter 8.

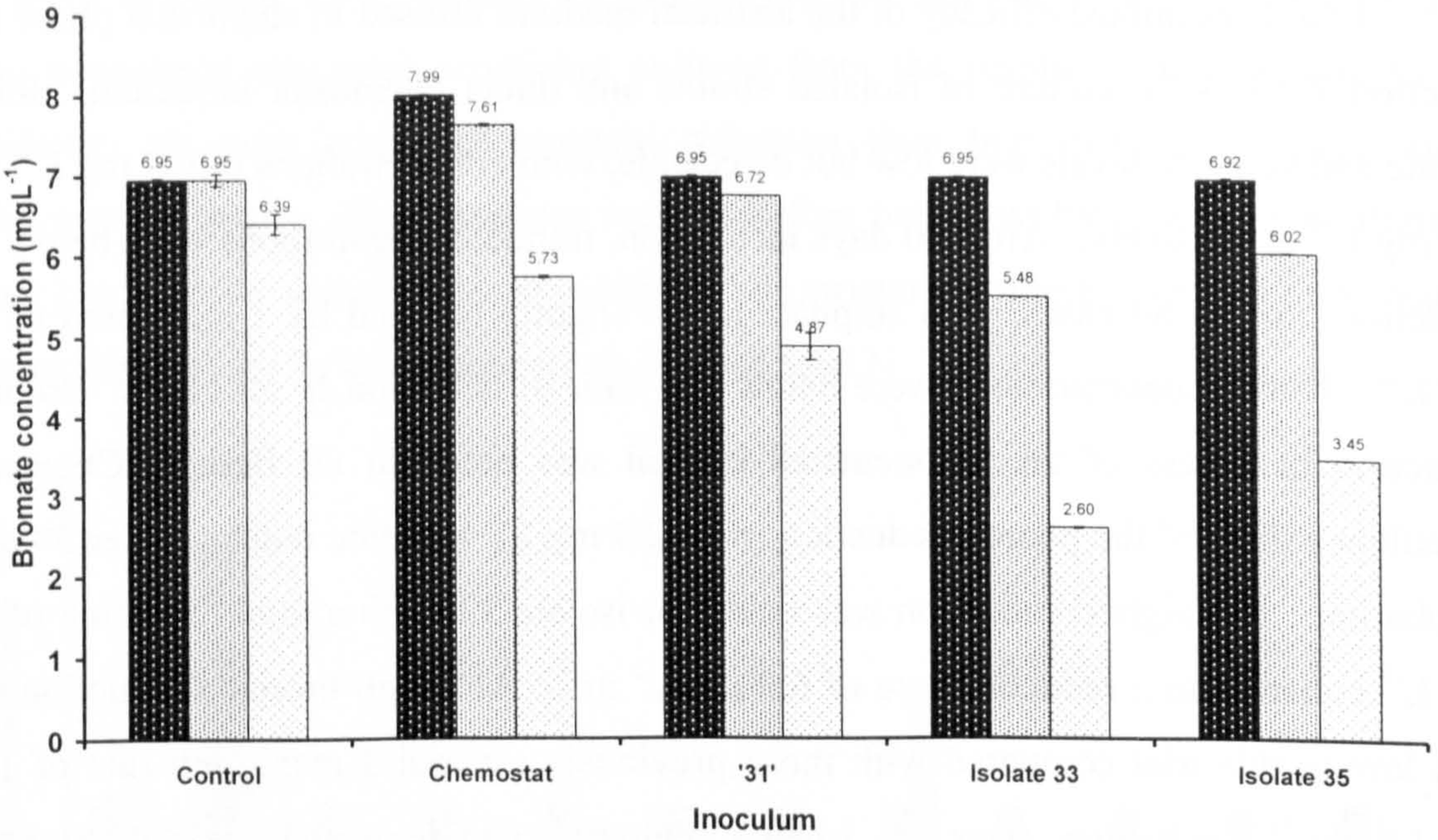


Figure 7.54 - Reduction of bromate by chemostat inoculum and selected isolated strains during batch trial L using an artificial low nitrate/sulphate medium. Initial (day 0) values given as black bars, day 8 as grey bars and final (day 30) values as light bars.

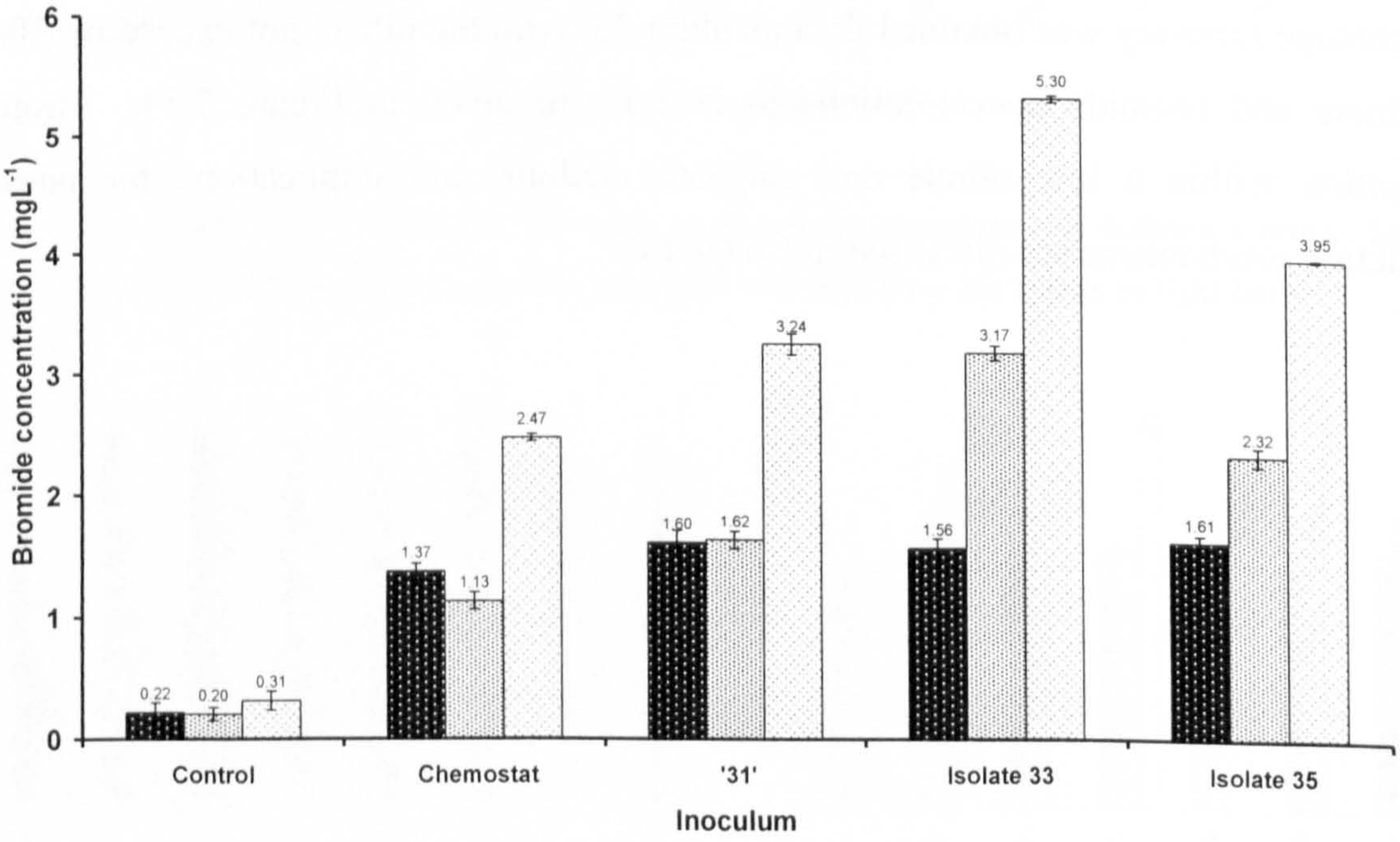


Figure 7.55 - Increase in bromide concentrations during batch trial L using an artificial low nitrate/sulphate medium. Initial (day 0) values given as black bars, day 8 as grey bars and final (day 30) values as light bars.

Bromate reduction capability of 40 strains isolated during plate culture trials (Section 7.2.2) was investigated in trial M. In all cases nitrate reduction to < 0.4 mg L^{-1} from an initial concentration of 6.4 mg L^{-1} was obtained, with no detectable nitrite production after 40 days. Slight decreases in sulphate concentration were noted in some flasks, but this was never in excess of 1.5 mg L^{-1} from an average initial concentration of 53.7 mg L^{-1}. Bromate reduction was observed in all cases, but comparison with reduction noted in an unsterile uninoculated control flask such as during batch trial D (1.3 mg L^{-1} decrease in bromate concentration over 36 days) suggests some isolates were not exhibiting greatly enhanced reduction rates. However other isolates showed a higher rate of reduction, for example isolate U reduced 5.1 mg L^{-1} bromate over the 40 day period, a reduction rate of 5.3 µg L^{-1} hr^{-1}. Bromide concentrations increased over the period, with calculated bromine recoveries for all isolates trialled within the range 99.3 – 104.7 % suggesting total conversion of bromate to bromide. Bromate concentrations for batch trial M is given in Figure 7.46.

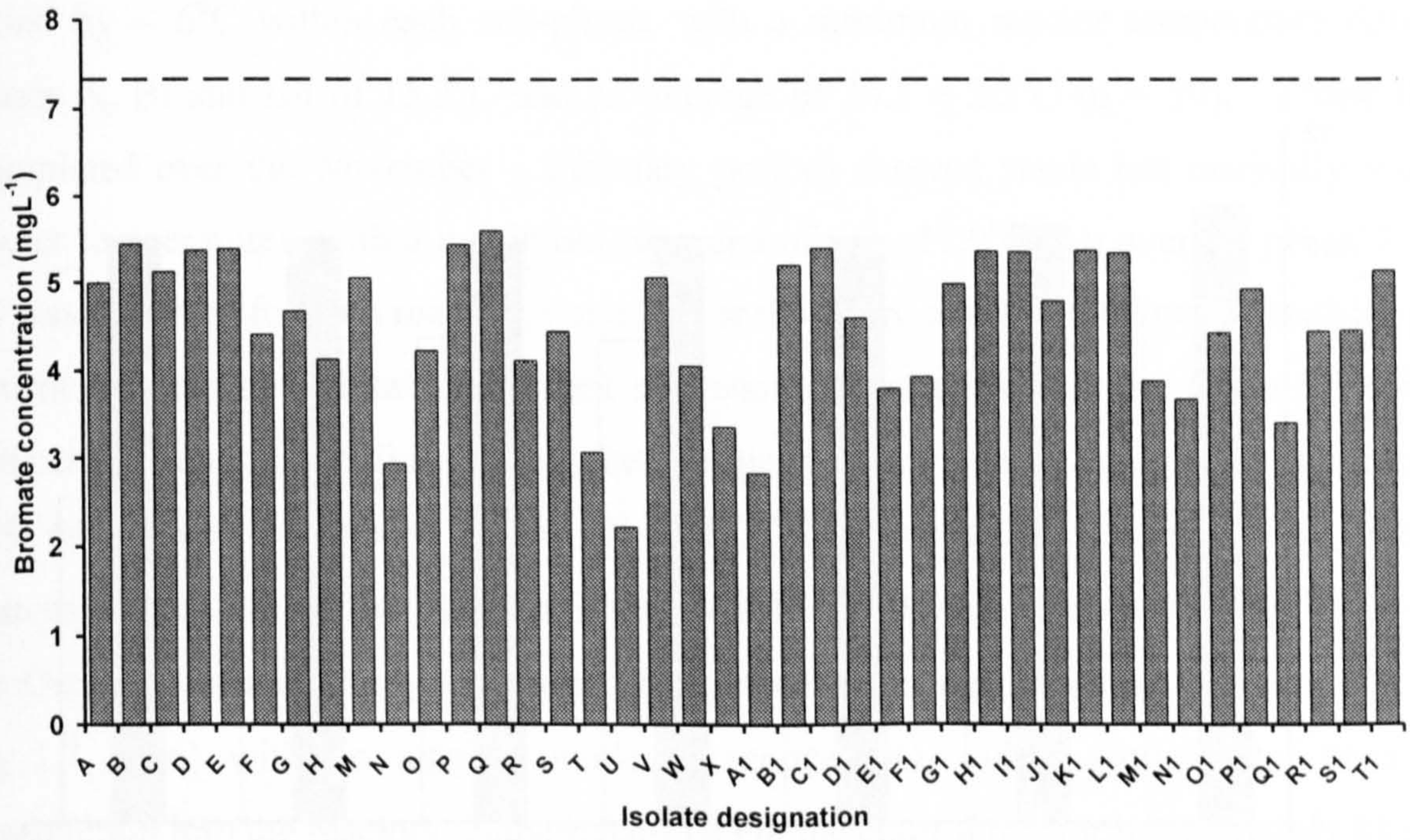


Figure 7.56 – Batch trial M: Bromate reduction in batch culture flasks of selected plated colonies. Average initial (day 0) bromate concentration indicated (– – –)

Following preservation of isolates in the proprietary 'Protect' bacterial preservation system, selected cultures (17, 31, 33, 35, 59 and 60) were unfrozen and

trialled for bromate reduction capability in batch trial N. Cultures 17 and 31 were directly derived from chemostat inoculum, 33 and 35 from repeated plate sub-culturing, and 59 and 60 were directly derived from samples taken from the pilot-scale trial (Section 7.2.4). An initial nitrate concentration of approximately 8 mg L^{-1} was reduced to ≤ 1.3 mg L^{-1} after 30 days incubation in all cases but culture 59, where 3.4 mg L^{-1} nitrate remained. Nitrite was not observed in any flask above the limit of detection. Bromate concentrations reduced in all cases, but cultures 17, 31, 33 and 35 showed no greater decrease than that observed in the uninoculated control (1.8 mg L^{-1}), suggesting these cultures were not actively reducing bromate. Only cultures 59 and 60 showed higher bromate reduction than control values, with 4.0 and 4.5 mg L^{-1} reduction respectively after 30 days, equating to a bromate reduction rate of 6.2 µg L^{-1} hr^{-1} for culture 60. Bromide increase was not observed for any isolates apart from 59 and 60, leading to calculated bromine recoveries within the range 92.1 – 93.8%. Bromate concentrations for batch trial N are given in Figure 7.57.

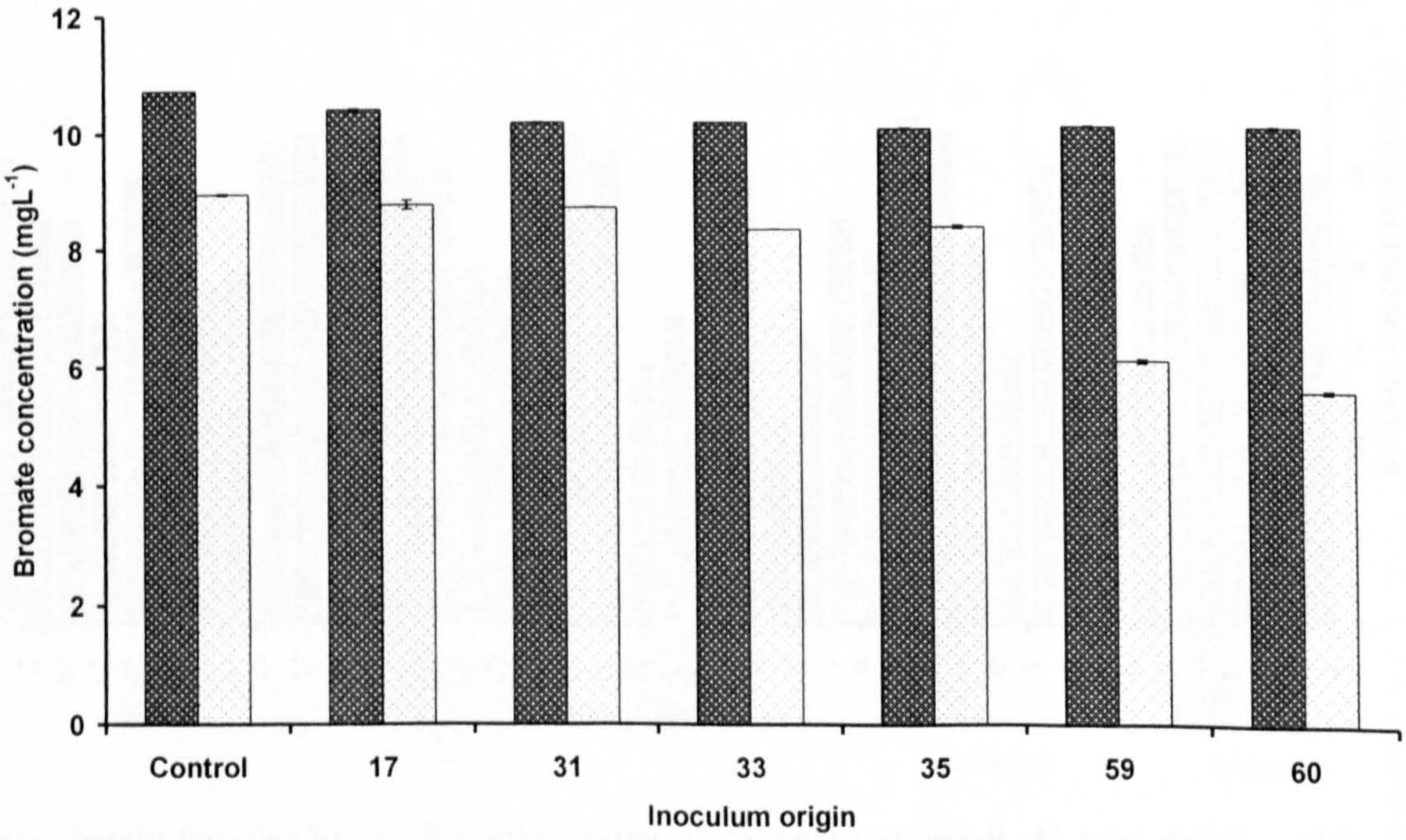


Figure 7.57 – Batch trial N: Bromate reduction in batch culture flasks of selected agar colonies following freezing in proprietary 'Protect' bacterial preservation system. Initial (day 0) values given as black bars and final (day 30) values as light bars.

7.2.4 Pilot-scale groundwater bioremediation

7.2.4.1 General operating conditions

Bromate groundwater bioremediation at pilot-scale was investigated in an inoculated two-vessel fixed-film bioreactor on a test and control basis at a range of retention times. Steady state samples were taken from both within the reactor (supernatant) and from the effluent line. Results from the two sets of readings were similar, with the example of both bromate and bromide data all being within ± 6% during Phases Bi/ii. Supernatant readings provided the more complete dataset so supernatant results have been used throughout. The reactors were operated under continuous flow conditions throughout phase B, with only occasional minor perturbations (less than 24 hours) caused by blockage of influent pipes. Operation of the reactors during the experimental period was under ambient temperature conditions. Reactor temperature decreased within this period due to the time of year, with a maximum of 24.2°C observed on day 33 and minimum of 10.0°C on day 146. However, reactor temperature only varied by < 6°C within each sub-phase, with a minimum reactor temperature during phases A, Bi and Bii of 15.2°C and an average of 20.1 ± 2.2°C (n = 19). Phase Biii (completed over the November – February period) showed stable but markedly lower reactor temperatures, with a maximum recorded of only 11.9°C. An average pH of 7.3 ± 0.4 was observed for both reactors 1 and 2 over the entire experimental period with no pH control required to maintain consistent pH readings, largely within the range 6.5 – 8.0. Temperature and pH readings during the trial period are given in Figure 7.58 and Figure 7.59 respectively. Influent anion, pH and TOC concentrations from the two water sources utilised during the trials are given in Table 7.20, with averaged values given for the Orchard Garage supply. Influent DO concentration during Phase B was 6.1 ± 1.4 mg L^{-1} (n=6) with no attempt made to reduce DO within groundwater prior to introduction into the reactors. Supernatant DO in the control reactor between days 81 and 97, measured immediately following sample collection, was within the range 0.4 – 1.1 mg L^{-1}. This concurred with DO data obtained during the laboratory-scale chemostat trials, and again suggested largely anoxic conditions were present within a bromate reducing bioreactor under steady state conditions.

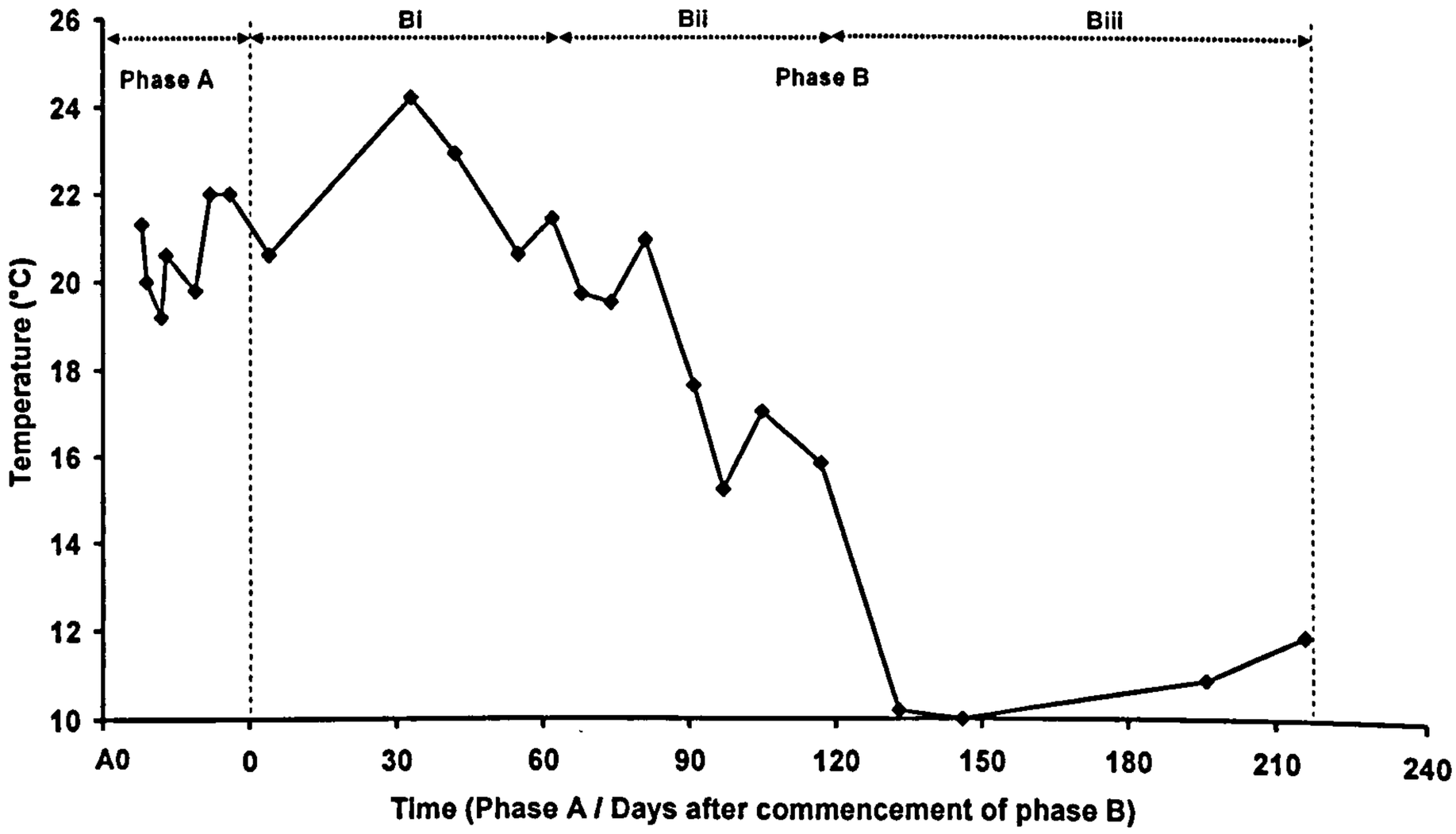


Figure 7.58 – Pilot-scale reactor supernatant temperature over the pilot-scale trial experimental period

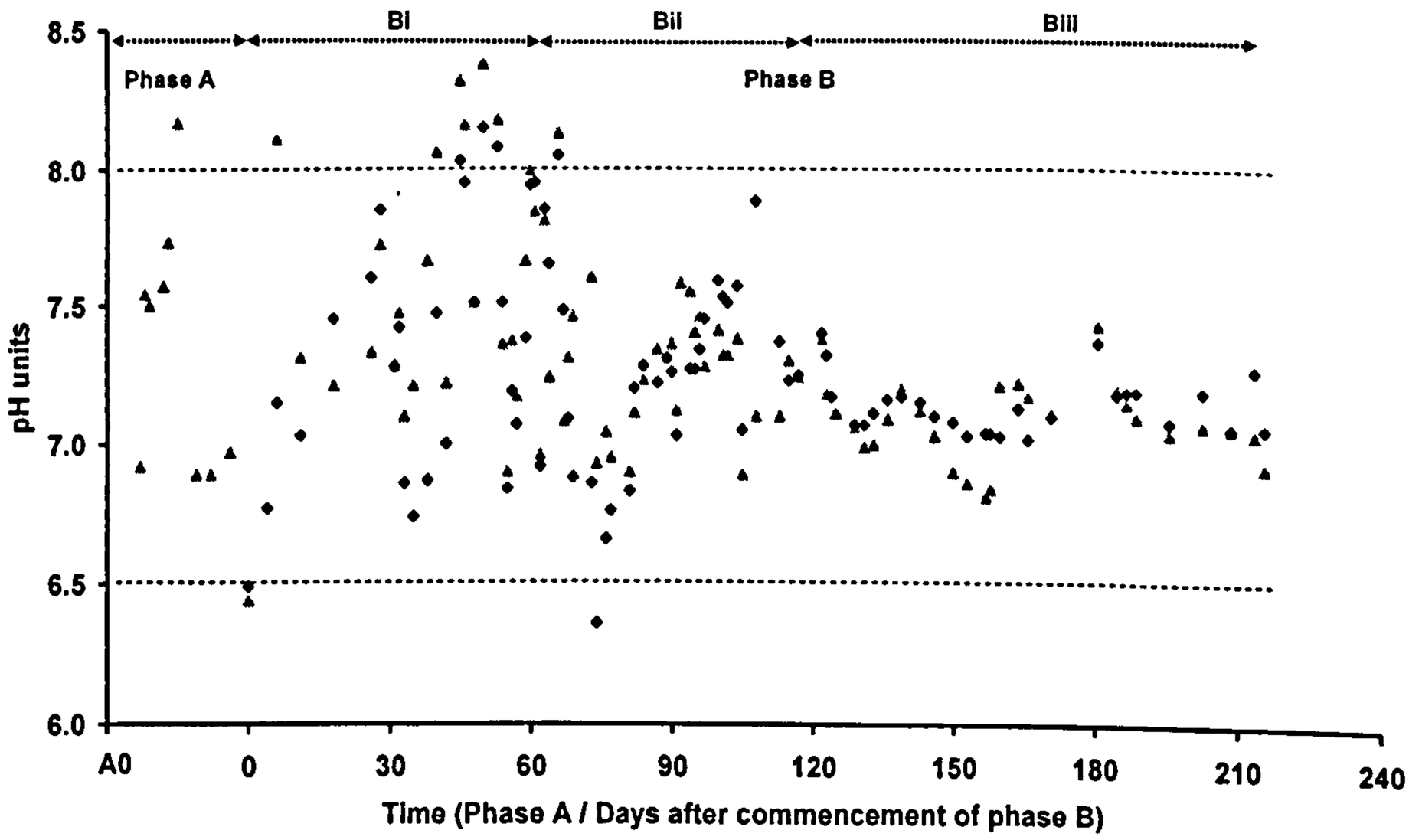


Figure 7.59 - Supernatant pH within pilot-scale reactors 1 (♦) and 2 (▲) over the pilot-scale trial experimental period

Table 7.20 - Selected properties of influent groundwater supplies over the pilot-scale trial experimental period

	House Lane supply (Phase A only)*	Orchard Garage supply (Phase B only)**
pH	6.9	7.7 ± 0.4
Total Organic Carbon (mg L^{-1})	19.9***	47.2 ± 2.7****
Bromate as BrO_3 (mg L^{-1})	1.4	1.1 ± 0.2
Bromide as Br (mg L^{-1})	16.6	2.5 ± 0.5
Total oxidised Nitrogen as NO_3 (mg L^{-1})	8.6	31.5 ± 3.7
Nitrite as NO_2 (mg L^{-1})	0.08	≤ 0.06
Sulphate as SO_4 (mg L^{-1})	74.8	23.9 ± 2.5

* Single sample collected 7 May 04 ; ** pH n=15;TOC n=4; Bromate n=17; other anions n=20;
*** no additional glucose; **** Spiked with glucose (mg L^{-1} expressed as glucose = 118.0 ± 6.8 mg L^{-1})

7.2.4.2 Phase A - Inoculation and batch suspended growth period

Phase A was carried out in Reactor 2 only, under batch suspended growth conditions. Biomass readings during this period increased from 0.039 g dry wt L^{-1} (Day A0) to 0.078 g dry wt L^{-1} (Day A22). An increase in bromate and nitrate concentrations over days A0 – A2 from the initial values of 26.6 mg L^{-1} and 9.3 mg L^{-1} to 28.5 mg L^{-1} and 10.9 mg L^{-1}, respectively, can be explained by addition of inoculum containing bromate and nitrate on days A1 and A2. Following this short lag phase, both bromate and nitrate reduction was occurring by day A5. Nitrate concentrations declined over the first 5 days, with a maximum of 3 mg L^{-1} remaining. Nitrite concentrations did not rise above 0.2 mg L^{-1}, suggesting the occurrence of total denitrification. Bromate concentrations exhibited a continuing decrease over the remaining period of batch operation. Simultaneously a stoichiometric increase in bromide concentration from an initial level of 10.4 mg L^{-1} was observed, a calculated percentage recovery (Days A0 – A21) of 101.6% showing total reduction of bromate to bromide. This observation confirms that the stoichiometric reduction of bromate with no intermediate production was occurring in the pilot-scale reactor. Anion concentrations during Phase A are given in Figure 7.60.

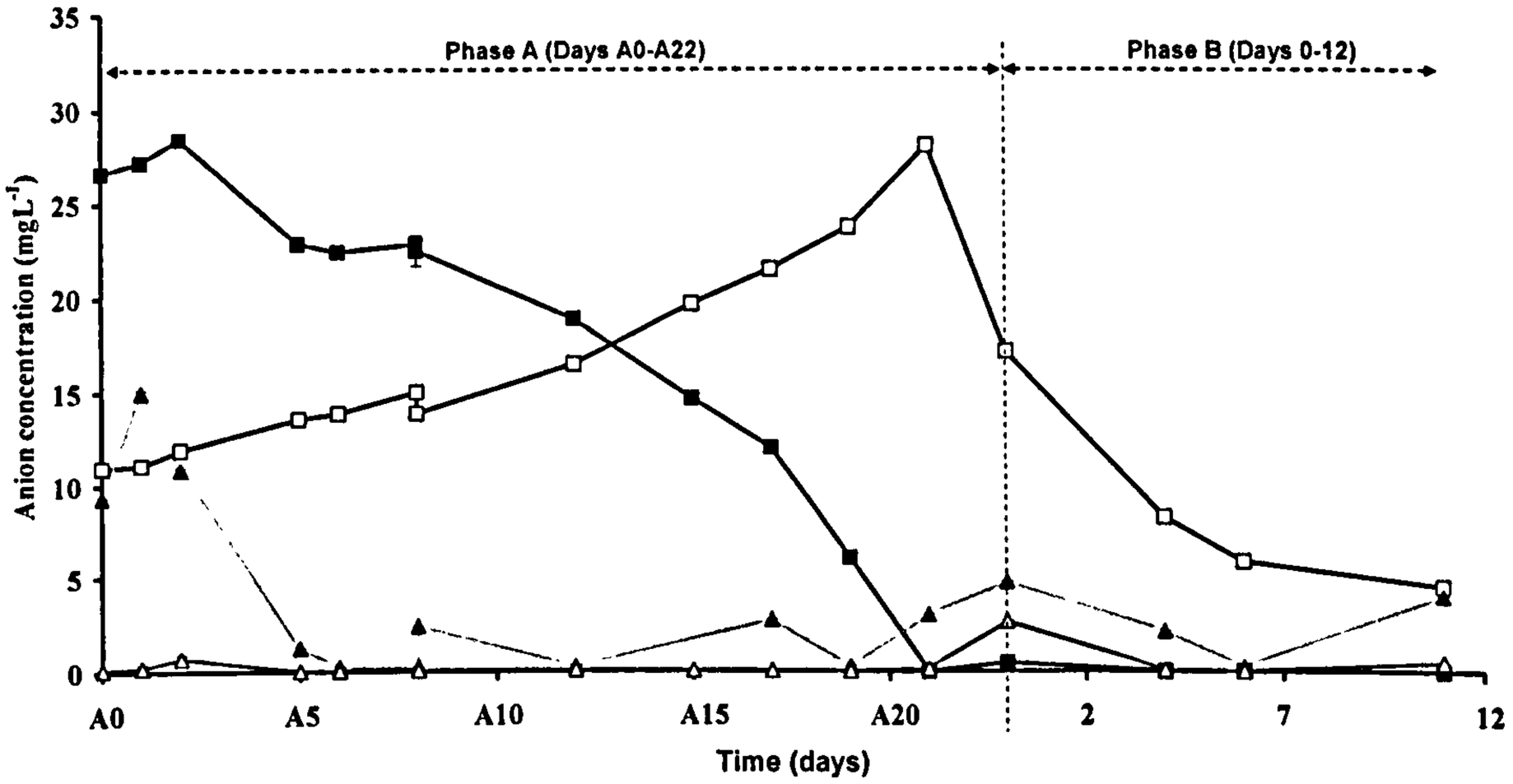


Figure 7.60 – Pilot-scale reactor 2 supernatant bromate (■), bromide (□), nitrate (▲) and nitrite (Δ) concentrations during Phase A (Batch operation) and Phase B days 0-12 (Continuous flow operation)

7.2.4.3 Phase B – Continuous flow fixed-film reactor operation

A. Reactor 1

The initial experimental aim for reactor 1 was operation at an RT of 80 hours. However, following commissioning an increased susceptibility to washing out of the biomass was noted when compared with reactor 2, measured as a decrease in optical density and increase in supernatant bromate concentration. During Phase A, a biomass layer would have formed on the inner wall of reactor 2, even prior to addition of plastic media. This surface biofilm may have facilitated biomass maintenance under the continuous flow conditions of Phase B. There was insufficient time for a biofilm to form on any reactor 1 surface prior to commencement of continuous flow conditions. This appears to have made the reactor more prone to washing out of biomass until day 75, by which time it is likely a biofilm had formed on both reactor walls and the packing media. As a consequence the reactor was operated as a batch system between days 29 – 38. In addition, failure of the nutrient pump on day 42 led to two further periods of batch operation (between days 45 and 56 and days 65 – 74) to regenerate biomass. During this period, it was noted that reactor 2 recovered faster than reactor 1, and therefore did not

require the second period of batch operation for bromate reducing ability to be regained. Sustainable bromate and nitrate reduction was not achieved in reactor 1 until following the third period of batch operation, on day 75.

Reactor 1 was considered in steady state operation at an RT of 80 hours for days 81 – 97, during which period average percentage bromate and nitrate reduction of 85.0% and > 99.2% (below detection limit), respectively, was obtained. Nitrite concentrations during this period were always below 0.06 mg L^{-1}, suggesting total denitrification was occurring at all times. Bromide supernatant concentrations were in excess of influent (2.5 ± 0.5 mg L^{-1}), with an average of 2.8 ± 0.4 mg L^{-1} during the steady state period. Bromide concentrations during the initial 10 days of the experimental period were in excess of 4 mg L^{-1} due to carry-over from the startup conditions during Phase A within reactor 2. Bromate and bromide supernatant concentrations during operation at an RT of 80 hours in Phase B are given in Figure 7.61.

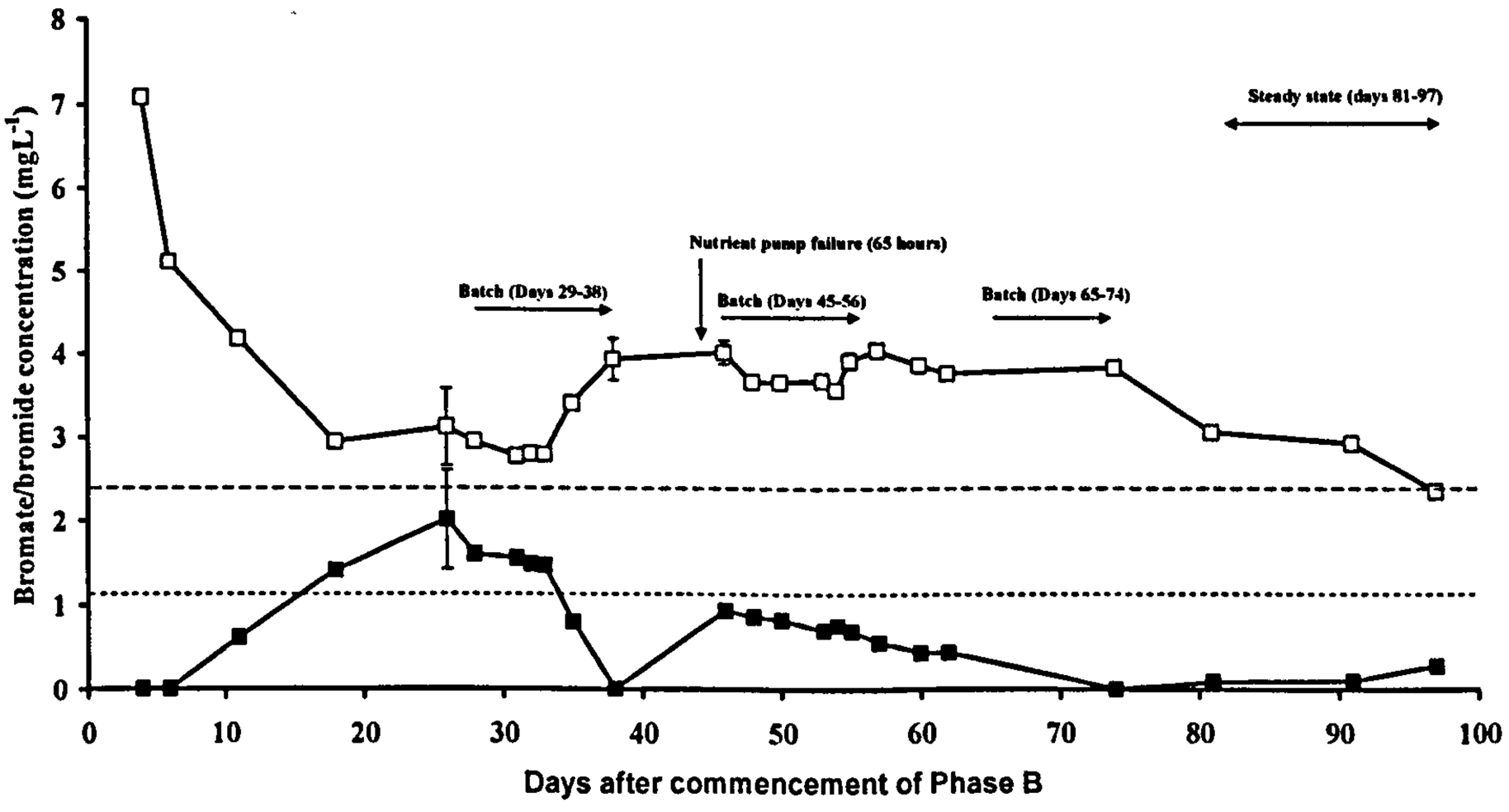


Figure 7.61 – Pilot-scale reactor 1 supernatant bromate (■) and bromide (□) concentrations during operation at an 80-hour RT in Phase B (Days 0 – 97). Average influent concentrations are given for bromate (---) and bromide (– – –), with periods of batch operation and nutrient pump failure indicated

On day 97, retention time in reactor 1 was raised to 100 hours, and this reactor was subsequently run under constant conditions (steady state from day 117) until completion of the trial on day 216. Operation in this manner allowed an evaluation into stability of the bromate reducing system over an extended timespan. During this period ambient temperature fell from a maximum of 17.0°C on day 105 to 10.0°C on day 146, so stability of the culture under these less favourable conditions would also provide evidence for robustness of the system. Over the 99-day steady-state period supernatant nitrate concentrations were within the range 0.3 – 1.9, suggesting almost total reduction from an average influent concentration of 31.5 ± 3.7 mg L^{-1}. Nitrite concentrations with one exception (0.2 mg L^{-1}) were always below the 0.06 mg L^{-1} limit of detection. A minimum supernatant sulphate concentration of 8.4 mg L^{-1} was observed, although this was variable with no reduction observed at some timepoints. Bromate reduction was noted throughout the period of operation, with supernatant concentrations within the range 0.2 – 0.6 mg L^{-1} (average influent of 1.1 ± 0.2 mg L^{-1}). Bromide supernatant concentrations were always in excess of influent, with the range 2.2 – 4.3 mg L^{-1} reflecting variable influent concentrations. Figure 7.62 shows bromate and bromide concentrations over the period of extended operation. Although temperature did not have an observed effect on nitrate reduction in reactor 1 (average percentage reduction of 97.2 ± 1.5 mg L^{-1}), bromate reduction followed supernatant temperature closely with reduction efficiency decreasing in line with the ambient temperature. Figure 7.63 shows this correlation between temperature and percentage bromate reduction over the period of extended running. Although not under steady-state conditions for the entire period, reactor 2 was also running at a 100-hour RT. This reactor did not exhibit comparable alteration of bromate reduction capability with percentage bromate reduction no lower than 86% at any time. Selected points for reactor 2 supernatant bromate reduction are also plotted on Figure 7.63. Again it is possible that reactor history, with an initial period of batch operation to allow biofilm growth, proved advantageous for later operation of reactor 2 by cushioning some effects of temperature.

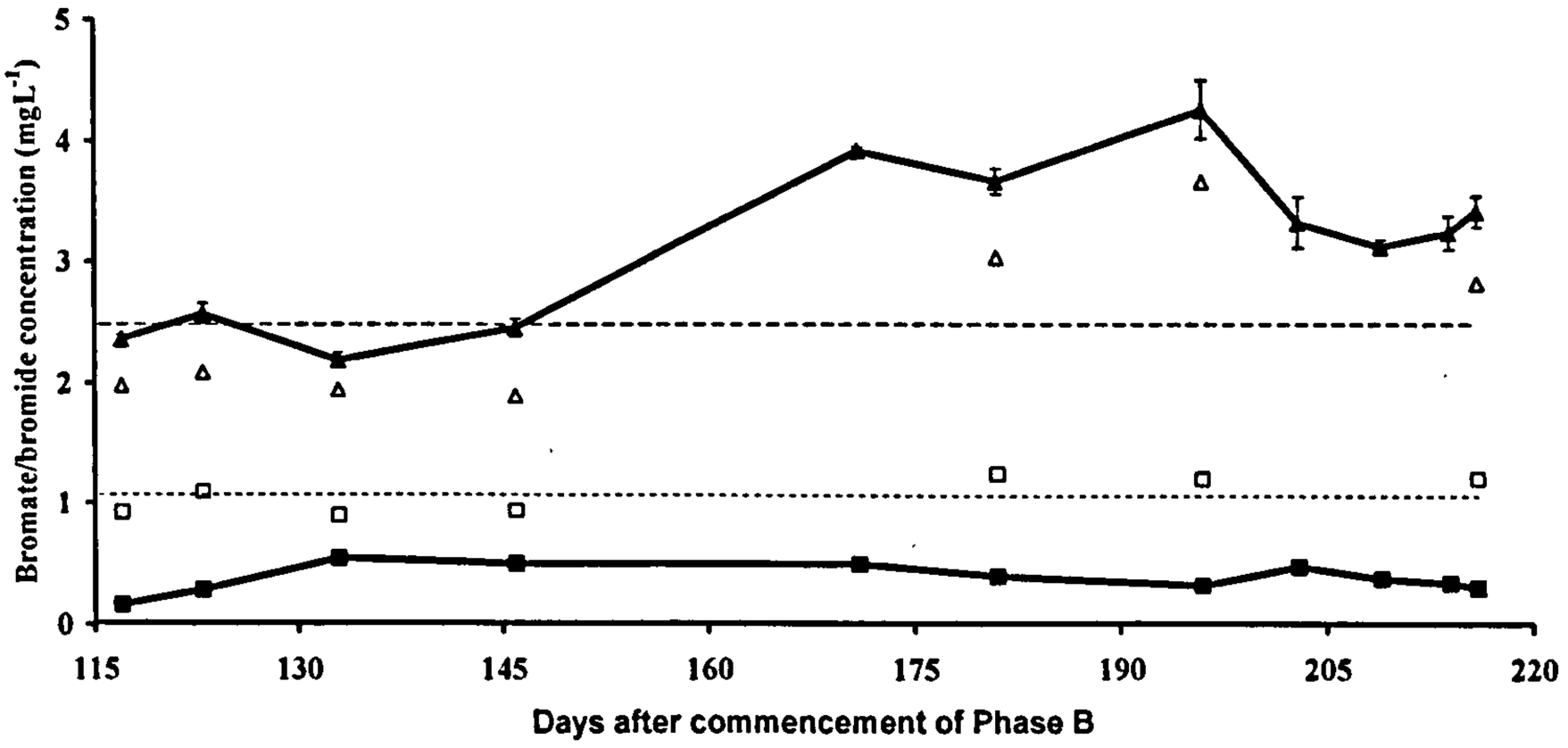


Figure 7.62 - Bromate (■) and bromide (♦) supernatant concentrations within pilot-scale reactor 1 over steady state phase B extended running period (100-hour RT). Individual influent bromate (□) and bromide (◊) concentrations and averaged influent bromate (- - -) and bromide (— — —) concentrations also shown

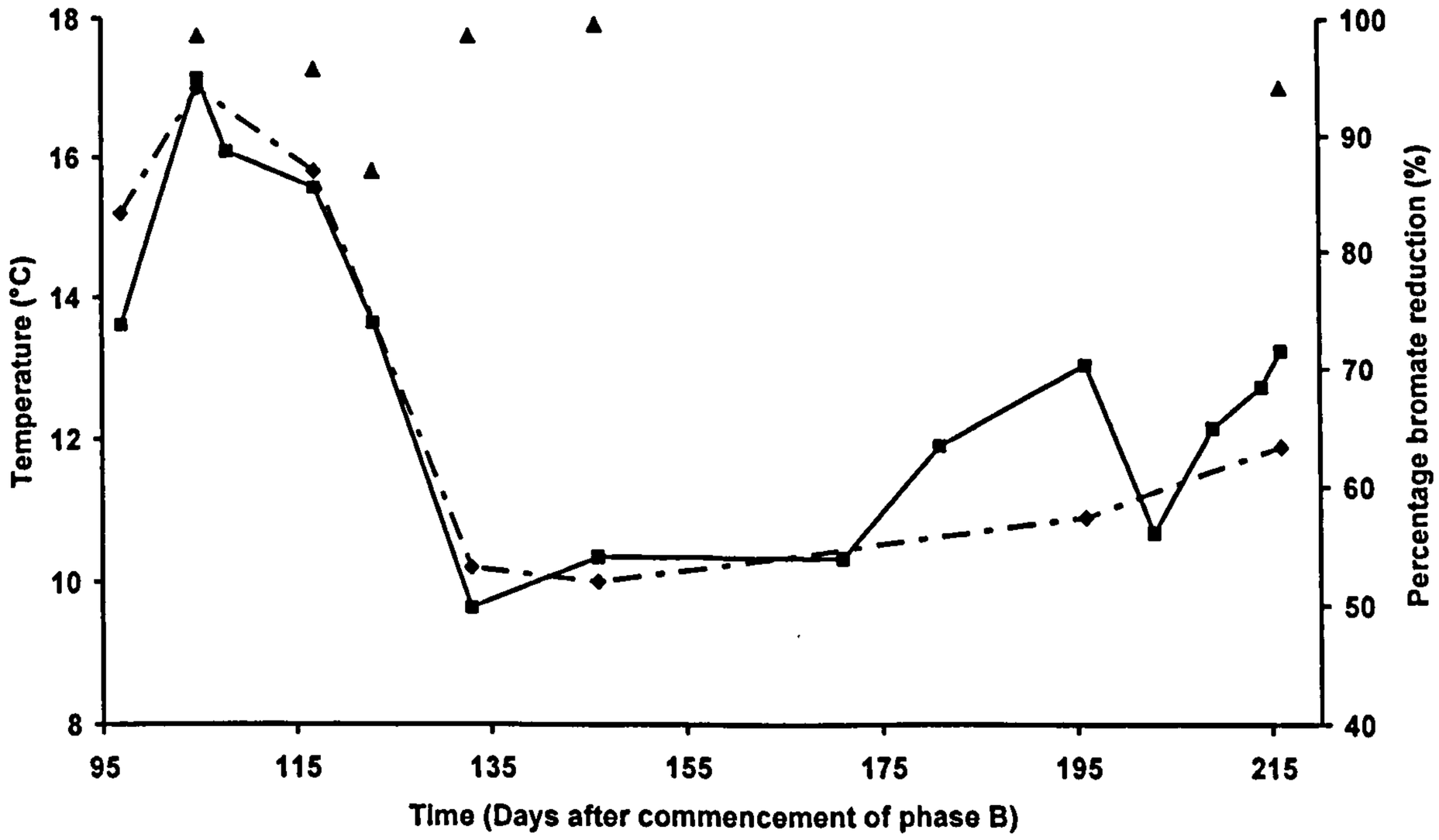


Figure 7.63 – Pilot-scale reactor 1 supernatant temperature (♦) and percentage bromate (■) reduction over phase B extended running period (100-hour RT). Selected reactor 2 supernatant percentage bromate reduction points also indicated (▲).

B. Reactor 2 - Phase Bi (80-hour retention time)

Subsequent to addition of plastic media and commencement of continuous flow conditions on day 0 (80-hour RT), bromate reduction from an average of 1.1 ± 0.3 mg L^{-1} influent to the 0.01 mg L^{-1} detection limit was observed on days 4 – 18. Between days 18 and 26 an average supernatant concentration of 0.5 ± 0.02 mg L^{-1} was achieved, with subsequent steady state supernatant concentrations of 0.2 ± 0.1 mg L^{-1} between days 26 and 42. The nutrient pump failure on day 42 led to the requirement for a period of batch operation between days 45 and 56. However, biomass recovery was rapid during this period, with only 3 reactor volumes (240 hours) required to regain previous reduction rates. Figure 7.64 shows bromate and nitrate reduction between days 45 and 62, immediately following the period of nutrient pump failure. Bromide concentrations were in excess of 3 mg L^{-1} at all times during Phase Bi (influent concentration of 2.5 mg L^{-1}), with supernatant nitrate concentrations not exceeding 4 mg L^{-1} at any time apart from during the period of nutrient pump failure.

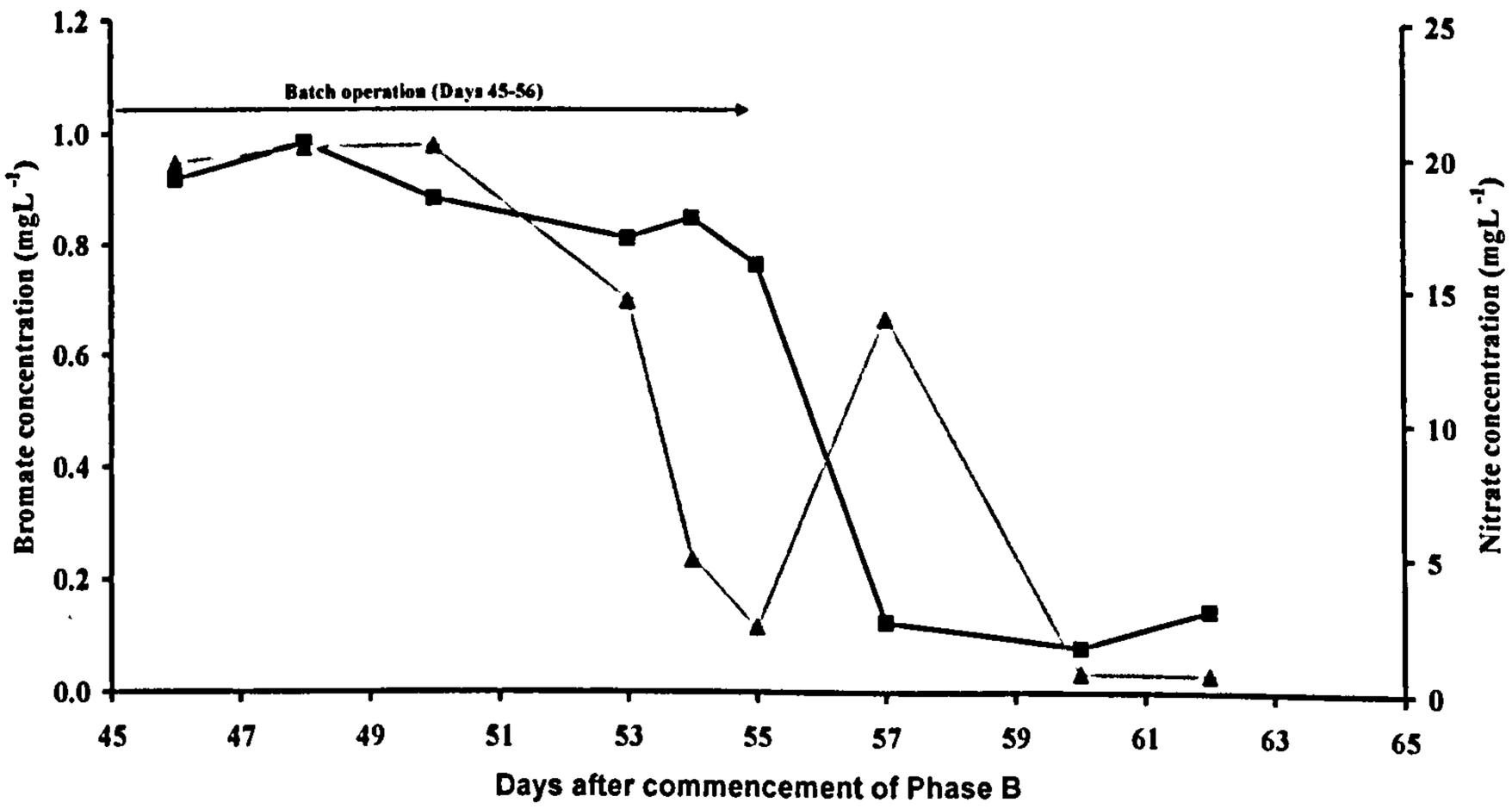


Figure 7.64 - Effect of nutrient pump failure and subsequent temporary batch operation on pilot-scale reactor 2 supernatant bromate (■) and nitrate (▲) concentrations between days 45 – 62 (Phase Bi)

C. Reactor 2 - Phase Bii (Effect of retention time)

Reactor 2 was operated during phase Bii at an 80-hour RT between days 63 – 68 prior to retention time trials, and subsequently at a 100-hour RT between days 100 – 117. Use of these retention times allowed comparison with the control reactor (Reactor 1) to investigate reproducibility of the system, and results are given in Table 7.21, confirming the two reactors were exhibiting similar performance characteristics at both retention times.

Table 7.21 - Comparison of pilot-scale reactor 1 and 2 supernatant characteristics at 80- and 100-hour retention times during phase Bii

	80-hour RT		100-hour RT	
	Reactor 1 *	Reactor 2 **	Reactor 1 ***	Reactor 2 ***
pH	7.2 ± 0.3	7.3	7.3	7.3
Total Organic Carbon (mg L^{-1})	14.3 ± 11.8	12.4	n/d	n/d
Bromate as BrO_3 (mg L^{-1})	0.2 ± 0.1	0.1	0.2	0.04
Bromide as Br (mg L^{-1})	2.8 ± 0.4	3.8	2.4	2.5
Total oxidised Nitrogen as NO_3 (mg L^{-1})	≤ 0.3	≤ 0.3	1.5	0.9
Nitrite as NO_2 (mg L^{-1})	≤ 0.06	≤ 0.06	≤ 0.06	≤ 0.06
Sulphate as SO_4 (mg L^{-1})	21.5 ± 3.9	21.1	14.1	18.5

* n=3; ** Single sample collected 15 Sept 2004; *** Single sample collected 3 Nov 2004

Percentage bromate and nitrate reduction within reactor 2 at the longest (100-hour) RT were 95.7% and 97.4% respectively. Lower retention times of 80, 60 and 40 hours led to little alteration in bromate or nitrate reduction, with percentage removals of 86.9 – 90.3% and 98.4 – 99.2% respectively. Under a 20-hour RT minimal alteration in nitrate reduction capacity was noted (97.9%), although a slight reduction in efficiency to 76.5% was observed at the 10-hour RT. In tandem, an increase in nitrite production was observed, leading to supernatant concentrations of 2.7 mg L^{-1} and suggesting total denitrification was not occurring. A loss in bromate reduction capacity at a 20-hour RT was observed, although 50.2% reduction of influent concentrations was still noted. Under a 10-hour RT, this loss of capacity became much more marked, leading to only 11.5% reduction. Sulphate reduction was noted at all retention times apart from 10 hours,

with a maximum reduction of 15.3 mg L^{-1} at a 20-hour RT from an average influent of 23.9 ± 2.5 mg L^{-1}. Figure 7.65 shows bromate and bromide concentrations and Figure 7.66 gives nitrate and nitrite concentrations during phase Bii. TOC concentration in the supernatant was always >10 mg L^{-1}, indicating that overall carbon levels were always in excess.

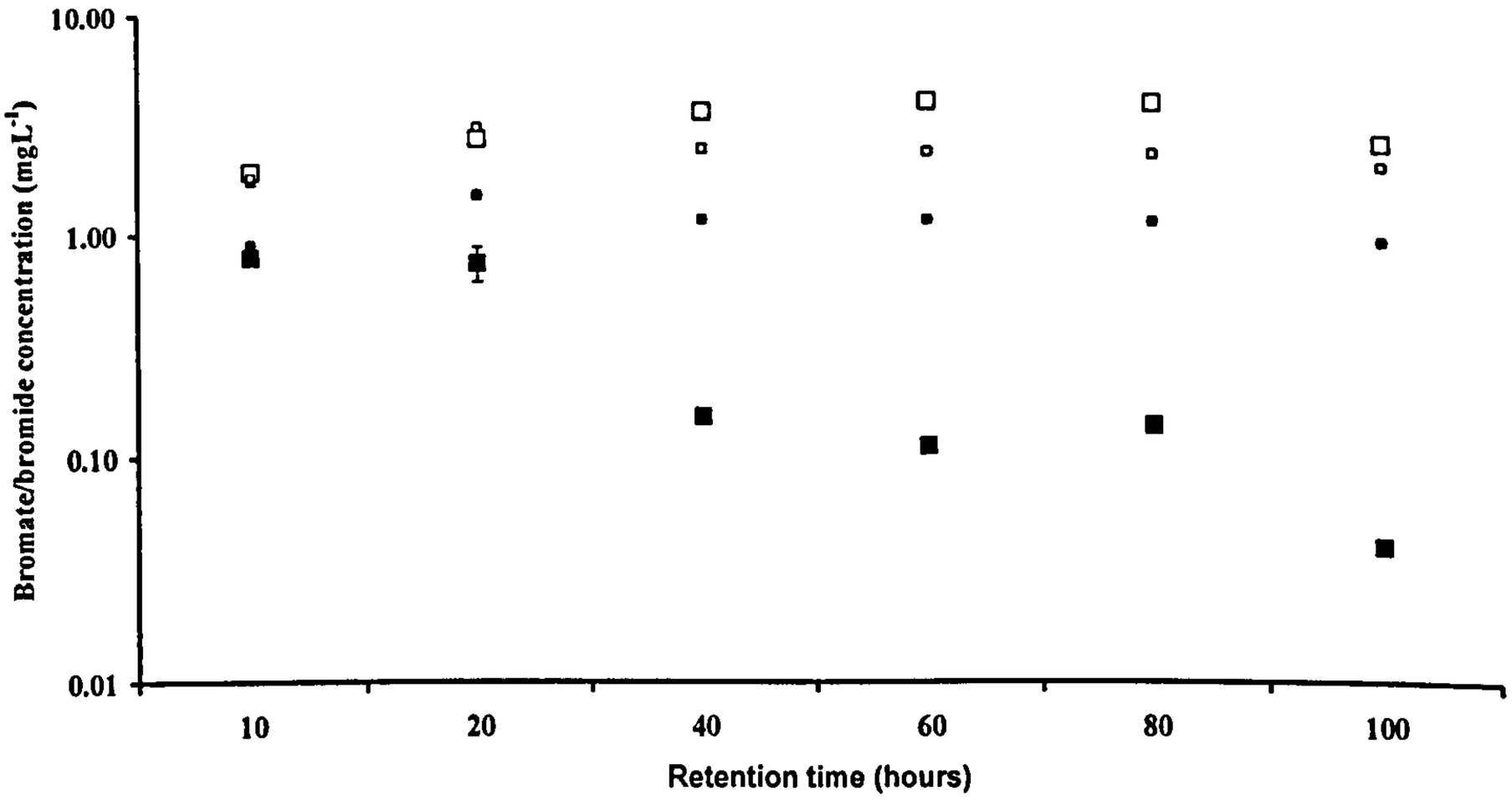


Figure 7.65 – Pilot-scale reactor 2 bromate (■) and bromide (□) concentrations during phase Bii at retention times from 10 hours (RT10) to 100 hours (RT100). Supernatant concentrations indicated by large symbols and influent concentrations by smaller symbols

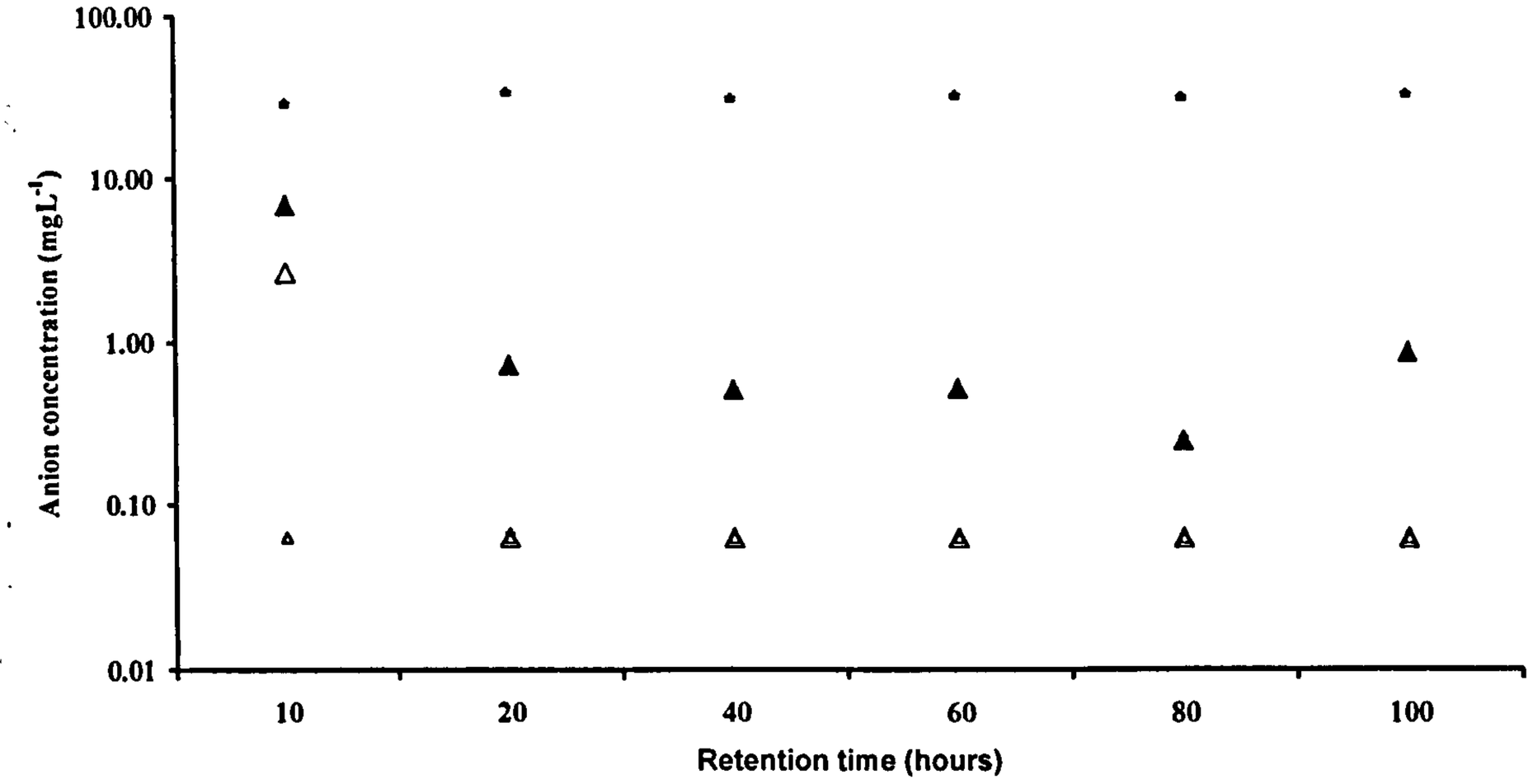


Figure 7.66 – Pilot-scale reactor 2 nitrate (▲) and nitrite (Δ) concentrations during phase Bii at retention times from 10 hours (RT10) to 100 hours (RT100). Supernatant concentrations indicated by large symbols and influent concentrations by smaller symbols

In summary, this trial confirmed that bromate contamination can be removed by acclimatised biomass within a pilot-scale bioreactor, utilising an unspiked bromate-contaminated groundwater and glucose as carbon source. A range of retention times were studied to ascertain optimum operating efficiency of the system, with the choice of 10 – 80 hours based on results gained during the laboratory-scale chemostat trials. This was intended to maximise potential for obtaining bromate reduction in this previously untested pilot-scale fixed-film system. Influent groundwater contained considerably more nitrate than bromate (an average of 30.7 mg L^{-1} nitrate as NO_3^- as opposed to 1.08 mg L^{-1} bromate) so conditions could be thought to favour predomination of denitrifiers. However, despite the removal of > 97% nitrate at all but the 10-hour RT, in excess of 50% bromate contamination was also removed concomitantly. At the 10-hour RT bromate reduction declined sharply to only 17.5% whilst denitrification showed a much less pronounced trend, with a decrease in reduction from 97.9% (20-hour RT) to 76.5% (10-hour RT).

8 DISCUSSION

In this chapter, Section 8.1 provides interpretation of wastewater studies, covering implications of any effects caused by bromate dosing on wastewater sludge biomass, and also the behaviour of bromate within a wastewater environment during sorption trials. Data obtained from investigations into groundwater bromate bioremediation are combined in Section 8.2 to give an overall picture of bromate reduction rates, factors affecting bromate bioremediation and possible biological pathways occurring during these processes. Recommendations are then made as to potential processes and operating conditions for full-scale aquifer bioremediation, with effectiveness of such techniques for remediation at aquifer scale also addressed (Section 8.3). Finally, other possible applications for technologies highlighted in the current study are outlined in Section 8.4.

8.1 BROMATE CONTAMINATION WITHIN WASTEWATER

With bromate input to wastewater treatment process likely in the vicinity of a bromate-contaminated aquifer, wastewater trials aimed to elucidate whether there would be any detrimental effect to biological processes within the treatment chains, and give evidence for behaviour and ultimate fate of bromate within these systems.

8.1.1 Effect of bromate on wastewater sludge

Significant alteration of respiration rate (measured as SOUR) within MBR sludge following bromate spiking during respiration inhibition trials 1 and 4 was not noted at a dose below 2 g L^{-1}. The lack of any respiratory effect strongly suggests that bromate does not significantly affect overall biological processes of the microbial biomass at concentrations found within the contamination plume. At the gross contamination level of 2 g L^{-1} a significant reduction in respiration rate did occur with biomass unacclimatised to bromate contamination. However, following acclimation to continuous bromate dosing of up to 100 mg L^{-1} this effect was ameliorated, with no significant difference between spiked and unspiked biomass samples noted. There are no comparable data available in the published literature, with the only toxicity information available concerning mutagenicity of bromate to microorganisms. Bromate was shown to

be slightly mutagenic to the bacterium *Salmonella typhimurium*, with weakly positive results obtained at a concentration of 3 mg per agar plate (cited in Depository Services Program (Canada), 1999). However, related *Salmonella* strains and other bacteria, including the common *Escherichia coli* and *Bacillus subtilis* strains, gave negative results in the same trial.

Limited information has been published on toxicity of chlorate to microorganisms which, as a related oxyanion, is pertinent to the current study. In a literature review of chlorate toxicity, van Wijk and Hutchinson (1995) summarised studies undertaken using aquatic microorganisms. Results suggested a wide variation of toxicity between species, with a range of 0.1 – 817 mg L^{-1} noted for the lowest-observed effect concentration (LOEC) on colony growth. During the trials, only one of the wide range of aquatic species investigated (the brown alga *Ectocarpus variabilis*) was sensitive to chlorate, producing a LOEC of 0.4 mg L^{-1}. Subsequent work investigated LOEC values for respiration inhibition in two test species, *Bacillus subtilis* and *Pseudomonas putida* (van Wijk *et al.*, 1998). Inhibition relative to control was measured over a short (2 minute) timespan. Results showed that no effect was apparent at chlorate concentrations below 200 mg L^{-1}. LOEC values were 389.7 – 780.3 mg L^{-1} and 748.5 – 780.3 mg L^{-1} respectively for the two test species. These results are directly comparable with the current study, where no significant effect on sludge bacteria was noted at or below 200 mg L^{-1} bromate contamination, but respiration inhibition occurred at the 2 g L^{-1} concentration. Toxicity of chlorate has been proposed to be not via a direct toxic effect, but the conversion of chlorate to chlorite by the nitrate reductase enzyme (van Wijk and Hutchinson, 1995). Chlorite addition produced a respiration inhibition LOEC range of 21.6 – 592.9 mg L^{-1} for the two species studied by van Wijk *et al.* (1998), although growth inhibition tests showed chlorite toxicity more clearly (LOEC range 0.7 – 14 mg L^{-1}). However, it is unlikely formation of bromite is the method of toxicity in the current study, as bromite is a short-lived and unstable intermediate (Jhanji and Gould, 1991).

No significant effect of bromide dosing at concentrations up to 200 mg L^{-1} was noted in respiration inhibition trial 2. There is limited information available regarding

bacterial toxicity of bromide. One study observed that strains of *Salmonella typhimurium* tested negative for mutagenic effects following incubation with 0.1 – 10 mg potassium bromide per agar plate (Zeiger *et al.*, 1992), but no studies were available regarding respiratory effects. Given the widespread use of bromide as a tracer during *in-situ* groundwater trials (ie. Smith *et al.*, 2001) with no reported effects on soil microorganisms, direct impacts of bromide dosing on microbial activity are unlikely. Bromide (as both potassium and sodium bromide) has been utilised in antimicrobial preparations for sanitisation of food handling areas, spa water, cooling water, and paper mill water systems (US EPA, 1991; US EPA, 1993). However, the method of action for these preparations does not involve direct bromide toxicity, and activators such as chlorine gas or sodium hypochlorite are also required. These react with bromide to produce hypobromous acid, which is the active ingredient (US EPA, 1993). Hypobromous acid is a powerful oxidising agent that forms stable nitrogen-halogen bonds with proteins, thus interrupting metabolic processes in living organisms (Conley *et al.*, 1987). However, given that no acute toxicity of bromide addition was observed in the current study, it is unlikely formation of hypobromous acid was a significant factor within the test system.

The combination of elevated bromate and bromide concentrations is found in all areas of the contaminated aquifer plume. Trial 3 investigated the possibility that a combination of these two contaminants, neither of which individually showed any significant effect on specific oxygen uptake rate at concentrations up to 200 mg L^{-1}, could together induce an alteration in bacterial respiration at typical aquifer contamination levels. No significant difference in SOUR was noted at bromate and bromide concentrations up to 30 mg L^{-1} and 180 mg L^{-1} respectively. As concentrations trialled were comparable to the maxima likely to be encountered following contaminated water ingress from the contaminated aquifer into sewage treatment processes, this result strongly suggests no gross respiratory effect on biological processes would be noted following release of this bromate-loaded water into a treatment system.

To further investigate any potential effects of bromate ingress into a real sewage treatment process, the pilot-scale MBR system (Section 7.2.4) was utilised for dosing

trials on acclimatised biomass. Although a 2 mg L^{-1} bromate dosing event caused slight perturbation within the nature and composition of MBR sludge biomass, recovery was rapid. Even with a subsequent increase in bromate dosage to 100 mg L^{-1}, acclimated biomass was not significantly affected, with a degree of tolerance apparently built up that was not evident in equivalent unacclimatised biomass. More importantly, neither hydraulic parameters nor macroscopic performance of the plant were affected, even during the initial dosing event. The only long-term effect noted in the study, an increase in SMP carbohydrate and COD content, could possibly be explained by effects of ionic composition. It is known that increased concentrations of cations (ie. magnesium and potassium) can cause deflocculation of activated sludge flocs (Bruus *et al.*, 1992), and some EPS components are bound by very weak forces (Keiding and Nielsen, 1997). Bromate dosing was as potassium bromate and, although deflocculation was not observed, the significant increase in SMP parameters may be explained by a decreasing stability of EPS under high potassium loadings. This would therefore suggest neither direct nor indirect toxicity of bromate at the concentrations trialled.

The scope of this dosing study appears to be unique within the published literature, so no comparative data could be analysed. However, from the results of the current studies it appears unlikely that bromate ingress into sewage treatment processes would significantly affect operation of the overall plant.

8.1.2 Bromate behaviour within a wastewater environment

The initial aim of sorption trials was to quantify the magnitude of bromate adsorption within sludge biomass. Although no studies of anion adsorption to sewage sludge were available, activated carbon has been shown to reversibly sorb nitrate by ion exchange processes (Kirisits *et al.*, 2000). With influent nitrate concentrations within the range 9.9 – 39.2 mg L^{-1}, nitrate uptake by GAC was observed to be between 2.0 – 4.4 mg g carbon^{-1}. However, almost total nitrate recovery (97%) following washing with 100 mg L^{-1} sulphate solution was obtained, indicating this was sorption by ion exchange processes and not abiotic nitrate reduction. Chlorate (Gonce and Voudrias, 1994) and perchlorate (Brown *et al.*, 2002) have also been shown to undergo reversible sorption via

ion exchange to GAC. Similar sorption of bromate to biomass following ingress of bromate-loaded water into a sewage treatment process could initially reduce influent concentrations but, due to the reversible nature of the process, would only provide temporary loss from solution.

Trials 1 and 2 both showed that bromate concentrations reduced following incubation of bromate laden water with freeze-dried MBR biomass at ambient temperature over a 72-hour period, which would be consistent with sorption processes. However, a concurrent and stoichiometric increase in bromide concentration provided evidence that fate of the spiked bromate was in reduction to bromide and not reversible ion exchange processes. No desorbed bromate was detected in any samples during trial 2 following a 72-hour desorption period. Desorption of bromide within the range 0.064 – 1.5 mg L^{-1} was noted over the same period, with greater bromide concentration at higher initial bromate concentrations.

Bromate behaviour within a wastewater sludge matrix has not been widely investigated, with only one study briefly considering the process (Almeida, 2004). This trial utilised settled sludge from a full-scale sewage works, with a similar methodology applied as with the current study. The dried sludge:solution ratio used was 1:10, with an initial bromate concentration of 1 mg L^{-1}. No data were given on the conversion of bromate to bromide and possible reduction mechanisms were not explored, but results showed overall bromine stoichiometry did not alter during the 48-hour experimental run and the author concluded that little bromate adsorption was occurring (Almeida, 2004).

Bromate sorption and reduction has been widely reported in conjunction with use of GAC as a potable water remediation strategy, with trials outlined in the literature review chapter (Section 2.7.4). Many studies have been process-orientated, only investigating bromate removal and not bromide production. However, overall bromine recovery within a GAC system has been investigated by a limited number of authors. Marhaba (2000) calculated that only 70% and 59% recovery were obtained from continuous flow pilot- and laboratory-scale GAC columns respectively spiked with 50 µg L^{-1} bromate, and Asami *et al.* (1999) obtained 82% recovery from GAC batch trials

with a 250 μg L^{-1} initial bromate concentration. In the latter study, one suggestion was that the 'lost' bromide was either adsorbed to the GAC (which was not quantified) or was present as the putative intermediate compound hypobromite. Other authors have achieved mass balances closer to the current study, with Siddiqui *et al.* (1996d) and Huang and Chen (2004) obtaining 95% and 83 – 96.2% recovery respectively using continuous flow columns. Influent bromate concentrations were 25 – 100 μg L^{-1} for the former trial and 80 μg L^{-1} in the latter. Batch trials carried out by Miller *et al.* (1996) in 4-litre flasks using distilled water with a 110 μg L^{-1} bromate spike obtained 101% recovery, suggesting all bromate was reduced to bromide within this controlled situation. The current study achieved in excess of 94% bromate recovery as bromide in all cases during trial 2, taking into account the cumulative effects of bromide increase and subsequent bromide desorption. Therefore, almost complete reduction of bromate to bromide was occurring in all cases, with little production of unmeasured intermediate compounds such as hypobromite or bromite.

Reduction of bromate by GAC is known to be a chemical and not biologically-mediated process, with some authors even attributing a decrease in bromate reduction capacity to biofilm growth on the GAC surface (Asami *et al.*, 1999; Marhaba, 2000). The reduction process is thought to be via adsorption to the activated carbon followed by chemical reduction to first hypobromite and then bromide (Equation 8.1 and Equation 8.2) (Yamada, 1993).

$$\equiv C + BrO_3^- \rightarrow BrO^- + \equiv CO_2$$

Equation 8.1

$$\equiv C + 2BrO^- \rightarrow 2Br^- + \equiv CO_2$$

Equation 8.2

This mechanism has also been confirmed by other authors (Siddiqui *et al.*, 1996d; Bao *et al.*, 1999; Marhaba, 2000; Huang and Chen, 2004). A potential process diagram was given in Asami *et al.* (1999), and is reproduced in Figure 8.1. Chlorite (ClO_2^-) has also been observed to undergo a similar chemical reduction mechanism, forming chloride

(Cl^-) (Gonce and Voudrias, 1994). It is known GAC can be successfully produced by activation of sewage sludge biomass (Martin *et al.*, 2002). Therefore, although no published work is available regarding chemical bromate reduction by sewage sludge, similar mechanisms may lead to reduction observed in the current trials.

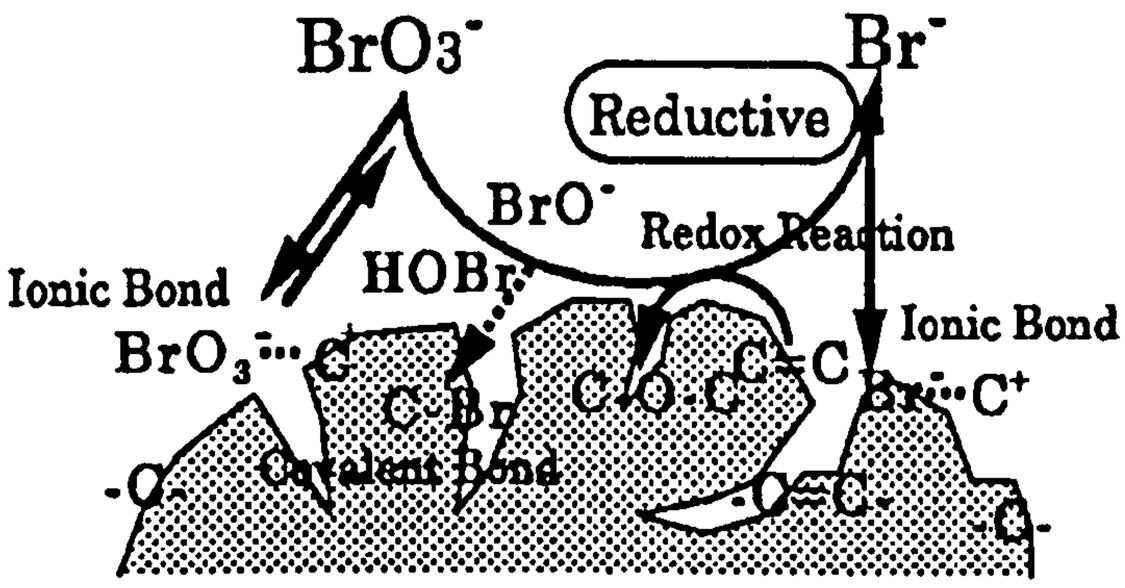


Figure 8.1 - Possible bromate reaction on new Granular Activated Carbon (GAC) (Asami *et al.*, 1999)

Microbial reduction of other oxyanions including nitrate and sulphate has been reported within sewer systems, with anoxic conditions allowing sulphate reduction to undesirable hydrogen sulphide gas (Yang *et al.*, 2004). Nitrate can be dosed into sewer systems to eliminate sulphate reduction, as the added nitrate is reduced preferentially by microbes within the raw sewage (Bentzen *et al.*, 1995). As a related oxyanion, bromate reduction could be via a comparable microbial mechanism. Therefore, the nature of the bromate reduction observed was investigated in trial 3. This trial demonstrated that bromate reduction still occurred following bacterial inactivation by either a physical (autoclaving) or chemical (formaldehyde addition) method, thus supporting the hypothesis that bromate reduction to bromide on a sewage sludge biomass matrix was mediated by a chemical reduction process. In a previous trial, Almeida (2004) dosed a bromate spike of 1 mg L^{-1} into 20-litre batch reactors containing screened sewage sterilised by formaldehyde (1%). Under anaerobic conditions, both sterilised and unsterilised reactors reduced bromate to < 0.1 mg L^{-1} within the first hour of a 24-hour trial. The occurrence and also the rapid nature of this reduction following bacterial inactivation appeared to confirm the involvement of a chemical mechanism.

In the current study, reduction of 42.4% and 52.5% (of initial bromate concentrations) were achieved following physical and chemical sterilisation processes respectively. A higher percentage reduction (67.6%) was achieved during trial 2 at a comparable initial bromate (Figure 7.11b), but this did not constitute a significant difference to either set of sterilised samples (Mann-Whitney; $p = 0.1160$). In addition, bromate reduction was observed to commence rapidly during trial 2. For example, at the lowest bromate concentration, 76.6% reduction of the initial 0.3 mg L^{-1} spike had occurred within two hours. The occurrence of rapid stoichiometric bromate reduction to bromide which is not halted by sterilisation suggests a chemical reduction mechanism when in contact with sludge biomass. Physical sorption of bromate was not observed, although some bromide sorption was noted (maximum 1.5 mg L^{-1} with a 30 mg L^{-1} bromate spike). Limited bromate reduction by opportunistic denitrifying bacteria in unsterilised samples cannot however be ruled out, and this may account for the slight (non-significant) decrease in percentage bromate reduction noted following sterilisation. Further trials would be necessary to fully elucidate this possibility.

8.2 GROUNDWATER BROMATE BIOREMEDIATION

Overall success of the groundwater bromate bioremediation trials is discussed and interpreted in this section, with parameters including rates of bromate reduction, rate-determining parameters and potential mechanisms mediating groundwater bromate bioremediation discussed and related to data obtained.

8.2.1 Effect of bromate influent concentration on reduction rate

Microbial bromate reduction, which has only been observed in a limited number of trials to date (Hijnen *et al.*, 1995; Hijnen *et al.*, 1999; Kirisits and Snoeyink, 1999; Kirisits, 2000; Kirisits *et al.*, 2002; van Ginkel *et al.*, 2005a; van Ginkel *et al.*, 2005b) was achieved in all three groundwater bioremediation trials completed within the current study. By contrast to when in contact with sewage sludge biomass, a biotic reduction mechanism was also confirmed during batch flask trials D and E, where bromate reduction was halted following filtration and sterilisation of the media, but reinoculation

by a microbial culture reinitiated reduction ability. Bromate reduction rates were calculated (units of μg L^{-1} hr^{-1}) for all trials, with specific reduction rates (units of μmol g dry wt^{-1} hr^{-1}) also obtained for the laboratory-scale chemostat results. These data have been presented in the relevant sections, with representative reduction rates achieved in each trial also summarised in Table 8.1.

Table 8.1 - Representative bromate reduction rates from bioremediation trials

Trial description		Reduction rate (μg L^{-1} hr^{-1})	
		Bromate	Nitrate
Suspended growth laboratory-scale chemostat	Phase I	27.8	1858.7
	Phase IIa	240.5	455.4
	Phase IIb (start)	84.9	193.8
	Phase IIb (end)	1086.5	216.5
	Phase IIIb	676.0	89.6
Suspended growth batch trials	Trial F	12.1	156.1
	Trial K	17.1	n/d
Pilot-scale fixed-film bioremediation system	Phase A	59.4 (Days A2-A21)	122.9 (Days A1-A6)
	Phase Bii	37.5	1672.3

Bromate reduction rates have not been widely calculated within the published literature, but rate studies investigating perchlorate reduction have been more extensive. Perchlorate reduction rates in continuous-flow packed bed reactors were reviewed by Logan (2001), who collated from seven individual trials a range of 0.042 – 1200 μg L^{-1} hr^{-1}. Influent perchlorate concentrations were 0.13 – 1500 mg L^{-1} and reactor retention times also varied widely, within the range 1.08 – 600 minutes. This range of reduction rates encompasses those observed during current trials, suggesting both that perchlorate reduction can be carried out at similar rates to bromate reduction and that comparison between the two processes is therefore valid.

There are many factors which can affect reduction rate, including intrinsic bacterial kinetics, substrate differences, and within a fixed film system media surface area and biofilm thickness (Logan, 2001). Other potentially rate-determining factors are also discussed in this chapter. It was noted by Logan (2001) that perchlorate removal rates

should be first order with respect to perchlorate concentration, regardless of substrate or inoculum utilised. To test this hypothesis, regression analysis on the collated data was undertaken. Due to the variation between inlet and outlet perchlorate concentrations, a log-mean concentration (C_{lm}) was calculated (Equation 8.3) and used instead of influent level. Results were plotted on a log-log graph, and are reproduced in Figure 8.2.

$$C_{lm} = \frac{C_{\text{inf}} - C_{eff}}{\ln(C_{\text{inf}} / C_{eff})}$$

Equation 8.3

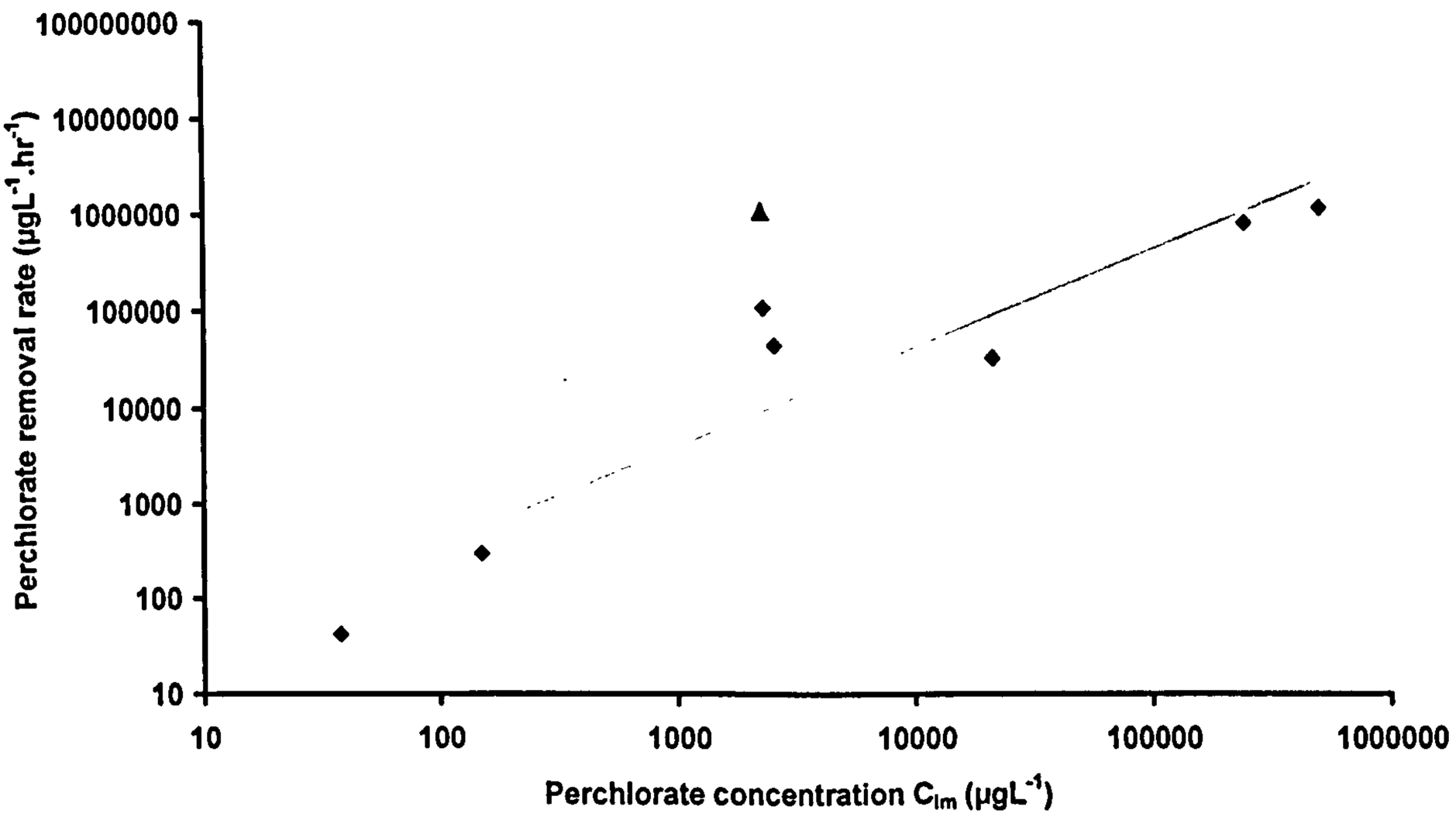


Figure 8.2 - Perchlorate degradation rates as a function of initial perchlorate concentration. Outlying point (▲) not included in regression line. (Data compiled by Logan, 2001)

Regression analysis revealed that, with the exception of one point (▲) which exhibited substantially larger kinetics, the regression line showed reasonable fit (R^2 = 0.87). The slope, which for a first order relationship should be unity, produced a value of 1.02 ± 0.17. This confirmed that rate was first order with respect to perchlorate concentration.

Only one trial has attempted to correlate reduction rate with bromate concentration. Hijnen *et al.* (1999) studied low bromate concentrations (15 – 35 μg L^{-1}), achieving a low maximum bromate reduction rate of only 1.4 μg L^{-1} hr^{-1} at 20°C compared with an equivalent denitrification rate of 108 μg L^{-1} hr^{-1}. However, it was speculated that, with linear extrapolation bromate reduction rate may increase to the same order of magnitude as the nitrate reduction observed. The plot obtained of reduction rate as a function of initial concentration is reproduced in Figure 8.3. Although C_{lm} values were not used in this study, a rough estimate of slope gives a value of 1.2, suggesting that first order kinetics may be expected within a bromate reducing system.

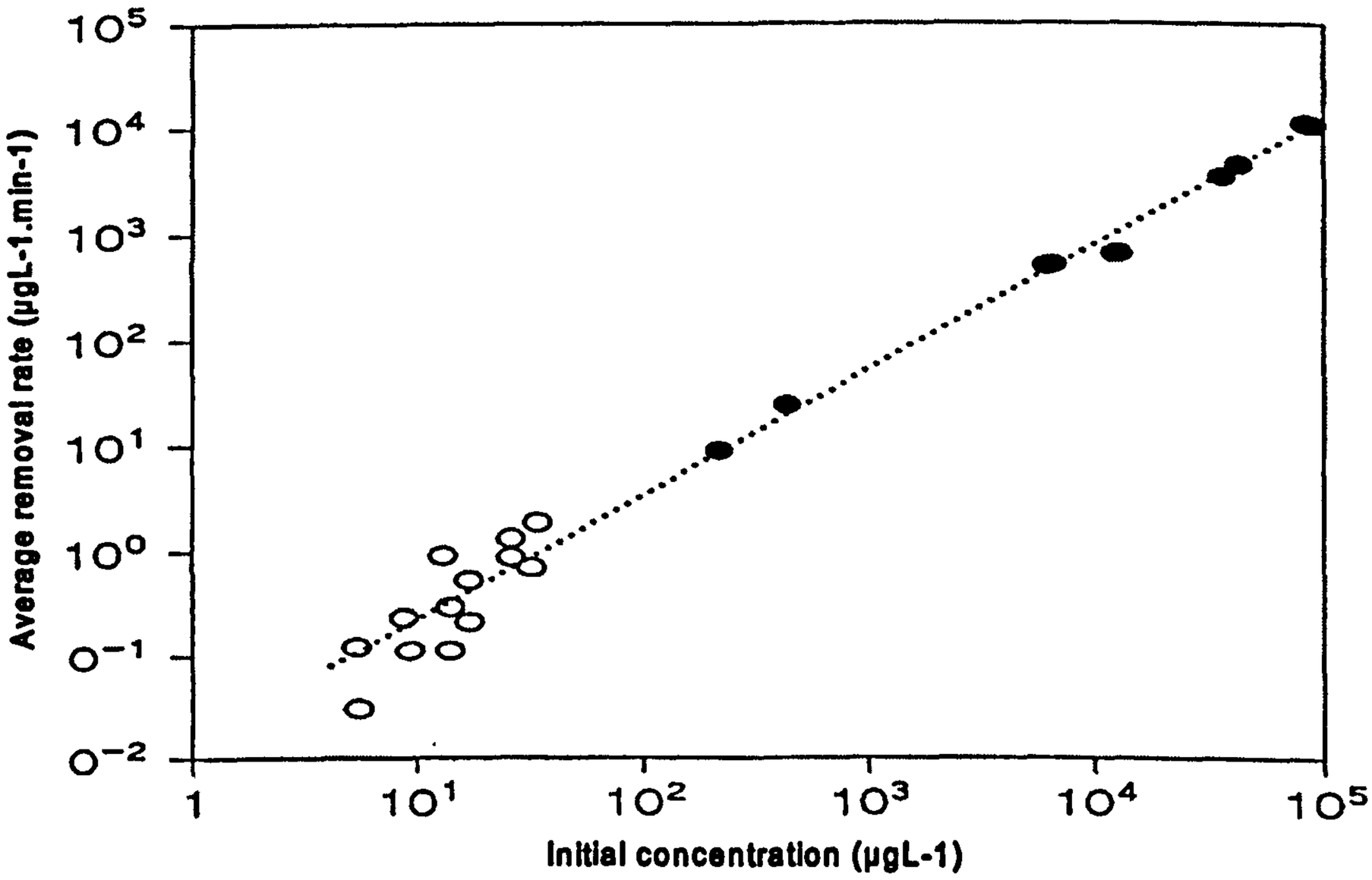


Figure 8.3 - Bromate (○) and nitrate (●) removal rates as a function of initial bromate concentration (Reproduced from Hijnen *et al.*, 1999). Regression line based on all plotted data. Note units of μg L^{-1} min^{-1}.

To further analyse bromate reduction rates, examination of published data was undertaken during the current study, with C_{lm} values and reduction rates calculated for all collated results (Appendix B). Data from both previous (open symbols) and current trials (filled symbols) were then plotted on a log-log graph, given in Figure 8.4. The graph of

'rate profiles' obtained suggests a relationship between bromate concentration and reduction rate, and that previous trials generally follow this same relationship. However, there are two sets of outliers. The data obtained by Hijnen *et al.* (1995) show lower rates than would be expected, and the three points within the dashed circle show substantially higher rates. The reason for the slow rates obtained by Hijnen *et al.* (1995) is not clear. Two of the high-rate points indicated were obtained in the current study during phase IIb and III chemostat trials, one point indicating rate with a groundwater influent and the other with the artificial low nitrate/sulphate medium. The third point (open diamond) was obtained by van Ginkel *et al.* (2005b) using a continuous flow column inoculated with activated sludge and fed with bromate (294 mg L^{-1}) and acetate (385 mg L^{-1}). During this trial, use of an artificial medium allowed the observation that bromate reduction was occurring in the absence of other electron acceptors. The authors concluded that bromate reduction in this case was being utilised for growth, and that the mechanism was not via co-metabolic processes. This was the first, and to date is the only published report of bromate use as the sole electron acceptor by microbes capable of coupling bromate reduction with growth.

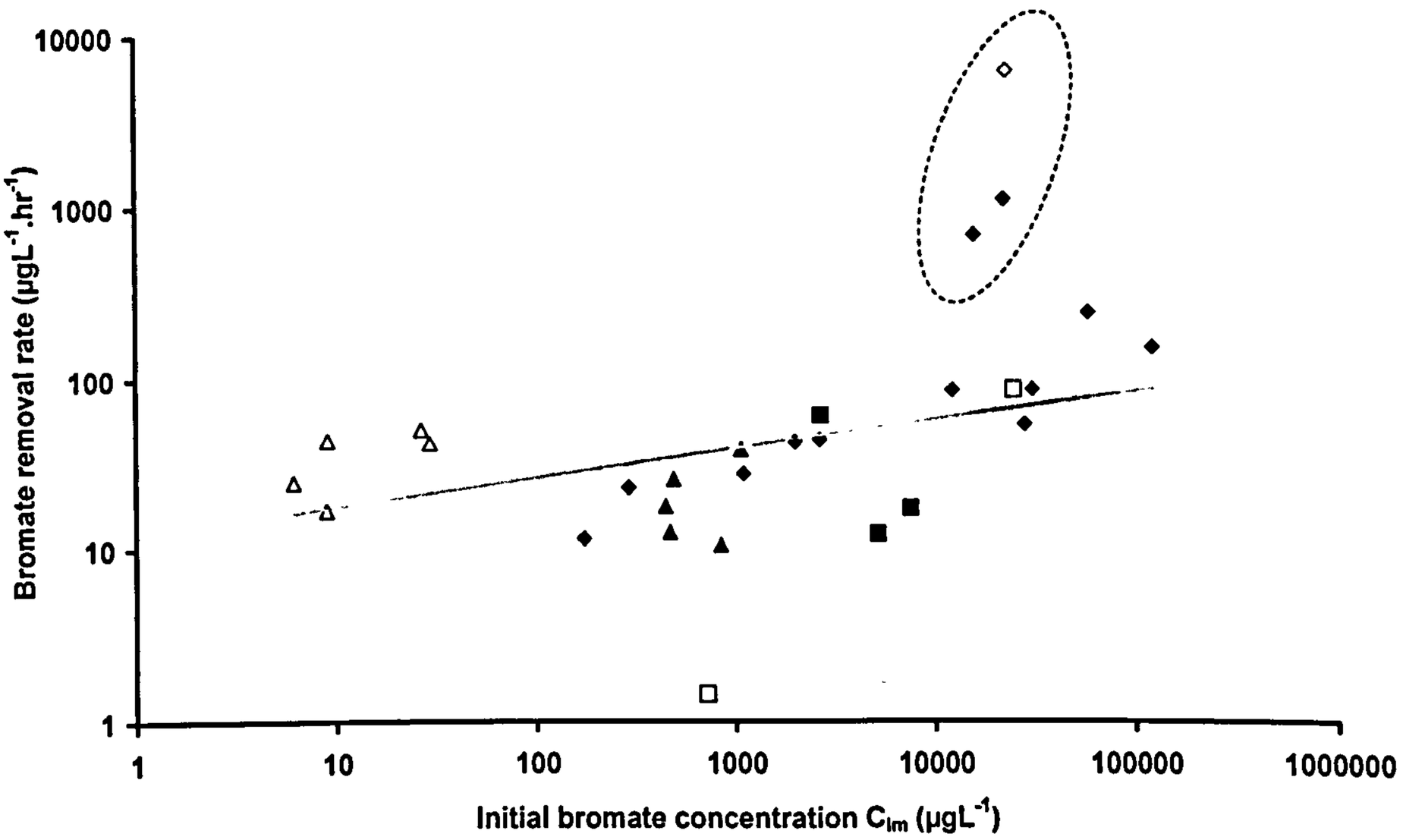


Figure 8.4 - Bromate reduction rates as a function of initial bromate concentration. Data from chemostat (♦) and pilot-scale (▲) continuous flow trials during the current study, plus other suspended growth (◊) and fixed-film trials (Δ) are shown (Hijnen *et al.*, 1999; Kirisits and Snoeyink, 1999; van Ginkel *et al.*, 2005b). Batch trial results from the current study (■) and previous trials (□; Hijnen *et al.*, 1995; van Ginkel *et al.*, 2005b) are also indicated. Regression line based on all continuous flow data apart from outlying points highlighted in the text.

The similarity apparent in Figure 8.4 between chemostat phase IIb/III 'rate profiles' and those obtained by van Ginkel *et al.* (2005b) suggests a similar microbial population was predominating during latter chemostat trials. It is possible that, following the groundwater pump failure in chemostat phase IIb (Section 7.2.1.2), a shift in microbial population occurred, with significantly increased bromate reduction kinetics resulting. This shift was sustained into phase IIIb following adjustment to a medium containing low nitrate (3.6 mg L^{-1}) and sulphate (3.1 mg L^{-1}), sulphate concentrations being directly comparable to those utilised (3.2 mg L^{-1}) by van Ginkel *et al.* (2005b). However, only within the chemostat system was this shift observed, with batch trials (filled squares) and early chemostat phases all exhibiting a 'rate profile' more indicative of that observed in previous studies (Hijnen *et al.*, 1995; Hijnen *et al.*, 1999; Kirisits and Snoeyink, 1999) and concluded by the authors to be co-metabolic in nature. Transfer of

high-rate bromate degrading chemostat inoculum to the pilot-scale bromate bioremediation system (filled triangles) also appears to have favoured reversion to lower rate bromate reduction. The highest reduction rate obtained under continuous flow conditions, at the 20-hour RT, was 37.5 μg L^{-1} hr^{-1}. The 40-hour RT exhibited a slightly reduced overall reduction rate of 25.1 μg L^{-1} hr^{-1}, despite having a higher percentage reduction. However, the aim of this trial was to investigate realistic bromate and nitrate concentrations and not achieve maximal bromate reduction rates. Limiting bromate concentrations in this system may have simply repressed reduction rate by high rate bromate degrading bacteria in the culture. In addition, although not quantified, transfer of inoculum from the pilot-scale to chemostat bioreactors was observed to rapidly revert to 'high-rate' bromate degradation characteristics, suggesting this capability was not lost when cultured within the pilot-scale bioreactor.

Regression analysis of available data (excluding outlying points) suggested that, unlike with perchlorate reduction, first-order kinetics with respect to bromate concentration is not observed within a 'co-metabolic' bromate reducing system. A slope value of 0.14 ± 0.047 was obtained. However, the fit of the regression line was poor (R^2 = 0.33), which may reflect inclusion of both suspended growth and fixed film data due to a paucity of relevant studies. It is also possible that bacteria exhibiting high-rate bromate degrading activity, such as those obtained during chemostat phase IIb/III, may follow first order kinetics. However, sufficient data are not currently available for this hypothesis to be tested.

Specific reduction rates can give a measure of the efficiency of a bacterial reduction process, and were calculated in the current study for bromate (qBr) and nitrate (qN) during all three phases of chemostat trials. During chemostat phase I, specific bromate reduction rates were poor compared with equivalent rates for nitrate, suggesting low bromate reduction efficiency and a largely denitrifying culture. For example, the highest qBr value obtained during this phase (2.2 μmol Br g dry wt^{-1} hr^{-1}) led to a qN/qBr ratio of 138.0 (Table 7.8). During phase II an improvement in bromate reduction was achieved prior to the period of groundwater supply failure, with a maximum qBr value of 30.3 μmol Br g dry wt^{-1} hr^{-1} (80-hour RT). A corresponding lowering of the qN/qBr ratio

to 3.9 suggested an increased bromate reduction efficiency within the culture. However, nitrate reduction was still predominating. Following the groundwater pump failure (Phases IIb and III), bromate reduction efficiency further increased, with qBr values of 41.8 – 120.9 μmol Br g dry wt^{-1} hr^{-1} obtained, leading to a stable qN/qBr ratio of < 2 in all but phase III temperature manipulation trials. Figure 8.5 shows qBr values during chemostat trials (♦) with the phase IIb/III 'high-rate' bromate reducing culture circled.

No comparable values are available for specific bromate reducing cultures, with only two studies providing sufficient data for calculation of qBr values. Data for these fixed-film (Δ) and batch (□) trials are given in Figure 8.5. The qBr range obtained by Hijnen *et al.* (1995; 1999) for bromate reduction within denitrifying batch and fixed-film cultures (0.8 – 6.9 μmol Br g dry wt^{-1} hr^{-1}) was equivalent to those calculated for phase I during current studies (0.3 – 2.2 μmol Br g dry wt^{-1} hr^{-1}). A similar value was reported by van Ginkel *et al.* (2005b) for batch denitrifying cultures, where a maximum qBr value of 3.8 μmol Br g dry wt^{-1} hr^{-1} was obtained. No biomass growth data were provided by van Ginkel *et al.* (2005b) for the specific bromate reducing continuous-flow culture, but specific bromate reduction rates were measured in a batch culture inoculated with these strains. A qBr value of only 10.6 μmol Br g dry wt^{-1} hr^{-1} was obtained in this case, agreeing with observations during current trials that high bromate reduction rates under continuous-flow conditions are not maintained upon reintroduction into batch culture.

Specific reduction rates are not routinely calculated during nitrate and perchlorate reduction trials, with reduction rates providing a more applied measure of process efficiency. However, Hollo and Czako (1987) did report a maximum qN value of 752.5 μmol N g dry wt^{-1} hr^{-1} for a pilot-scale fluidised bed denitrification reactor. Malmqvist *et al.* (1991) calculated specific chlorate reduction rates for a chlorate reducing culture propagating on acetate. Specific rates calculated of 336 – 404 μmol Cl g volatile suspended solids $(VSS)^{-1}$ hr^{-1}, and those obtained by Hollo and Czako (1987) were both roughly comparable to qN values obtained during phase I and IIa chemostat trials. Lower maximum qBr values were obtained in current studies, even for the 'high-rate' Phase III bromate reducing chemostat culture. This provides evidence bromate reduction is a less efficient form of anaerobic respiration than either denitrification or chlorate reduction.

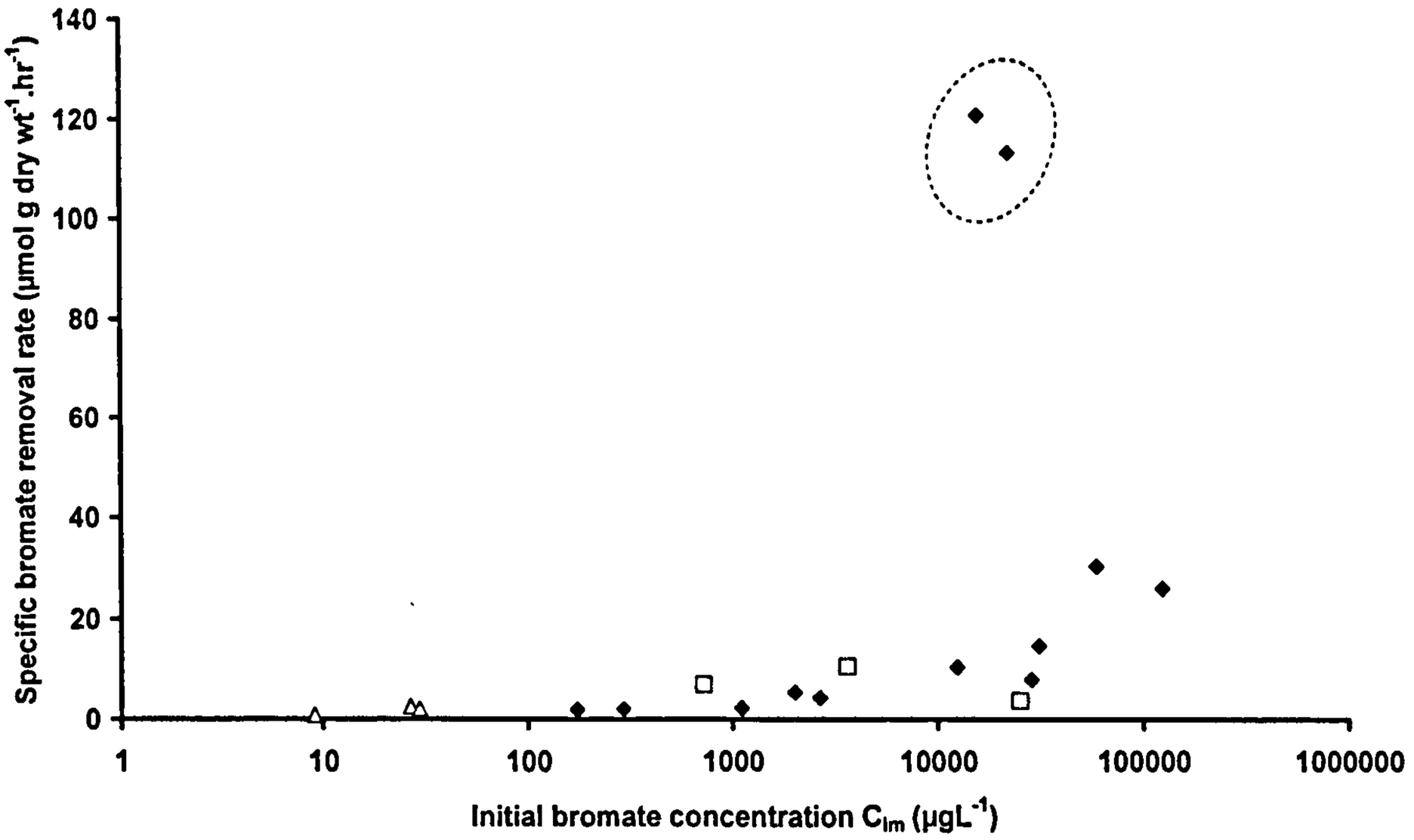


Figure 8.5 - Specific bromate reduction rates as a function of initial bromate concentration. Data from chemostat (♦) trials during the current study, plus previous fixed-film (Δ) and batch (□) trials are shown (Hijnen *et al.*, 1995; Hijnen *et al.*, 1999; van Ginkel *et al.*, 2005b).

Chemostat and batch trials showed that, at high bromate influent concentrations, inhibition of the microbial consortium occurred, resulting in a lower bromate reduction rate compared with that expected. Chemostat phase IIa trials, which investigated bromate spiking in the range 1 – 128 mg L^{-1}, revealed a fall in reduction rate at the highest bromate concentration (128 mg L^{-1}). Reduction rate within the total system (80-hour RT) fell from 240.5 µg L^{-1} hr^{-1} with a 64 mg L^{-1} bromate influent to only 149.9 µg L^{-1} hr^{-1}. This rate decrease was also correlated with a fall in both biomass and TOC usage within both reactors. Reactor 1 exhibited the most obvious biomass change as, having shown steady biomass concentrations (0.031 – 0.042 g dry wt L^{-1}) at all other bromate concentrations, exhibited an abrupt drop to only 0.013 g dry wt L^{-1} at the 128 mg L^{-1} bromate influent (Figure 7.24). Total TOC consumption (Figure 7.26) also fell to only 54.1% of a 115.8 mg L^{-1} (as glucose) influent. A similar decrease in biomass and TOC usage at the 80 mg L^{-1} bromate influent level was noted in many of the phase I results (Section 7.2.1.1), one example being a lowering of biomass concentration from 0.104 to

0.085 g dry wt L^{-1} with influent bromate concentrations of 0.2 and 80 mg L^{-1} respectively (Figure 7.19). There was no evidence of an effect at the 64 mg L^{-1} influent concentration in chemostat phase IIa, so it appears inhibition starts to become apparent within the range 64 – 80 mg L^{-1}. Inhibition of bromate reduction at high bromate concentrations has been observed by other authors. van Ginkel *et al.* (2005b) noted total bromate reduction by biomass derived from activated sludge in batch culture with initial bromate concentrations of 15.4 – 76.8 mg L^{-1} under excess carbon conditions. With an initial bromate concentration of 192 mg L^{-1}, approximately 160 mg L^{-1} was reduced. However, at the highest bromate concentration trialled (384 mg L^{-1}), reduction of only 60 mg L^{-1} bromate occurred. The authors hypothesised production of a toxic intermediate could explain the observed effects, and suggested bromite could accumulate, although no supporting experimental evidence was given. Potentially toxic effects of bromate were also noted on sludge bacteria (Figure 7.8), although in this case the dose required to attain an inhibitory effect was much higher (> 200 mg L^{-1}), possibly either due to the more fundamental nature of the parameter being measured (respiration inhibition), or a buffering effect protecting bacteria within biomass flocs. The cause of inhibition in both cases, whether direct bromate toxicity, indirect toxicity via an intermediate or even as a result of potassium addition, is unknown. However, results suggest bromate reduction rates are retarded, and therefore bromate contamination may not be fully biologically remediated in groundwater by heterotrophic suspended growth processes at concentrations higher than 64 – 80 mg L^{-1}.

8.2.2 Bromate reduction mechanisms

As microbially-mediated bromate reduction is a sparsely studied topic, little information is available regarding possible mechanisms. A review of available literature (Chapter 2) suggested more than one reduction mechanism to be feasible, with studies of perchlorate reduction mechanisms also giving an insight into comparable processes. Early investigations concluded that perchlorate reduction was not mediated by specific perchlorate reducing microbial strains, but instead via co-metabolism using nitrate reductase enzymes within denitrifiers (Xu *et al.*, 2003). However, the subsequent discovery of specific perchlorate reducing strains, some of which are unable to reduce nitrate (Wolterink *et al.*, 2002; Wolterink *et al.*, 2003; Xu *et al.*, 2004) has led to elucidation of the separate perchlorate and nitrate reduction pathways now reported (Section 2.8.2). Investigations into bromate reduction mechanisms are currently at the early stages achieved for perchlorate reduction approximately 10 years ago. Initial studies (Hijnen *et al.*, 1995; Hijnen *et al.*, 1999; Kirisits and Snoeyink, 1999) all suggested bromate reduction to be carried out by pathways similar to those utilised for denitrification, mediated by nitrate reductase and not linked to energy conservation for growth. It was even suggested a specific pathway was unlikely to exist (Hijnen *et al.*, 1995). However, more recent trials have confirmed the existence of a specific bromate reducing pathway, and that this pathway can also be utilised for microbial growth (van Ginkel *et al.*, 2005b). The identity of bacterial strains mediating this pathway has not currently been investigated. However, current trials have studied a range of properties of bromate reduction, and from data collated further information regarding mechanisms involved can be obtained.

It is thought the most energetically favourable possibility for bromate reduction with glucose as electron acceptor would be direct anaerobic respiration (Butler *et al.*, 2004) using Equation 2.12. During phase I and IIa chemostat trials and all batch trials the high ratios of nitrate to bromate reduction (qN/qBr) suggested the presence of a similar denitrifying culture to that noted by Hijnen *et al.* (1995; 1999). Within this system, the relatively high denitrification rate suggested a majority of any direct glucose utilisation would occur via anaerobic respiration for nitrate reduction (Equation 8.4).

$$C_6H_{12}0_6 + 4NO_3^- \rightarrow 6CO_2 + 2N_2 + 6H_2O$$

Equation 8.4

Batch trial B, which used phase I chemostat inoculum, exhibited slightly enhanced bromate reduction with glucose addition than using either acetate or ethanol carbon sources. This may indicate that direct glucose utilisation by denitrifiers was enhancing bromate reduction by co-metabolism. However, other results suggested the major route for anion reduction was not via direct glucose utilisation. Periodic analysis for glucose within chemostat supernatant using the test strip methodology (Appendix A) did not detect glucose above the limit of detection (1 mg L^{-1}) in any sample. Batch trial G also showed that glucose concentrations dropped dramatically within a batch culture system, with an initial 190 mg L^{-1} reduced to < 1 mg L^{-1} after only 48 hours. The simultaneous rapid rise in absorbance (0.031 to 0.097 g dry wt L^{-1}) coupled with acetate and ethanol formation (Section 7.2.3.3) indicated that fermentative microbes were predominating during this initial period. With a period of high glucose availability, such as at the commencement of a batch trial, fermentation by microbes able to mediate the process could produce a range of products such as ethanol and acetate. The overall reaction occurring during fermentation of ethanol is given in Equation 8.5.

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + CO_2$$

Equation 8.5

Bromate reduction by anaerobic respiration could potentially then occur with fermentation products via reactions such as that given in Equation 8.6 for ethanol.

$$CH_3CH_2OH + 2BrO_3^- \rightarrow 2CO_2 + 2Br^- + 3H_2O$$

Equation 8.6

Alternatively, fermentative metabolism using bromate as an electron acceptor could occur, leading to an energetically more efficient reaction than that normally followed during fermentation (Equation 8.7). The energy yield for this pathway would be

4 moles of adenosine triphosphate (ATP) per mole glucose consumed compared with 2 moles ATP for Equation 8.5, thus giving any fermentative bacteria with this capability a selective advantage over other strains.

$$3C_6H_{12}O_6 + 4BrO_3^- \rightarrow 6CH_3COOH + 6CO_2 + 6H_2O$$

Equation 8.7

It has been suggested (Butler *et al.*, 2004) that bromate reduction could also be mediated by biotic autotrophic mechanisms. Nitrate can be biotically autotrophically reduced by reduced inorganic species such as Mn^{2+}, Fe^{2+} and HS^- (Korom, 1992). For example, microbial denitrification using Fe^{2+} has been reported (Guoy *et al.*, 1984; cited in Korom, 1992), with the bacterium *Gallionella ferruginea* able to reduce nitrate to nitrite and abiotic reduction mediating further reduction to N_2. Nitrate reduction by reduced sulphate has also been observed. Ferrous disulphide (FeS_2), commonly known as pyrite, has been shown to reduce nitrate in a reaction mediated by the bacteria *Thiobacillus denitrificans* (Equation 8.8) in an area near Hanover, Germany (summary in Korom, 1992). A comparable reaction for bromate could be suggested as in Equation 8.9.

$$5S^{2-} + 8NO_3^- + 4H_2O \rightarrow 5SO_4^{2-} + 4N_2 + 8OH^-$$

Equation 8.8

$$3S^{2-} + 4BrO_3^- \rightarrow 3SO_4^{2-} + 4Br^-$$

Equation 8.9

However, this pathway could only occur following sulphate reduction to form the reduced sulphur species. Therefore, whilst it cannot be ruled out by current studies, microbial bromate reduction by reduced sulphur species is considered unlikely as sulphate reduction was only generally noted following removal of bromate.

One final potential biotic bromate reduction mechanism, investigated by van Ginkel *et al.* (2005a), is autotrophic reduction by hydrogen. Although unlikely to be

present in current studies, promising results have been obtained utilising microbes able to mediate this pathway. Use of a chlorate-acclimatised autotrophic hydrogen-fed gas-lift bioreactor led to reduction of up to 800 mg L^{-1} influent bromate after only 10 days acclimation with a 6 – 48 hour RT. In this study, which utilised considerably higher bromate concentrations than current studies apparently without occurrence of inhibition, the maximum bromate removal rate of 104.2 mg L^{-1} hr^{-1} was substantially higher than even the high rate bromate reduction (6132 µg L^{-1} hr^{-1}) obtained by van Ginkel *et al.* (2005b) using a heterotrophic bioreactor. The following reaction was proposed for reduction of bromate in this system (Equation 8.10).

$$BrO_3^- + 3H_2 \rightarrow Br^- + 3H_2O$$

Equation 8.10

The high bromate reduction rate achieved suggests that autotrophic bromate removal may exhibit different kinetics to that observed in heterotrophic bioreactors. This agrees with Logan (2001) who observed that, of the two perchlorate reducing autotrophic bioreactor trials reviewed, both exhibited enhanced 'rate profiles' relative to comparable studies completed with organic carbon sources. However, the temperature utilised by van Ginkel *et al.* (2005b) was higher (30 – 36°C) than that for the equivalent heterotrophic study (20°C) and, as it is currently the only published study investigating autotrophic bromate reduction, no confirmation of this encouraging result is possible.

With bromate reduction shown in current studies to be dependent on influent glucose concentration in both phase I (Section 7.2.1.1) and phase IIIa (Section 7.2.1.3) chemostat trials, it is considered that heterotrophic mechanisms are the most likely source of observed bromate reduction. However, it is possible that this heterotrophic bromate reduction within the systems trialled is mediated by a wide range of microbial strains. Examination of (per)chlorate reducing bacteria has revealed a pylogenetically diverse membership, spread across four sub-classes (α, β, γ and ε) of the Proteobacterial phylum and exhibiting diverse metabolic capabilities (Coates and Achenbach, 2004). Therefore, a wide range of pathways may be utilised within any microbial consortium. Only by investigation of individual isolates can specific pathways be fully elucidated.

Despite the possibility of co-existence of different reduction pathways within the bioreactors, analysis of bromate reduction rate data (Section 8.2.1) suggested that two distinct microbial consortia were present during current studies. During initial (Phase I/IIa) chemostat trials and all batch trials, it is suggested a co-metabolic bromate reduction mechanism mediated by denitrifying bacteria predominated, due to similarities in 'rate profile' (Figure 8.4) with previous studies (Hijnen *et al.*, 1995; Hijnen *et al.*, 1999; Kirisits and Snoeyink, 1999). Microbes isolated by Hijnen *et al.* (1995) following the bromate remediation batch trials were all shown to be denitrifying *Pseudomonas* species, only exhibiting bromate reduction in the absence of nitrate. Results obtained from batch trial F also suggested the occurrence of a co-metabolic mechanism. In this trial, linear decreases in bromate reduction were noted over the experimental period (Figure 7.46 and Figure 7.47). Linear regression over the initial 16-day period shows a good fit, with R-squared values of 0.997 and 0.971 for chemostat and isolate 35 inoculum respectively. A similar, linear reduction was also observed over an 11-day period by van Ginkel *et al.* (2005b) during bromate reducing batch trials with an overdose of acetate and 38.4 mg L^{-1} bromate (Figure 8.6). The authors concluded from these data that bromate degradation observed in batch culture was not catalysed by microbes propagating on bromate and acetate and were therefore mediated by co-metabolic processes. It was argued that growth-linked bromate reduction in batch culture would be indicated by a lag phase and subsequent increasing rate over the trial period, an example of which is given by Figure 8.7 (van Ginkel *et al.*, 1995).

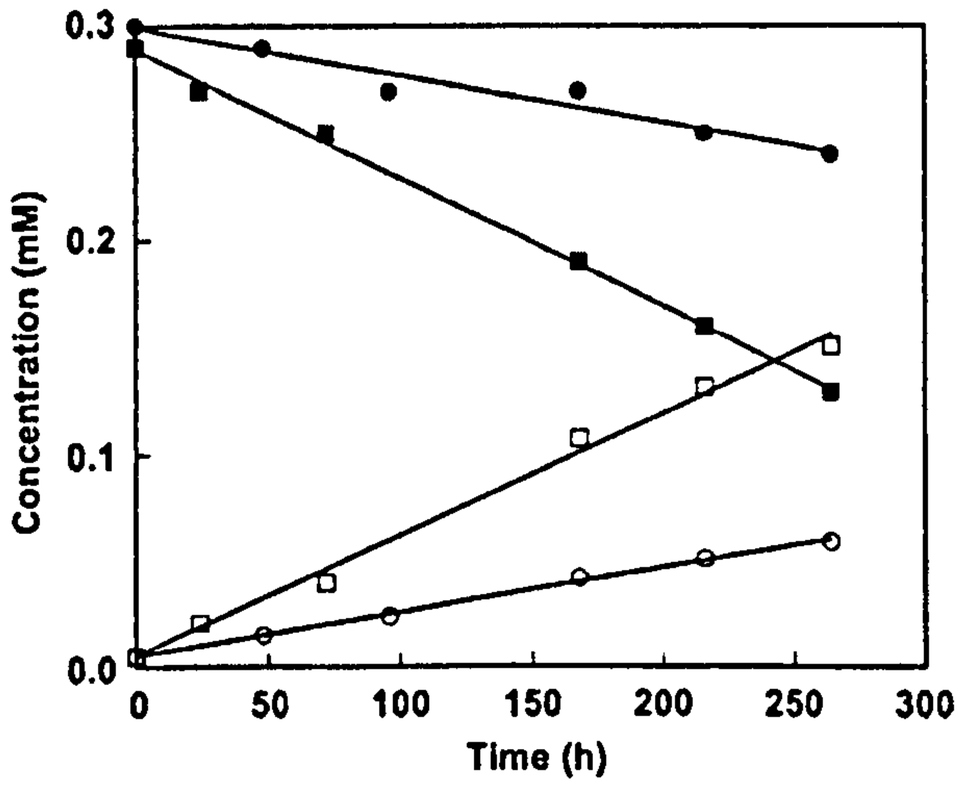


Figure 8.6 - Reduction of bromate (■,●) and formation of bromide (□,○) by two sludge-derived inocula in batch cultures with 38.4 mg L^{-1} initial bromate and an overdose of acetate (van Ginkel *et al.*, 2005b)

Chemostat phase IIa, with continuous enrichment of the culture under favourable conditions, achieved a specific rate increase from 1.8 to 30.3 µmol Br g dry wt^{-1} hr^{-1} (80-hour RT). A comparable improvement of the molar ratio (qN/qBr) from 85.5 to only 6.0 was also observed, despite maintenance of nitrate influent at 38.2 mg L^{-1}. This improvement confirms the efficacy of a chemostat system exerting continuous selection pressures, and may also suggest that enrichment of the culture by strains able to reduce bromate without also conducting denitrification had occurred. Whilst Hijnen *et al.* (1999) also used a continuous-flow bioreactor (ethanol-fed) for bromate reduction trials, only very short periods of bromate spiking were maintained within this denitrifying system. Therefore, little enrichment of bromate reducing ability would have been expected to occur relative to denitrification activity within the timescale of these trials. However, despite the observed increases in bromate reduction rate during chemostat phase IIa, inspection of the 'rate profile' data (Figure 8.4) still suggested the presence of a substantially co-metabolic bromate reducing population.

With the period of groundwater feed failure in Phase IIb chemostat trials, a sharp increase in TOC content of both reactors was observed (Section 7.2.1.2). Biomass concentrations also increased rapidly, peaking at 0.084 and 0.150 g dry wt L^{-1} (40 and 80-hour RT respectively). Growth rate of typical fermentative microbe strains (*Escherichia*

coli sp.) are within the region 0.30 – 0.35 hr^{-1} (Prescott *et al.*, 1999; Gonzalez *et al.*, 2002), with comparable maximum growth rates (known as μ_m) reported for denitrifiers (0.39 hr^{-1}) propagating on glucose (Mazierski, 1994). Growth rate of perchlorate reducers propagating on glucose-glutamic acid was reported by Logan *et al.* (1998) to be 0.17 hr^{-1}. By contrast, bromate reduction in current studies was only sustained under suspended growth conditions with a minimum RT of 40 hours in current studies, suggesting a growth rate of ≤ 0.025 hr^{-1} for a sustainable bromate reducing consortium. This indicates the rapid biomass increase noted during the period of glucose excess to have been largely mediated by fermentative strains and/or denitrifiers instead of bromate respiring microbes. Although not measured, it is therefore likely the availability of fermentation products within the solution increased. Fermentation may have occurred via either direct fermentation to products such as ethanol (Equation 8.5), or fermentation utilising bromate as electron acceptor leading to acetate production (Equation 8.7). However, although the exact mechanism cannot be elucidated from this incident, 'rate profile' data (Figure 8.4) shows a subsequent favourable shift in the microbial consortium which was sustained following restoration of the groundwater supply. It is possible an excess of fermentation products during this period may have elicited this shift leading to enrichment of bacteria, such as those capable of utilising fermentation products (ie. Equation 8.6) but not glucose for anaerobic respiration, previously present in the culture but only in low numbers.

8.2.3 Carbon usage by a bromate reducing population

Denitrification has been successfully achieved using a range of carbon sources including ethanol (Dahub and Lee, 1992; Moreno *et al.*, 2005), methanol (Gauntlett and Craft, 1979) and acetate (Mohseni-Bandpi and Elliott, 1998). Glucose (Williams *et al.*, 1978; Bengtsson and Bergwall, 1995) has been used for denitrification trials, but no indication was given as to whether carbon utilisation was via direct glucose respiration or a fermentative pathway. The majority of perchlorate reduction trials have utilised the potential fermentation product acetate as carbon source and, although a wide variety of organic substrates can be used for growth of perchlorate reducing strains (Xu *et al.*, 2003), other authors have demonstrated that complex sugars such as glucose are not directly utilised by some isolated (per)chlorate degraders. Bruce *et al.* (1999) isolated a

perchlorate reducing strain from paper mill waste able to use fermentation products including acetate and lactate, but unable to directly utilise glucose. Coates *et al.* (1999) obtained a similar result following characterisation of thirteen isolates, all of which could metabolise acetate and lactate but not glucose. Van Ginkel *et al.* (1995) investigated glucose metabolism within a chlorate reducing batch system. It was noted glucose was rapidly consumed, so that after 3 days an initial 1800 mg L^{-1} glucose dose was reduced to below the limit of detection (not given). A concomitant increase in acetate and formate was observed, indicating the occurrence of glucose fermentation. Growth-linked chlorate reduction with stoichiometric chloride production was achieved in this trial, but did not commence until day 5, by which time no glucose remained for direct utilisation. Figure 8.7 shows the results obtained by van Ginkel *et al.* (1995), who concluded growth of chlorate-reducing strains was only occurring on fermentation products including acetate and formate, and not directly on glucose.

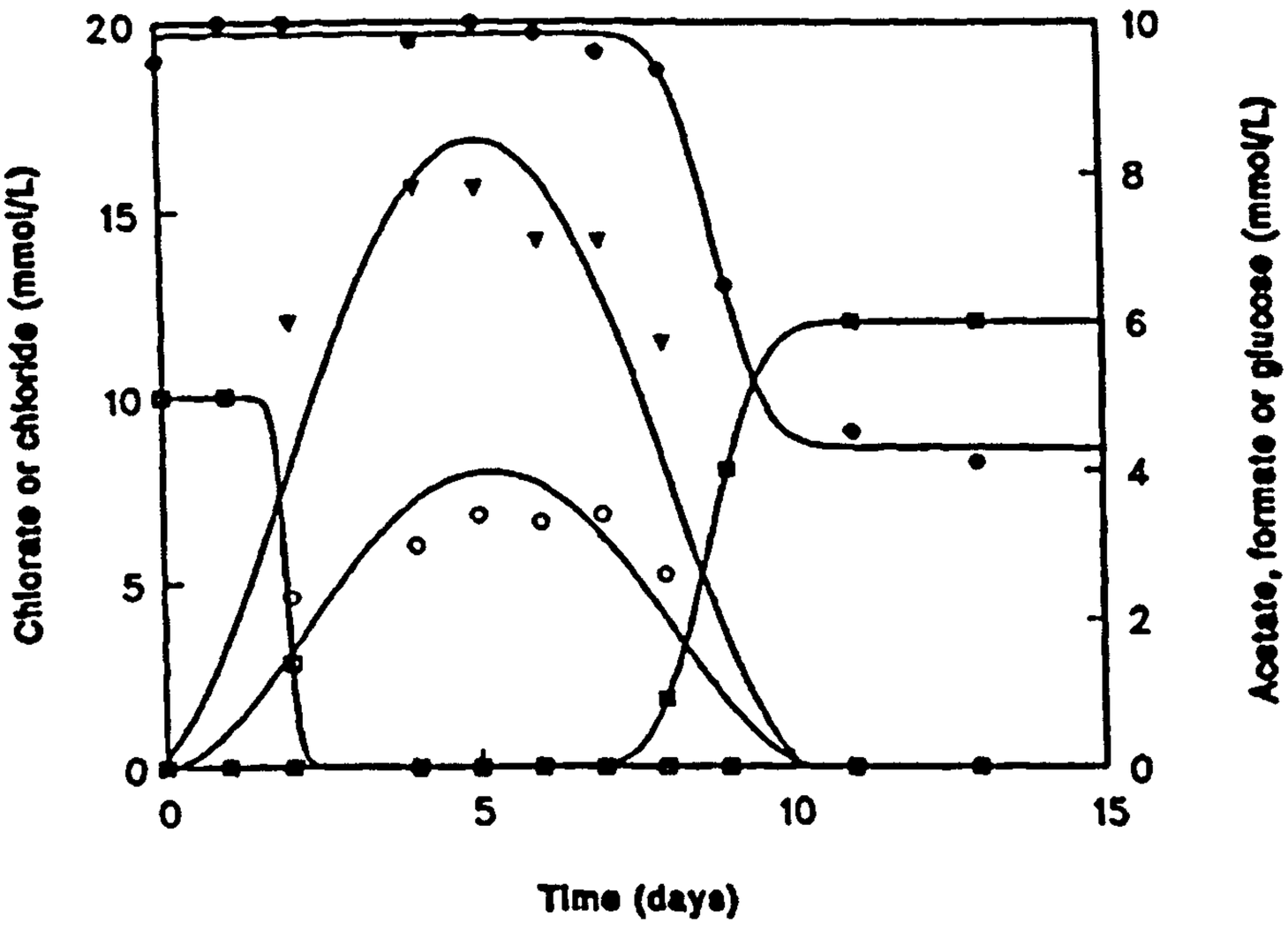


Figure 8.7 - Conversion of glucose (□) into acetate (○) and formate (▼), and subsequent utilisation of these fermentation products for chlorate (●) reduction to chloride (■) in batch culture (van Ginkel *et al.*, 1995)

All published studies to date investigating microbial bromate reduction have utilised potential glucose fermentation products as carbon source. Ethanol was dosed in

excess (Hijnen *et al.*, 1995; Hijnen *et al.*, 1999) during investigations into bromate reduction within a denitrifying consortium. Ethanol concentrations of 50 – 100 mg L^{-1} (batch trials) and 40 mg L^{-1} (continuous-flow trials) were used, with 0.2 – 5 mg L^{-1} and 15 – 35 µg L^{-1} bromate spikes respectively. BAC trials carried out by Kirisits *et al.* (2001), dosing a roughly equal mixture of pyruvate and lactate at total concentrations (as carbon) of 0.6 – 3.6 mg.L^{-1}, observed that denitrification (1.6 mg L^{-1} nitrate influent) increased with carbon addition up to almost 100%. Bromate reduction (of a 21 µg L^{-1} influent) also increased to the limit of detection with carbon doses above 1.0 mg L^{-1}. Results suggested denitrifying strains were propagating on the pyruvate/lactate carbon source in this case, thus enhancing bromate reduction by co-metabolism. Acetate addition has also been trialled, with van Ginkel *et al.* (2005b) dosing excess acetate in both batch and continuous flow cultures.

It is known that synergistic relationships between respirative and fermentative bacteria are important mechanisms allowing total carbon usage within the subsurface environment (Section 2.8.1). Therefore, with no studies having demonstrated bromate reduction by direct utilisation of glucose, it is possible that glucose fermentation could be required prior to utilisation. Batch trials I and K investigated bromate reduction by isolated strains (Trial I from dilution-to-extinction cultures and Trial K from plating studies). In both cases bromate reduction rate by isolates was lower than equivalent rates noted with a direct chemostat inoculum (Section 7.2.3.5). This was initially taken as evidence for failure of the isolation process. However, another explanation could be the inability of isolated non-fermentative bromate reducing strains to efficiently utilise glucose in the absence of a separate fermentative microbial consortium. This provides evidence that a microbial consortium would be necessary or at least beneficial for efficient bromate reduction using glucose as carbon source.

Studies of isolated (per)chlorate reducing strains have shown some to be incapable of fermentation (Coates *et al.*, 1999; Bruce *et al.*, 1999; Wolterink *et al.*, 2002), thus demonstrating microbial (per)chlorate reduction on a glucose carbon source would require a separate fermentative consortium. A similar conclusion was reached by van Ginkel *et al.* (1995), who argued that fast-growing glucose fermenting strains were

present in glucose-augmented batch cultures, and that it was only the presence of these bacteria which enabled growth of chlorate reducers utilising fermentation products. Other trials have also noted a necessity for a microbial consortium. Miller and Logan (2000) observed a mixed culture was required for autotrophic perchlorate reduction within a continuous flow packed bed bioreactor. The authors isolated a perchlorate reducing bacterium (*Dechlorimonas sp.* JM) from the bioreactor. However, it was not an autotrophic strain and was unable to grow without a carbon source. Therefore, to allow autotrophic reduction other microbes must have been essential to the overall mechanism. Giblin *et al.* (2000b) also noted that, of four strains isolated from an autotrophic perchlorate reducing reactor, none was able to directly reduce perchlorate to chlorate. It was speculated the function of these strains may be in removing residual DO, thus allowing proliferation of unidentified anaerobic perchlorate reducers.

Batch trial G suggested that bromate reduction following fermentation was only partially related to acetate or ethanol utilisation, as little decrease in their concentration was noted despite continuance of bromate reduction. This contrasts with the acetate utilisation by chlorate reducers (Figure 8.7) observed in van Ginkel *et al.* (1995). However, it is likely that other, unmeasured fermentation products were also present, one example noted by van Ginkel *et al.* (1995) being formate. These products may have instead been preferentially utilised by the microbial consortium. During chemostat phases IIb and III, a roughly constant percentage TOC utilisation (ie. 75.5% during Phase IIb, 80-hour RT) was observed, regardless of the influent glucose concentration. Phase IIIa exhibited a maximum TOC utilisation of 75.2% even with a 100 mg L^{-1} glucose influent. These results suggest a proportion of carbon present in solution (approximately 25%) was unavailable for utilisation by the high-rate microbial consortium. Prior to the chemostat groundwater pump failure in phase IIa, a higher TOC utilisation of 78.7 – 90.7% was noted. This suggests the microbial consortia present within the 'high-rate' and co-metabolic cultures may exhibit subtly different carbon requirements. It is therefore possible that a relatively fastidious bromate reducing strain was enriched within the high-rate bromate reducing chemostat culture. However, further trials would be necessary to more fully elucidate carbon requirements of the enriched strains.

8.2.4 Use of isolation and batch study techniques for bromate bioremediation

Isolation of bromate reducing microbial strains was attempted by agar plate techniques and also a dilution-to-extinction trial during current studies. Agar plate culture produced a range of isolates, subsequently trialled for bromate reduction in batch flask trials (J, K, M and N). In all batch trials low bromate reduction rates were achieved, with examples of maximum rates for trials M and N of 5.3 µg L hr^{-1} and 6.2 µg L hr^{-1} respectively. These rates were substantially lower than those obtained within the chemostat system, where reduction rates in excess of 500 µg L hr^{-1} were routinely obtained during latter phases. The batch trial bromate reduction rates obtained, even with a direct chemostat inoculum, were more representative of those achieved during phase I chemostat trials (0.9 – 27.8 µg L hr^{-1}). This suggests that growth in batch culture provides different selection pressures within the flasks, thus leading to results more indicative of early stage chemostat growth than later acclimatised and enriched phases.

The observed disparity in bromate reduction rates between the two systems may be due to differences between chemostat and batch culture. Under batch culture conditions, initial concentrations of glucose, bromate and nitrate are high and nutrient limitation is unlikely to be a major controlling factor. This allows a short exponential growth phase with microbial growth rates at their maximum (μ_m), thus favouring the fastest-growing strains within a microbial consortium. Nutrient exhaustion is the final stage in a batch culture, eventually leading to starvation conditions. Conversely, a chemostat culture under steady influent conditions is a self-regulating system, with a continuous input of nutrients. Substrate limitation allows stable operation, with growth rate (μ) less than maximal (ie. $\mu < \mu_m$) (Pirt, 1975). This may eventually allow enrichment and predomination of a strain more suited to the medium, but exhibiting slower growth kinetics. Therefore, within a mixed culture or semi-purified inoculum, the strains dominating batch cultures may be different to those in a continuous culture system. This difference has been noted previously in bromate bioremediation studies. It was concluded by van Ginkel *et al.* (2005b) that bromate reduction observed within batch cultures (Figure 8.6) was mediated via co-metabolism of bromate by denitrifying bacteria. Only when a continuous-flow column was constructed and operated was

growth-linked bromate reduction obtained, suggested to be due to culture of different microbial consortia within the two systems. Kirisits (2000) also observed low bromate reduction rates (≤ 0.02 µg L hr^{-1}) with bromate reducing isolates placed in batch culture. Results from the current study suggest similar effects leading to differing selection pressures operating within batch and continuous flow trials, thus invoking divergent bacterial populations. Batch cultures may have favoured opportunistic high growth rate denitrifying or fermentative microbes, outcompeting any high rate bromate reducing strains. This again suggests that μ_m for high-rate bromate reducing strains is substantially lower than that of typical groundwater bacteria. Therefore, although batch flasks were proven as a useful indicator of low-rate bromate reducing ability, possibly via co-metabolism, the observed limitations of the technique also need to be taken into account during interpretation of results.

Use of agar plating was successful in producing isolated strains, although the limitations of batch trials outlined above suggested that efficacy of any isolated high-rate bromate reducing strains was not fully investigated. However, colony growth was slow, with an 8-week period required for production of sufficiently visible colonies. Many of the isolated colonies also did not respond well to subsequent agar sub-culturing. This suggests the agar medium trialled was either deficient in one or more nutrients, or simply sub-optimal for growth of bromate degrading strains. Agar composition is critical for successful isolation of anion degrading microbes, with no single medium and set of growth conditions able to permit growth of all microorganisms present in a natural population (Stanier *et al.*, 1986). An early attempt to isolate perchlorate reducing strains were reported to be unsuccessful (Attaway and Smith, 1993) but advancements in isolation techniques meant that, by 1999, 6 isolates had been reported (Coates *et al.*, 1999). Six years later over 40 strains have been isolated (Coates and Achenbach, 2004). The only documented study for isolation of bromate reducers using plating techniques observed growth after only 3 weeks, with 9 potential isolates produced (Kirisits, 2000). Further trials would be required to investigate and optimise medium composition, with development of techniques for confirming high-rate bromate reducing ability also required. In addition, alternative methods such as dilution-to-extinction (Batch trial J)

may allow isolation of strains unamenable to agar plate growth as, although the majority of bacteria can grow on solid agar media, not all strains have or can be successfully isolated using plating techniques (Stanier *et al.*, 1986).

8.2.5 Other factors affecting bromate bioremediation

8.2.5.1 Dissolved oxygen

Inspection of the Gibbs Free Energy changes for the major redox reactions anticipated to occur within a bromate reducing system (Section 2.8.1) shows oxygen will be reduced, via aerobic respiration, in preference to all other anions. As many denitrifying strains are facultative anaerobes, they will preferentially utilise oxygen until supplies become limiting. The concentration of DO at which this occurs varies between species. There are reports of strains able to utilise nitrate at DO concentrations as high as 6.9 mg L^{-1}, but the majority switch to nitrate reduction at much lower DO levels (Korom, 1992). For example, little denitrification was reported to occur within both fixed-film and suspended growth bioreactors with DO in excess of 2 mg L^{-1}. Perchlorate reducers have been reported as similarly sensitive to oxygen, with concentrations less than 2 mg L^{-1} enough to inhibit perchlorate reduction by the strain *Azospira suillum* (Chaudhuri *et al.*, 2002). Raising influent DO concentration from 2.1 to 3.8 mg L^{-1} was shown by Kirisits *et al.* (2000) to reduce bromate removal by 11 – 15% with a 20 µg L^{-1} bromate influent during BAC trials. Higher DO concentrations (3.8 – 13.6 mg L^{-1}) produced no effect on bromate reduction. Effluent DO concentrations were below limits of detection (0.1 mg L^{-1}) with influent values of 2.1 – 5.8 mg L^{-1}. Hijnen *et al.* (1995) noted an absence of bromate reduction under aerobic conditions, when batch flasks were continuously aerated. Van Ginkel *et al.* (2005b) also stated bromate reduction did not occur in the presence of oxygen, but concentrations trialled were not given. Equivalent autotrophic trials with a hydrogen electron donor noted addition of 20% oxygen halted all bromate reducing ability, although the reversible nature of this procedure demonstrated that strains responsible were not strict anaerobes (van Ginkel *et al.*, 2005a).

During current studies, nitrogen sparging of influent chemostat supplies lowered influent DO concentrations to $\leq$ 4.4 mg L^{-1}, with supernatant DO concentrations all

measured as < 2 mg L^{-1}. Although the influence of DO on bromate reduction rate was not directly studied, bromate degradation during phase IIb/III chemostat trials confirmed that high-rate reduction can occur with low but measurable influent DO concentrations, with the DO being further lowered within the reactor by the microbial consortium (influent and supernatant phase III averages of 2.9 mg L^{-1} and 1.4 mg L^{-1} respectively). This concurs with Xu *et al.* (2003) who concluded that, whilst the exact effect of oxygen on perchlorate reduction within bioreactors is still unclear, it does not generally appear to be a problem for steady-state systems. Bromate reducing strains are not permanently inactivated by oxygen exposure as, despite no culture removed from a bioreactor being handled under strictly anaerobic conditions during current studies, bromate reducing ability was always maintained upon reincubation. It appears likely that bromate reducers may be facultative anaerobes and able to utilise both bromate and oxygen, which is analogous to many denitrifying strains (Cartmell, 1997). Pilot-scale fixed film trials did not attempt reduction of DO levels, with an average influent concentration of 6.1 mg L^{-1} observed. This fixed-film system was therefore shown to have a relatively high tolerance to influent DO concentrations, suggested by Kirisits and Snoeyink (1999) to be due to DO gradients within a biofilm (Figure 8.8). Outer layers of the biofilm were hypothesised to reduce DO levels, thus protecting the bromate reducing culture by maintaining low DO concentrations within deeper layers. Alternatively, low influent flow rates coupled with excess carbon dosing may have facilitated maintenance of low DO concentrations throughout the reactor, with residual DO largely removed by aerobic microbial processes at or near the influent supply port.

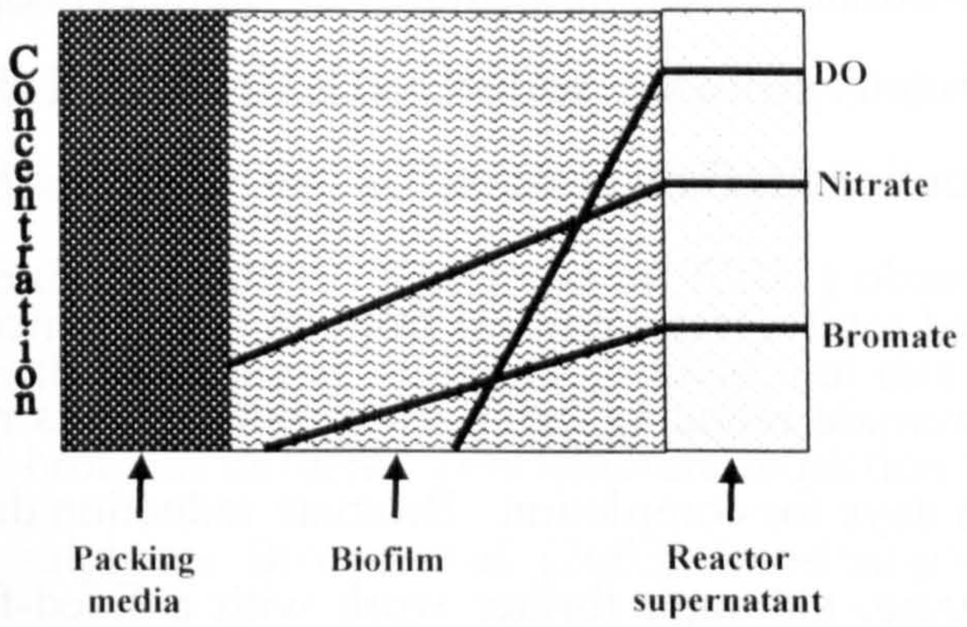


Figure 8.8 - Possible depletion traces of bromate, nitrate and dissolved oxygen on a fixed-film biofilm (Adapted from Kirisits and Snoeyink, 1999)

8.2.5.2 Competing anionic electron acceptors

Denitrification also has a higher Gibbs Free Energy yield to bromate reduction (Equation 2.11), so this electron acceptor could be presumed to exert an effect on efficacy of bromate reduction. Nitrate has been shown to inhibit perchlorate reduction, taken as evidence for preferential utilisation of nitrate as an electron acceptor over perchlorate, by strains capable of utilising both species (Coates and Achenbach, 2004). However, the interspecies variability in effects of nitrate on perchlorate reduction outlined in Section 2.8.3 suggests the presence of differing reduction mechanisms in different cultures. In the current study, nitrate was effectively removed in all trials regardless of bromate reducing ability. The speed at which removal was observed to occur in batch trial G (< 4 hours) however suggested an abiotic removal mechanism in batch flask samples. Further evidence for an abiotic nitrate removal mechanism, in contrast to bromate reduction which was shown to be biologically-mediated, was provided in batch trials D and E where nitrate removal (from approximately 6 mg L^{-1} to $\leq$ 0.7 mg L^{-1}) was still observed in both filtered and also sterilised samples (section 7.2.3.2). The form of this removal is not clear, but one possibility is abiotic reduction by iron species. Abiotic bromate reduction in the presence of iron is known to occur (summarised in Section 2.7.2), and abiotic reduction of nitrate to ammonia has also been observed within aquifers in the presence of iron-containing green rust compounds (Hansen *et al.*, 1996). Davison (1995) suggested that trace concentrations of iron (as Fe^{2+}) found naturally in groundwater were

sufficient for abiotic nitrate reduction. However, further studies would be required to confirm the relative importance of nitrate reduction mediated by biotic (via opportunistic environmental denitrifiers) and abiotic pathways within batch flasks, and the potential occurrence of abiotic removal in continuous flow systems.

Hijnen *et al.* (1995) obtained total nitrate removal from an initial concentration of 10 mg L^{-1} in suspended growth bromate reducing batch trials, but this was not initiated until day 2 and required a further 6 days for completion. Bromate reduction did not occur until following removal of all nitrate, although further work with a fixed-film system showed that bromate could be reduced in the presence of nitrate (Hijnen *et al.*, 1999). In this case, it was suggested the fixed-film reactor reduced bromate only in biofilm areas where nitrate was completely removed, a similar conclusion to Kirisits and Snoeyink (1999). In the current study, speed of nitrate removal within batch flasks suggested that bromate reduction would not be retarded by the presence of nitrate in this system past an initial period suggested by batch trial G to be as short as 4 hours. However, in the same trial bromate reduction also commenced with no lag period (Figure 7.48), showing that bromate and nitrate could be reduced concomitantly in a suspended growth system. This was confirmed in chemostat phase IIa, where bromate reduction (up to 15.0 mg L^{-1}) within reactor 1 (40-hour RT) was observed despite supernatant nitrate concentrations of up to 10.8 mg L^{-1}. The relatively poor bromate reduction (2.26 mg L^{-1} after 30 days incubation) observed using chemostat inoculum during batch trial L in a low nitrate medium may suggest a requirement for nitrate to induce bromate reduction by co-metabolism in a batch culture. However, with the high-rate bromate reducing chemostat culture in phase IIIb, maintenance of comparable bromate reduction rates (Table 7.13) despite a decrease of influent nitrate concentration from 11.0 to 3.6 mg L^{-1} showed that high-rate bromate reduction in the enriched continuous flow system was not dependent on nitrate concentration. This concurred with the culture obtained by van Ginkel (2005b), where high-rate bromate reduction was obtained in a low-nitrate artificial medium with a bromate-specific growth-associated enrichment culture.

Sulphate reduction was suggested by Gibbs Free Energy values to be less energy-yielding than bromate reduction (Section 2.8.1) and therefore unlikely to be favoured in

the presence of bromate. This was borne out in both chemostat and batch trials, where sulphate reduction was not observed until the removal of bromate to low levels (for example < 1.3 mg L^{-1} in batch trial G). van Ginkel *et al.* (2005b) also showed that sulphate addition did not affect bromate reduction, although mechanisms behind this were not further explored. Kirisits *et al.* (2001) observed a slight inhibition of bromate reduction with a 100 mg L^{-1} sulphate influent, but this was at maximum less than 10% of a 21 µg L^{-1} bromate influent. (Per)chlorate reduction has also been shown as unaffected by influent sulphate. Brown *et al.* (2003) dosed sulphate in a perchlorate reducing BAC bioreactor at concentrations of 1, 50 and 100 mg L^{-1} for approximately 14 days each. No detectable impact on perchlorate reduction was observed at any concentration. Current studies also confirmed that sulphate reduction capability was not lost under conditions unfavourable for sulphate utilisation within a mixed microbial consortium. During chemostat phase IIIa, an increase in influent glucose concentration from 52 mg L^{-1} to 100 mg L^{-1} rapidly initiated sulphate reduction, rising from no observed removal to an 18.5 mg L^{-1} decrease in supernatant concentration within a period of only 9 days. Following the subsequent glucose influent decrease, sulphate reduction again ceased.

Concomitant sulphate and bromate reduction was only observed in the pilot-scale bioreactor system. This observation could be explained by anion gradients in a biofilm similar to those hypothesised by Kirisits and Snoeyink (1999) for DO concentrations (Figure 8.8). Competitive inhibition between bromate and sulphate for the same reduction pathway may lead to the sequential reduction observed in the other studies. However, batch trial F suggested sulphate reduction to be mediated by a different pathway in the batch flask environment. With a mixed chemostat inoculum, sulphate reduction was observed following bromate reduction to the limit of detection (0.01 mg L^{-1}). By contrast, an isolated strain (culture 35) incubated under identical conditions exhibited no sulphate reduction, despite complete removal of all bromate from the flasks after 32 days. It is possible the bromate reducing culture 35 was incapable of sulphate reduction, with this pathway mediated by other microbial species with the mixed microbial consortium. Studies by Logan *et al.* (2001) on isolated (per)chlorate reducers concurred with this result, showing that none of the ten isolated strains were capable of

reducing sulphate. However, although the exact mechanism of sulphate reduction cannot be explained by current studies, it is clear the two are closely linked within a mixed culture environment.

Little information is available regarding competitive effects of other oxyanions on bromate reduction, and neither chlorate nor perchlorate reduction were investigated in current trials. van Ginkel *et al.* (2005b) analysed both chlorate and perchlorate reduction by the high rate bromate reducing culture obtained in that trial, and noted that neither were substantially reduced ($\leq$ 0.1 µmol g dry wt^{-1} hr^{-1}) in batch culture compared with bromate (17 µmol g dry wt^{-1} hr^{-1}). However, the ability of both (per)chlorate reducing strains (Wolterink *et al.*, 2002) and isolated (per)chlorate reductases (Kengen *et al.*, 1999) to reduce bromate has been noted, and this was further investigated by van Ginkel *et al.* (2005a) in a chlorate reducing autotrophic culture. Results showed that a chlorate:bromate molar ratio of 3:1 was required for total bromate removal (800 mg L^{-1} influent concentration). Lower ratios led to a loss of bromate reducing activity, thus confirming a dependence of bromate reduction by this culture on the presence of chlorate.

The complexity of anion utilisation preferences and characteristics by different microbial strains has previously been demonstrated by studies on (per)chlorate reducing strains (Section 2.8.3). However, operation of the enriched suspended growth chemostat using an artificial medium with no added nitrate or sulphate has demonstrated that bromate reduction can occur independently of these potentially-competing anions. With other authors only achieving bromate reduction by co-metabolism in the presence of other anions such as nitrate or chlorate (ie. Hijnen *et al.*, 1999; Kirisits and Snoeyink, 1999; van Ginkel *et al.*, 2005a), this observation has provided supporting evidence for the existence of a putative bromate-specific bacterial strain.

8.2.5.3 Temperature

Reactor temperature of a biological system is critical, with a 'rule of thumb' for microbial activity generally accepted as a doubling of growth rate for every 10°C rise, up to the optimum temperature for the strain. The temperature range of a typical mesophilic strain is between 20 – 50°C, although many can grow at lower temperatures if necessary

(Pelczar *et al.*, 1993). Attaway and Smith (1993) noted perchlorate reduction within an enrichment culture between the temperatures of 25 – 42°C, with an optimum of 42°C. At temperatures outside this range but within the extremes 15 – 45°C, growth was observed, but no perchlorate reduction occurred. Bromate reduction by autotrophic bacteria has been shown to exhibit a similar response to temperature, with a marked increase in reduction rate range observed between 10°C up to the optimum of 35°C (van Ginkel *et al.*, 2005a). Above 35°C rate swiftly decreased, with little activity observed at 45°C (Figure 8.9). This response is common in microbial systems, with temperature optimum usually found to be close to the top of the physiological range (Pelczar *et al.*, 1993).

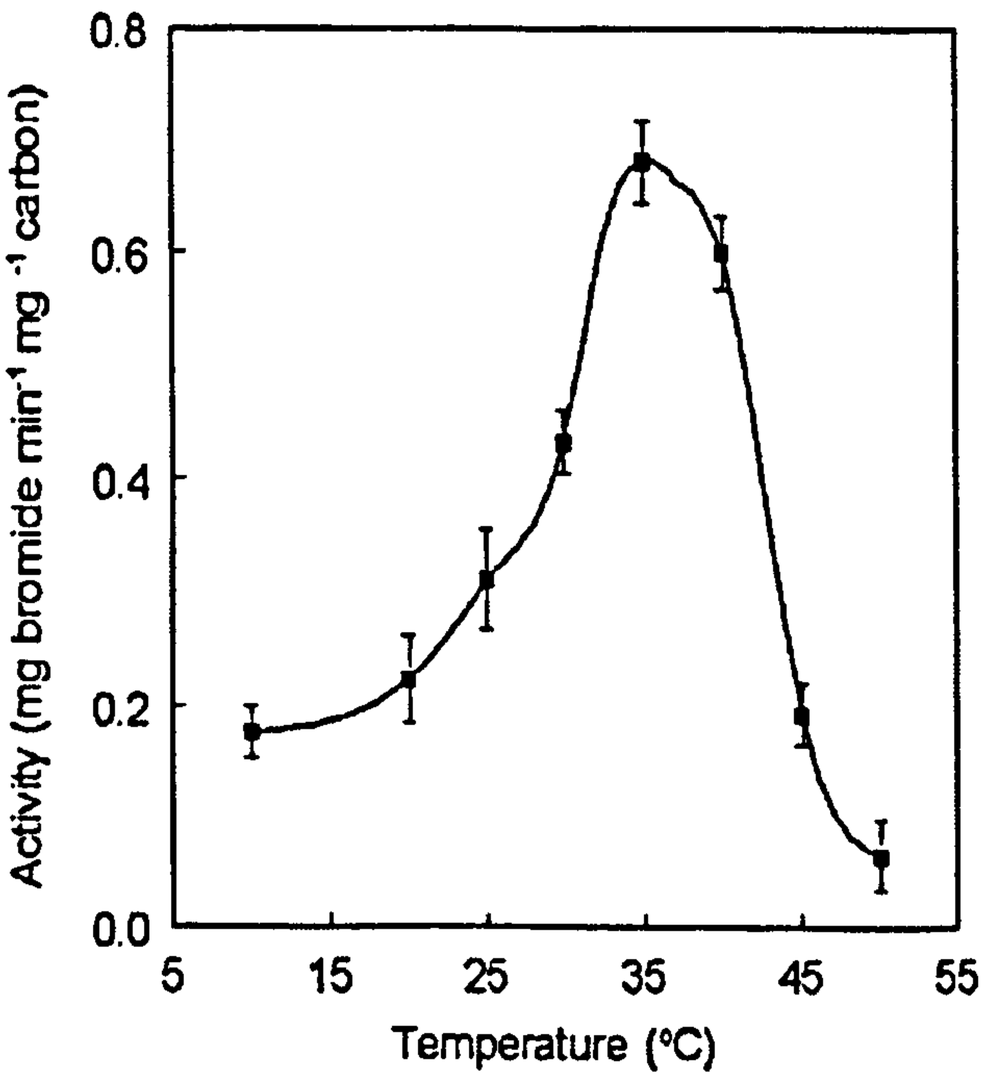


Figure 8.9 - Effect of temperature on the biological bromate reduction activity in an autotrophic bioreactor at pH 7.5 (van Ginkel *et al.*, 2005a)

In the current study, batch trial C examined bromate reduction at a range of temperatures from 6 – 44°C, which may be expected to encompass the physiological range of a mesophilic bromate reducing strain. Figure 8.10 shows bromate reduction rate as a function of temperature. Although all observed bromate reduction rates were low in this trial compared with equivalent batch trials, rate over the 32-day trial period was shown to increase higher temperatures, from 1.2 ± 0.3 μg L^{-1} hr^{-1} at 6°C to 3.3 ± 0.7 μg L^{-1} hr^{-1} at 37°C. At 44°C, the difference between bromate concentration within the six

samples (3.4 – 8.9 mg L^{-1}) after 32 days led to a large standard deviation, but a continuing general increase in rate was observed. The optimum temperature for this culture can therefore be concluded as in excess of 37°C and possibly as high as 44°C. This result indicates that both continuous-flow systems trialled were operated at sub-optimal temperatures, and that enhanced bromate reduction could be achieved by controlling temperature within an *ex-situ* reactor.

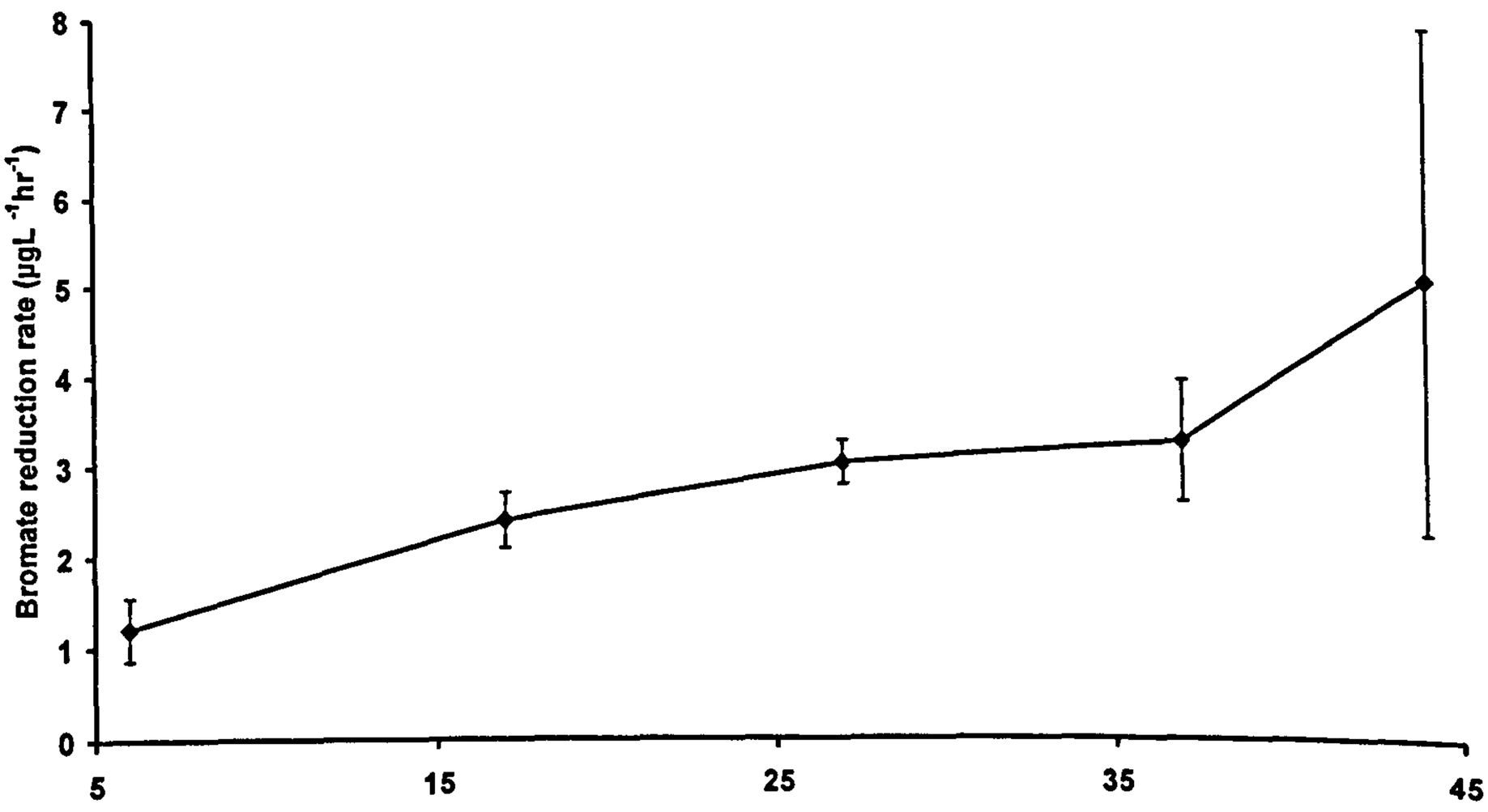


Figure 8.10 - Bromate reduction rate as a function of incubation temperature for groundwater batch trial C

The temperature dependence of pilot-scale reactor 1 over the phase B period (Figure 7.63) confirmed that temperature can have a marked effect on bromate reduction rate, even where nitrate reduction is not affected. Operation of the laboratory-scale chemostat system during phase I trials at 10°C resulted in low specific reduction rates (< 2.2 µmol Br g dry wt^{-1} hr^{-1}). A short (10-day) increase in temperature to 25°C within one reactor between phase I and II trials induced an increase in specific bromate reduction rate from 2.2 to 5.5 µmol Br g dry wt^{-1} hr^{-1} under otherwise identical conditions, suggesting a roughly doubling activity for a 15°C increase within this system. Phase II trials, conducted at 20°C, also exhibited substantially higher specific rates

(1.8 – 10.3 μmol Br g dry wt^{-1} hr^{-1}) at comparable influent bromate concentrations. Although other parameters such as influent water source and RT were also altered for phase II trials, it is likely that temperature played a role in the observed bromate reduction rate increases. Evidence of temperature dependence by the high-rate bromate reducing culture was observed during phase IIIa, where a lowering of reactor temperature from 20°C to 10°C elicited a specific rate decrease of 21.6 μmol Br g dry wt^{-1} hr^{-1} relative to the control, although no similar effect was noted at 15°C. It can therefore be concluded that temperature can affect bromate reduction rate in an *ex-situ* reactor system, with a higher temperature than those utilised in the current studies likely leading to enhanced bromate reduction within a bioreactor.

8.2.5.4 pH

The optimum physiological pH range for bacteria normally lies within the range 6 – 8, with a typical microbe required to keep an intracellular pH of around 7.5 (Pelczar *et al.*, 1993). A pH range of 6.8 – 7.2 was observed to be optimum for bromate reduction by Kirisits *et al.* (2000), with reduction efficiency falling by 25 – 30% at pH 8.2. van Ginkel *et al.* (2005a) concluded a pH range of 7 – 8 elicited the highest bromate reduction rate in an autotrophic bromate reducing bioreactor (Figure 8.11).

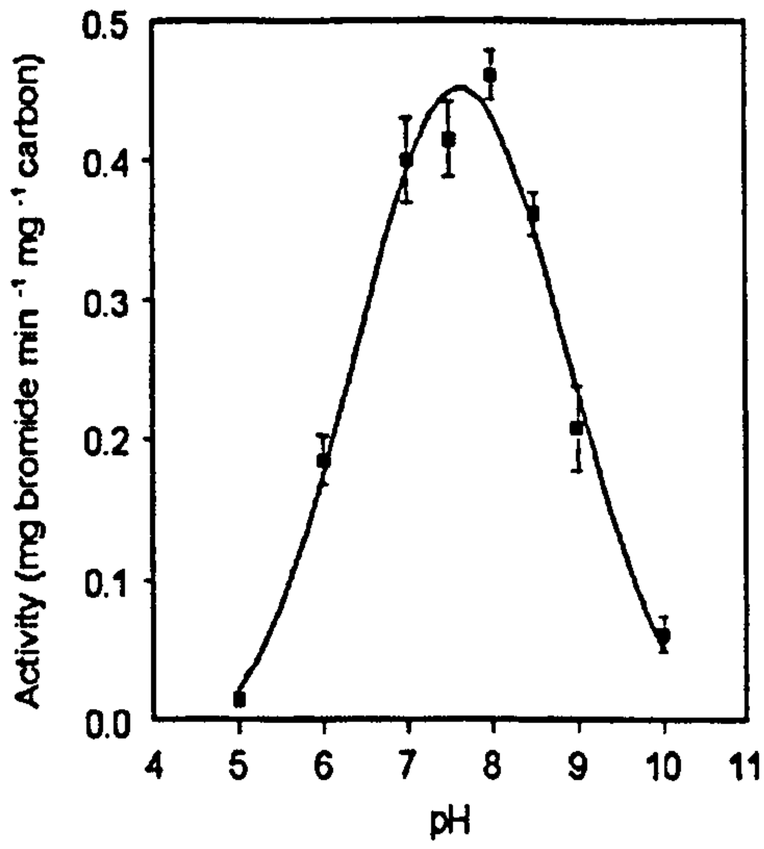


Figure 8.11 - Effect of pH on the biological bromate reduction activity in an autotrophic bioreactor at 30°C (van Ginkel *et al.*, 2005a)

Similar results have been obtained for nitrate where an optimum range of 7.0 – 8.5 was suggested by Gauntlett and Craft (1979) following a review of denitrification literature, and also perchlorate where a pH range of 6.5 – 9.0 did not elicit an alteration in biological reduction efficiency on BAC (Brown *et al.*, 2003). The optimum pH for perchlorate reducers is near neutrality (~ pH 7), but some strains have been shown to respire perchlorate with pH as low as 5.0 (Coates and Achenbach, 2004). Both the chemostat and pilot-scale bioremediation systems operation were operated with the aim of maintaining a pH range within the boundaries of 6.0 – 8.5, using pH control if necessary. A pH range of 6.5 – 8.0 was generally maintained during all chemostat trials, and an average pH value of 7.3 obtained during pilot-scale trials. pH control was not required apart from during the initial chemostat startup phase, with sufficient buffering capacity available within the reactors. It is unlikely the limited pH fluctuations (generally < 1.5 pH units) would have had a marked effect on bromate reduction capacity and pH, although necessary to be monitored, is not therefore considered a major controlling operational parameter.

8.2.5.5 Retention time

Retention times in current studies were within the range 10 – 100 hours for the pilot-scale bioreactor, and 20 – 80 hours for chemostat cultures. A comparable RT (48 hours) was utilised by van Ginkel *et al.* (2005b) for bromate reduction with acetate addition, with autotrophic bromate reduction investigated at retention times of 6 and 48 hours (van Ginkel *et al.*, 2005a). Other bromate reduction trials have used lower retention times. Hijnen *et al.* (1999) worked within the range 18 – 26 minutes and Kirisits and Snoeyink (1999) investigated 25 and 49 minute retention times, although in both cases influent bromate was also significantly lower (≤ 35 µg L^{-1} in both cases) than current studies. These lower RT values are comparable to those utilised in many perchlorate reduction trials. Kim and Logan (2001) fully reduced a 20 mg L^{-1} perchlorate in an acetate-fed fixed-film reactor with RT values of between 2.1 – 30 minutes. Brown *et al.* (2002) utilised a 25-minute RT to reduce 50 µg L^{-1} perchlorate within a BAC filter. A 30% reduction of 12 mg L^{-1} perchlorate influent was achieved by Logan and Lapoint (2002) with a short 1.5 minute RT, although the authors suggested a longer RT would

have improved reduction efficiency. Reduction of high perchlorate concentrations has been reported, with 500 and 1500 mg L^{-1} perchlorate reduced to below 100 mg L^{-1} with an RT of 0.5 and 1.2 hours respectively (Wallace *et al.*, 1998).

An RT of 20 hours in chemostat trials and 10 hours with the pilot-scale reactor both led to low bromate reduction in current studies, with < 50% removal from a 1.1 – 1.4 mg L^{-1} influent. The pilot-scale reactor particularly showed marked loss of bromate reducing ability at 10 hours, with high nitrite production (in excess of the current UK nitrite drinking water limit of 0.5 mg L^{-1}) and partial biomass washout also suspected. Optimum RT for bromate reduction was shown to be within the range 20 – 40 hours, with the higher end giving greater percentage bromate removal (87.0% against 50.2%) but less throughput and vice versa. With significant sulphate reduction observed to occur upon removal of all available bromate in chemostat trials (up to 21.8 mg L^{-1} from a 54.3 mg L^{-1} influent in phase IIIa), it could be anticipated that higher RT values may lead to increased sulphate reduction and therefore production of hydrogen sulphide. This is undesirable, both due to utilisation of the carbon source for sulphate reduction, and also liberation of the foul hydrogen sulphide 'rotten egg' odour which can be detected by humans at concentrations as low as 0.1 μg L^{-1} (Brown *et al.*, 2003). However, higher RT values did not generally lead to increased sulphate reduction within the pilot-scale bioreactor, with a maximum reduction (15.3 mg L^{-1}) at a low 20-hour RT.

Optimisation of RT within a continuous-flow system is critical to efficient functioning of the reactor. For stable operation, dilution rate of a chemostat should be less (and therefore RT higher) than the maximum growth rate of the microbe of interest (Pirt, 1975). With a growth rate for bromate reducing strains estimated at only ≤ 0.025 hr^{-1} during chemostat trials (Section 8.2.2), compared with a rate of 0.39 hr^{-1} exhibited by denitrifiers propagating on glucose (Mazierski, 1994), this difference may partially explain the difficulty of achieving and subsequently maintaining an effective high-rate bromate degrading culture in the presence of a faster growing denitrifying microbial consortium.

8.2.5.6 *Reactor history*

Bioreactor remediation systems may periodically be subject to perturbations of influent water quality and flow caused by operational changes or equipment failure, and also by natural seasonal alterations. For example, a full-scale trial fixed-film bioreactor operating for perchlorate remediation at a former military installation in Texas, USA experienced influent flow variations between 75 – 190 L min^{-1} and perchlorate concentrations within the range 2 – 34 mg L^{-1} over the first 110 days of operation (Polk *et al.*, 2001). To achieve stable and acceptable operation these perturbations should not elicit a variable or sustained negative response in effluent quality. In this case study no effect was observed; with perchlorate routinely removed to the limit of detection (< 5 µg L^{-1}). Reactor history has not been reported as a significant factor in either nitrate or perchlorate reducing bioreactors, with no reference uncovered in the published literature. However, within a bromate reducing BAC bioreactor, filter history was observed to have a measurable and sustained impact on operational efficiency. Kirisits *et al.* (2002) noted that, following a system perturbation which led to loss of bromate reducing ability during BAC bromate bioremediation trials, prior performance characteristics had not been fully regained even after almost an additional year of operation. Bromate reduction had decreased from 23 – 74% of a 20 µg L^{-1} influent immediately prior to perturbation, to 24 – 41% reduction 11 months later. Examination of microbial diversity using denaturing gel electrophoresis (DGGE) techniques showed a more diverse community in the undisturbed filter. The authors therefore concluded an alteration in microbial community persisted even following return of the original water quality conditions.

Reactor history was not observed to affect microbial population composition during phase I or IIa trials, although no sustained period of steady state operation was attempted. Chemostat phase IIb, by contrast, exhibited the most marked example of suspected population composition change, where the temporary groundwater feed failure elicited a permanent alteration in bromate reducing activity within both reactors (Section 7.2.1.2). The sustained and stable decrease in bromate reducing activity within both reactors in chemostat phase III following temporary utilisation of a 'high-nitrite' influent supply (Section 7.2.1.3) has also been highlighted. In this case bromate reduction

decreased whilst nitrate reduction was not affected both during and subsequent to a temporary increase of influent nitrite concentration to 36.4 mg L^{-1}. True denitrifying bacteria are unlikely to be affected by a nitrite increase as they contain mechanisms for nitrite reduction. However, bromate reducing strains may not contain such pathways, which would lead to a competitive disadvantage under high nitrite conditions. In this case the alteration noted was sustained, even following replacement of the high-nitrite influent supply. Bromate supernatant concentration remained stable within the range 10.8 – 11.9 mg L^{-1} over the following two-week period, compared with 0.7 – 0.8 mg L^{-1} prior to the incident. It is suggested that an altered microbial consortium stabilised in both reactors following this perturbation, thus causing the observed effects.

Glucose manipulations during chemostat phase IIIb also appeared to highlight effects of reactor history (Section 7.2.1.3). An increase in glucose influent from 52 mg L^{-1} to 100 mg L^{-1} elicited a concurrent increase in bromate reduction, measured as decreases in supernatant bromate concentration from 14.4 mg L^{-1} to the limit of detection (0.01 mg L^{-1}). Subsequent lowering of glucose influent back to 52 mg L^{-1} decreased bromate reduction, but supernatant concentration (8.6 mg L^{-1}) was still markedly lower than the initial level. Over the same period the control reactor lost efficacy, confirming the observed sustained increase in bromate reduction ability was largely due to the temporary glucose augmentation.

Reactor history was also critical to establishment of stable operation in the pilot-scale fixed-film bioreactor, with reactor 1 experiencing a tendency to culture failure following no initial batch period of operation (Section 7.2.4.3). That this susceptibility was prolonged past the initial startup period confirms operation of fixed-film bromate reducing systems can be affected by reactor history. Kirisits *et al.* (2002) also observed a possible correlation between BAC filter startup conditions and subsequent capacity for nitrite oxidation, with some filters establishing an undesirable ammonia-oxidising community instead of a nitrite-oxidising population. The absence of equivalent effects in published nitrate or perchlorate reduction studies suggests that bromate reducing strains are more sensitive to water quality perturbations than other bacteria within the microflora. This observation has implications for management of a full-scale bromate bioremediation

system. System perturbation by transient carbon increase may be required for 'kick-starting' a high-rate bromate degrading culture, but subsequent stable reactor operation would be a priority for maximum sustained reduction.

8.3 FULL-SCALE BROMATE BIOREMEDIATION

In this section results from the current study are applied to potential bromate bioremediation techniques to evaluate effectiveness for full-scale aquifer rehabilitation. The processes are then compared with known technologies for nitrate and perchlorate bioremediation.

8.3.1 Use of full-scale bioreactors for bromate bioremediation

The two major categories of unit process for bioremediation are *in-situ* and *ex-situ* techniques (Hyman and Dupont, 2001). *In-situ* groundwater treatment strategies utilise the aquifer as a bioreactor, with addition of exogenous agents to stimulate remediation. *Ex-situ* processes apply the remediation mechanism to abstracted groundwater within an engineered bioreactor. In addition, combined systems have been suggested. One combined approach is to integrate an above-ground remediation bioreactor with reinjection of treated groundwater to the aquifer for *in-situ* filtration and re-aeration (Hiscock *et al.*, 1991). Alternatively, a physical remediation method which separates but does not degrade the contaminant (e.g. ion exchange, membrane filtration), can be combined with bioremediation of the concentrated waste stream (Xu *et al.*, 2003).

8.3.1.1 In-situ bioremediation

In-situ bioremediation of nitrate pollution enhances natural attenuation via biological reduction by injection of a carbon source into the aquifer, thus increasing availability in a carbon-limited aquifer. A range of carbon sources have been reported, including methanol, ethanol, sucrose, formate and glucose (Mateju *et al.*, 1992; Smith *et al.*, 2001; Godbold, 2002). Nutrient mixtures can also be injected (Mateju *et al.*, 1992), although most groundwaters already contain adequate concentrations of minerals and trace metals necessary to support biosynthesis (Hiscock *et al.*, 1991). *In-situ* processes

have been used for biodegradation of petroleum contamination for over 30 years, with the first technique patented in 1974 and named the 'Raymond process' (Ritter and Scarborough, 1995). *In-situ* denitrification has been studied for at least two decades, one early example achieving 97% removal of an initial 14 mg L^{-1} groundwater nitrate concentration using 24 mg L^{-1} ethanol injection and a 2 – 5 day aquifer residence time (Chalupa, 1985; cited in Hiscock *et al.*, 1991). Although reports are scarcer, *in-situ* perchlorate bioremediation has also been investigated. Addition of acetate to an aquifer contaminated with 12 mg L^{-1} perchlorate resulted in removal to below the detection limit (4 μg L^{-1}) within 5 m of the injection well (Cox *et al.*, 2001).

No published literature on *in-situ* bromate bioremediation is currently available, with only one author even mentioning the possibility. Hijnen *et al.* (1999) suggested bromate removal could be achieved with passage of contaminated water through soil during artificial recharge or river bank filtration, in a similar manner to that observed for chlorate. However, this hypothesis was not tested. *In-situ* bromate reduction has been speculated as occurring within the contaminated study aquifer, but only circumstantial evidence was available. During the current study, successful use of source water from this aquifer as inoculum provided the first evidence indigenous aquifer microbial populations were indeed capable of bromate bioremediation. Development of a chemostat enrichment culture able to remediate a 48 mg L^{-1} bromate contamination level at a 40-hour RT showed that, even without bioaugmentation of the influent stream by an exogenous microbial source, latent bromate reducing ability was present. Subsequent investigation of environmental variables also provided information as to potential efficacy of an *in-situ* bromate bioremediation system.

Initial (phase I) chemostat trials noted the occurrence of bromate reduction at high (150 mg L^{-1}) carbon influent concentrations, within two groundwater sources exhibiting typical aquifer bromate contamination profiles (1.4 mg L^{-1} and 0.2 mg L^{-1} bromate). However, nitrate was also reduced simultaneously within both continuous flow systems, thus increasing carbon demand. Glucose concentrations of ≥ 40 mg L^{-1} were required to remove background influent nitrate, which was typically 30 – 40 mg L^{-1}, and with lower carbon addition nitrite formation was observed. Nitrite has been noted during *in-situ*

denitrification trials, with up to 0.6 mg L^{-1} measured in effluent flows during a methanol dosing trial to remove 80 mg L^{-1} nitrate (Kruithof *et al.*, 1985; cited in Hiscock *et al.*, 1991). However, a review of *in-situ* denitrification trials by Hiscock *et al.* (1991) did not note any further problems with aquifer nitrite accumulation. Reduction of nitrate simultaneously with perchlorate was noted in an *in-situ* trial at a former US military site, with perchlorate (16 – 27 mg L^{-1}) and nitrate (15 mg L^{-1}) both reduced to the study detection limits (Perlmutter *et al.*, 2000). Although evidence for specific bromate reduction independent of denitrification has been observed both previously (van Ginkel *et al.*, 2005b) and in current (Phase IIb/III) chemostat trials it is likely that, within the uncontrolled environment of an aquifer, those strains responsible could not compete with other aquifer bacteria such as denitrifiers. Therefore carbon requirements for nitrate reduction would also need to be factored into any *in-situ* bromate reduction trial.

With temperature in a typical aquifer system within the region of 10°C (Hiscock *et al.*, 1991), current studies showed *in-situ* bromate reduction would be expected to be sub-optimal. Phase I chemostat trials indicated that only low bromate reduction was observed at 10°C (Section 7.2.1.1), and lowering of reactor temperature from 20°C to 10°C during phase IIIa led to a decrease in bromate removal by the high-rate bromate reducing culture (Section 7.2.1.3). It is unlikely to be either economically or environmentally credible to raise the temperature of an aquifer, so reduction rates would be restricted to those observed at this temperature. Conversely, one advantage of *in-situ* processes is extended retention times, with one author calculating a 24 – 48 hour RT for a 12 – 15 m movement of groundwater in a denitrification trial (Janda *et al.*, 1988). With a relatively long 20 – 40 hour RT required within the pilot-scale bioreactor, this may be one factor limiting throughflow of an *ex-situ* method of operation. The extended retention times inherent with *in-situ* methodologies would allow time for bromate reduction to occur, and may serve to compensate for the reduction in efficacy at low temperatures.

In-situ bromate bioremediation is a possibility for aquifer remediation, and has the advantage of potentially low costs (Hyman and Dupont, 2001) due to the lack of infrastructure and extensive groundwater pumping requirements associated with an *ex-situ* system. However, all *in-situ* bioremediation methodologies have inherent

disadvantages which are not easy to overcome. Remediation efficiency can vary widely, with reports of between 10 and nearly 100% nitrate reduction noted in a review of relevant literature (Mateju *et al.*, 1992). Robustness of *in-situ* methods can also be a problem, with effectiveness being heavily dependent on physical factors such as type and heterogeneity of the soil (Barr *et al.*, 2002). A heterogeneous soil can potentially give patchy distribution of substrate and therefore reduction efficiency. This can make *in-situ* processes difficult to predict and control (Mateju *et al.*, 1992), with the complex nature of a natural system also possibly leading to production of undesirable by-products such as nitrite. A test system constructed by Mercado *et al.* (1988) utilised three injection wells to inject sucrose into an aquifer contaminated with 64.2 mg NO_3 L^{-1}, but achieved a maximum efficiency of only 10%. It was concluded that, of the three injection wells trialled, only one functioned correctly with one penetrating an area not actually hydraulically connected to the aquifer. More injection wells can increase treatment homogeneity (Mateju *et al.*, 1992), with Mercado *et al.* (1988) suggesting a 5 or 6 well system. However, this will necessarily increase complexity and cost of the operation. One final problem is the occurrence of clogging, as injection of carbon directly into the aquifer can lead to substantial biomass growth and blocking of aquifer pore spaces, thus decreasing efficiency. Clogging can be reduced by using slowly degrading carbon sources (Hiscock *et al.*, 1991) or intermittent injection cycles (Mateju *et al.*, 1992), but again complexity and therefore cost are also increased.

Current studies have shown bromate reduction at typical aquifer temperatures and nitrate concentrations (10°C and 30 – 40 mg L^{-1} respectively). Potential similarity of a bromate reduction methodology to denitrification systems may also allow relatively simple technology transfer. However, considerable further work to demonstrate efficacy in the new role would be required prior to regulatory approval for operation of field trials, which by their very nature are not isolated from the environment as within an *ex-situ* system. Despite current studies demonstrating development of an *in-situ* bromate bioremediation system may be feasible it is thought to be questionable whether, with the low incidence of known bromate aquifer contamination incidents, there would be either the economic or regulatory impetus for development of such a stand-alone system.

8.3.1.2 *Ex-situ bioremediation*

Whilst *in-situ* techniques have potential advantages relating to cost and retention time, *ex-situ* treatment systems have the important virtue of allowing more precise control over the entire system to attain maximal operating efficiency. Critical parameters already discussed in previous sections include pH, temperature, carbon source, carbon dosing concentration and retention time. These parameters can all affect composition of the microbial community within a reactor, and can be easily manipulated and optimised within an *ex-situ* bioreactor. Precise control within an *in-situ* process by contrast is difficult, if not impossible in some cases.

Full-scale *ex-situ* biological treatment unit processes are well developed and widely employed within the water industry. One of the most common applications is in the treatment of wastewater, for example by activated sludge and trickling filter systems. These have been in use for around a century, having been developed in 1914 and 1893 respectively (Metcalf and Eddy, 1991). The activated sludge process is an example of a suspended growth reactor, and the trickling filter a fixed film process. Both are most commonly used as aerobic treatments to reduce carbon loading in wastewater streams, but both have also been modified for nitrate reduction during wastewater treatment.

Anoxic wastewater denitrification systems are generally utilised in concert with aerobic processes, with the aim to remove nitrogen by a combination of nitrification to convert ammonia into nitrate, followed by nitrate reduction to nitrogen gas via denitrification. Approximately 40% of influent nitrogen in raw wastewater is in the form of ammonia created by the decomposition of organic matter. Only low levels of nitrate are typically detected, due to a lack of free oxygen for nitrification. Denitrification systems may require a nitrogen release step, commonly achieved by aeration to remove nitrogen gas attached to the biomass, plus clarification to remove excess sludge from the reactor. Unlike comparable drinking water nitrate removal, wastewater denitrification is rarely utilised as a stand-alone process without the related nitrification step.

Suspended growth (activated sludge) processes can be split into combined (single sludge) and separate (dual sludge) systems, the former combining nitrification and

denitrification in the same tank by selective aeration, and the latter performing the two stages in physically separate vessels. Activated sludge systems can be further split into a range of configurations depending on the sequence of unit processes. Configurations include aerobic-anoxic, aerobic-anoxic with partial bypass of the aerobic stage, and anoxic-aerobic (Vesilind, 2003). Both latter processes dispense with carbon addition as they utilise carbon already present within the wastewater. However, the aerobic-anoxic configuration does require carbon addition, due to utilisation of carbon during the initial aerobic nitrification stage. This system is therefore the most analogous to suspended growth drinking water denitrification. Completely mixed reaction chambers, as trialled in current studies, have been utilised. However, the recommended configuration for a full-scale suspended growth wastewater denitrification system is a plug-flow reactor. This configuration minimises short-circuiting within the chamber during relatively short retention times of 1 - 2 hours (Hammer and Hammer, 2004; Viessman and Hammer, 1985).

Fixed film systems generally operate as post-denitrification processes (following the nitrification step) and, as such require an exogenous carbon donor. Configurations reported include 'conventional' downflow sand media fixed-bed reactors (Jonsson, 2004), rotating biological contactors (Metcalf and Eddy, 1991) and fluidised bed reactors (Vesilind, 2003). One reported advantage over suspended growth systems is that attached growth reactors can also act as filters, thus eliminating the requirement for a separate clarifier and saving typically 35 – 50% of total reactor volume (Borrgaard, 1997). However, fixed-film reactors may require backwashing or 'bumping' of the media to remove headloss associated with attachment of nitrogen gas to the media (Vesilind, 2003), and also to remove attached biomass. Backwashing in relation to current studies is further discussed in Section 8.3.2.

One development of the fixed bed reactor utilised in wastewater denitrification is use of a floating media in the proprietary Biostyr® process. This utilises an upflow reactor with a submerged floating polystyrene 'Biostyrene' media (diameter of 3 – 3.5 mm). Five Danish Biostyr® plants were reported by Borregaard (1997), with an operational capacity of 2000 – 13000 m^3 day^{-1}. Simultaneous nitrification and

denitrification were achieved within the same unit by central aeration, leading to a lower anoxic zone and upper aerobic zone, and methanol was dosed to facilitate denitrification. At one plant, Frederikshavn, direct comparison with the on-site activated sludge system indicated comparable nitrogen removal (32.5 mg L^{-1} influent total N reduced to 8.9 – 9.6 effluent total N) and effluent suspended solids concentrations (3 – 3.7 mg L^{-1}) but with the smaller fixed-film reactor footprint.

Fluidised bed reactors use recirculated water to fluidise a sand medium, thus preventing clogging and channelling within the packing material and also maintaining a constant thickness biofilm by hydraulic shearing. The Dynasand filter is a proprietary fluidised bed filter which has been reported widely for wastewater denitrification (i.e. Hultmann *et al.*, 1994; Jonsson *et al.*, 1997). Simultaneous removal of nitrate and suspended solids is possible using this type of filter, with the fluidised medium providing adequate shearing forces for removal of both excess biofilm and also nitrogen gas bubbles from the sand media (Hultmann *et al.*, 1994). Low retention times of only 15 minutes can be achieved (Jonsson *et al.*, 1997), with the type of carbon source not affecting denitrification rate (Andersson *et al.*, 1991).

Full-scale wastewater denitrification filters have now been utilised in the US for more than 30 years. Comparable *ex-situ* technology has also been developed for bioremediation of nitrate contaminated waters for potable use. This application is now an established process, following studies spanning at least five decades (Eckenfelder, 1965; cited in Gauntlett and Craft, 1979). A trial by Gauntlett and Craft (1979) compared suspended growth, fixed-film and fluidised bed reactors using methanol as carbon source. Advantages of all three designs were noted, with suspended growth systems simple and consistent to operate, fixed-film reactors exhibiting low carbon consumption, and fluidised beds producing the highest rate per unit volume. Subsequent full-scale application of this work using an upflow fluidised bed reactor, the first European full-scale denitrification unit to be constructed, achieved 75% reduction of a 100 mg L^{-1} nitrate influent supply (Croll and Hayes, 1988). Other early full-scale biological denitrification systems were trialled in France, with both the 'Biodenit' and 'Nitrazur' systems commissioned at full-scale during 1983 (Mateju *et al.*, 1992). Using acetate and

ethanol respectively as carbon source, both were fixed-bed techniques. The Biodenit system utilised a GAC/sand mixture (2.7 mm diameter), and Nitrazur also incorporated an activated carbon media (3 – 6 mm diameter). Both processes had comparable nitrate removal efficiencies (72 – 74% of influent nitrate) and operated cost-effectively, with a cost comparable to nitrate removal ion exchange systems (Richard, 1989). Full-scale *ex-situ* systems have also utilised floating media in a similar fashion to the wastewater Biostyr® process. A German system known as 'Denipor' included a buoyant media, with a full-scale plant constructed at Monheim reported to operate at 90 – 95% nitrate removal efficiency (reported in Mateju *et al.*, 1992). A floating media was also utilised in the first US potable water treatment system, installed at the town of Coyle, Oklahoma in 1998 (Sanders *et al.*, 2004). The upflow reactor was packed with low density, high porosity mesh spheres, and was designed to treat up to 104 L min^{-1} groundwater contaminated with a maximum 73.1 mg L^{-1} nitrate-N influent. Carbon source was acetic acid supplied as food-grade vinegar, with additional phosphate supplied as monosodium phosphate. Other treatment consisted of a clarifier and slow sand filter, followed by chlorination and storage prior to potable supply. Results showed this system could remove in excess of 90% nitrate- and nitrite-N during periods of stable operation, but carbon dosing problems did lead to nitrite accumulation as high as 3.4 mg L^{-1}. A requirement for more effective analysis to improve carbon dosing was therefore identified. However, overall operation was successful and proved the concept also trialled in current studies, of a relatively simple groundwater-fed system utilising a readily-available carbon source.

Although perchlorate has only been recognised as a pollutant for a decade, the discovery of widespread pollution in the US and subsequent regulatory impetus has driven forward remediation studies. This is in contrast to bromate which has not been subject to the same urgent drivers. Pilot- and full-scale perchlorate reduction systems have now been developed, with both fixed-film and fluidised bed systems reported. An early trial in 1995 harnessed a suspended growth reactor to remediate heavily perchlorate-contaminated (> 5000 mgL^{-1}) military wastewater to < 0.5 mgL^{-1} , with brewers yeast utilised as carbon source (reported in Logan, 1998). However, later systems have moved to fixed-film designs more analogous to the pilot-scale bromate

reducing system trialled in current studies. In one pilot-scale trial, operation of a 6 metre-high fixed-film acetate-fed bioreactor remediated heavily perchlorate-contaminated groundwater (7 – 20 mg L^{-1}) to below the detection limit of 20 µg L^{-1} (Perlmutter *et al.*, 2000). Fluidised bed reactors have been trialled at full-scale, one system consistently removing over an 8-month period an average influent of 8 mg L^{-1} perchlorate and 1.5 mg L^{-1} nitrate at a flow rate of over 15 000 litres per minute to below the limit of detection (4 µg L^{-1} for perchlorate) using ethanol as carbon source (Hatzinger *et al.*, 2000). Another full-scale fluidised-bed reactor system, using acetate dosing, was shown to reduce up to 35 mg L^{-1} perchlorate to < 350 µg L^{-1} with an average flow rate of 115 – 130 L min^{-1} (Polk *et al.*, 2001). In addition to remediation of these highly-contaminated water sources, other authors have concentrated on lower concentrations more analogous to those encountered within bromate-contaminated waters investigated in current studies and therefore more suitable for comparison. One fixed-film system was developed to remove perchlorate from groundwater contaminated with 300 – 1000 µg L^{-1} perchlorate. Residence time of 30 minutes in this case was considerably lower than those utilised for bromate reduction. A range of carbon sources were trialled, with methanol concluded to be the most cost-effective. A proprietary floating polyurethane media was used in the reactor, and continuous recirculation similar to the pilot-scale reactor in current trials was employed. Reactor volume was 200 litres, and the entire system was designed to fit on a transportable skid of 1.3 m^2. During the startup phase, reactor contents were recirculated until reduction was noted, as during the current pilot-scale trial, and within one month of startup perchlorate was being reduced to < 9 µg L^{-1} (Hall, 2000).

Ex-situ laboratory- and pilot-scale trials within the current study have shown for the first time that *ex-situ* bromate bioremediation occurs using groundwater sourced directly from a contaminated aquifer. The manipulation of all critical parameters outlined above has also provided preliminary evidence of optimal conditions for operation of such a system. Although both suspended growth and fixed-film systems were observed to exhibit bromate reduction, it is recommended fixed-film systems be utilised. Suspended growth techniques have the disadvantage of retention time determining microbial residence time within the reactor. Due to the retention of a bacterial biomass on the

packing material, fixed-film techniques allow decoupling of these two parameters. This is ideal for waste streams that require slow-growing, specialised microbial populations for their efficient degradation (Hyman and Dupont, 2001), of which bromate is a possible example. Growth rates observed during current trials were effectively limiting RT to a minimum of 40 hours within the chemostat system. With the pilot-scale fixed-film bioreactor, a 20-hour RT was observed to still allow around 50% bromate reduction under non-optimised operational conditions. Other advantages of fixed-film systems over *in-situ* techniques include smaller reactor volumes and an increased robustness to perturbations in environmental conditions (Hyman and Dupont, 2001). With sensitivity of the chemostat system to reactor history observed during current studies this could be a crucial advantage of a fixed-film system, although optimisation of startup conditions would still be a priority. An alternative system not trialled during current studies would be a fluidised bed reactor. No bromate bioremediation study has to date investigated this technique but, with both full-scale perchlorate (Polk *et al.*, 2001; Hatzinger *et al.*, 2000) and nitrate (Hultman *et al.*, 1994; Croll and Hayes, 1988) trials successful, it may be possible to transfer knowledge gained to a bromate reducing system.

Overall, pilot- and full-scale perchlorate reduction trials have provided the basis for potential development of a comparable bromate reducing system. In developing such a system, knowledge gained from the above and other trials would likely prove invaluable in the maximisation of reduction efficiency and selection of effective operating conditions.

8.3.1.3 Combined processes

Process integration in contaminated land and groundwater remediation is common, a typical strategy being to combine a source removal technique with one or more plume management methodologies (Barr *et al.*, 2002). Processes can also be integrated into one remediation technique to enhance overall efficacy of the system. One problem with some of the physical bromate removal techniques (ie. filtration and ion exchange) is that bromate does not become reduced to innocuous bromide. Instead it ends up concentrated into a small volume of highly contaminated saline brine following

filtration or resin regeneration, disposal of which would be difficult and costly (Velizarov *et al.*, 2004). However, integration of a biological process to treat the brine produced by a physical removal technique can be effective. An ion exchange/bioreactor system has recently been approved by the California Department of Health Service for treatment of perchlorate contaminated water (Xu *et al.*, 2003). Biological nitrate removal has also been combined with ion exchange, with an upflow sludge blanket denitrification reactor reported to reduce the volume of ion exchange brine by 95% (van der Hoek *et al.*, 1988). The major problem encountered with this strategy is the high salt content of concentrated influent, typically up to 1% for reverse osmosis filtration and 7 – 12% (as sodium chloride) following anion exchange resin regeneration (Xu *et al.*, 2003). Microorganisms acclimated to freshwater environments are generally inhibited by sodium chloride concentrations greater than 1% (Pelczar *et al.*, 1993), and consequently may not be tolerant of these concentrated brine solutions. However, halotolerant perchlorate reducers directly isolated from high-salt environments have been reported to reduce perchlorate at 3% salinity, with continued acclimation increasing tolerance up to 11% salinity (Logan *et al.*, 2001). Salinity tolerance of the enrichment culture obtained during the current study was not investigated, and no halotolerant bromate-reducing strains are currently known. It is also unlikely that economic considerations would warrant development of such a system in the context of the study aquifer. However, the problem of bromate formation during ozonation and subsequent removal is much more widespread and an integrated physical/biological system may be more relevant. Whilst this avenue of investigation has not currently been explored, the success in isolation of halotolerant perchlorate reducers raises the possibility of analogous treatment for concentrated bromate brines.

Process integration by combining *ex-situ* and *in-situ* bioremediation was suggested for denitrification (Hiscock *et al.*, 1991), with the advantages in control of an *ex-situ* process combined with *in-situ* post-reactor filtration and reaeration within the aquifer. Following the current study, use of an *ex-situ* fixed-film bioreactor has been recommended for biological aquifer bromate reduction. However, disposal of the effluent stream emanating from such a system must also be considered. Use of treated

water for potable supply is not considered feasible without further research, due to the extensive secondary treatment required to remove residual biomass and carbon source. For example, the 'Biodenit' process involves a secondary treatment process train including filtration, coagulation, activated carbon adsorption, ozonation and chlorination prior to supply (Figure 8.12).

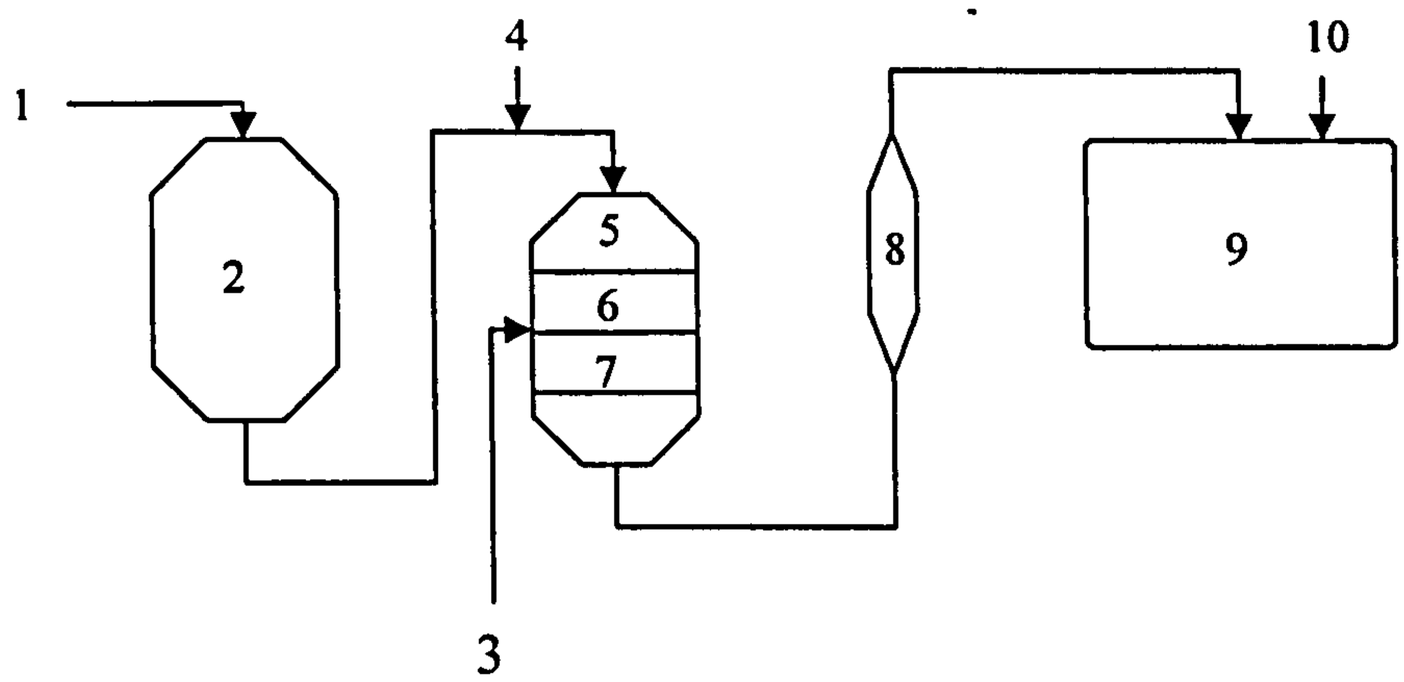


Figure 8.12 - General layout of Biodenit process. 1, Carbon-amended raw water inlet; 2, Bioreactor; 3, Air; 4. Filter; 5, Coagulant; 6, Activated carbon; 7, Sand; 8, Ozonation; 9. Treated water reservoir; 10, Gaseous chlorine and sodium thiosulphate addition (Mateju *et al.*, 1992)

For aquifer remediation purposes it is unlikely this approach would be cost-effective. Discharge to sewer is an option, and results of both laboratory-scale respiration inhibition and pilot-scale wastewater process dosing trials suggest no effect would be noted following discharge of either bromate- or bromide-loaded water into such a system, even at higher concentrations than likely to be encountered (≤ 200 mg L^{-1}). However, another option would be re-injection of treated water back into the contaminated aquifer. In addition to the advantages of this approach noted by Hiscock *et al.* (1991), reinjection would also provide a continual source of bromate-reducing microbes to the aquifer. This integrated system would therefore be analogous to bioaugmentation, whereby an aquifer is inoculated with strains known to be capable of degrading the contaminant present. Reports of successful bioaugmentation field trials were summarised in Hyman and Dupont (2001), with target contaminants including trichloroethylene and pentachlorophenol. One major disadvantage of *in-situ* bioaugmentation is that inoculated microbes are not indigenous to the contaminated site, which generally leads to a

competitive disadvantage within the complex aquifer environment. In addition public perception of bioaugmentation is often that introduction of an 'alien' species is detrimental, even within an already contaminated environment (Hyman and Dupont, 2001).

Development of indigenous high-rate bromate degraders within an *ex-situ* bioreactor environment during the current study has raised the possibility of an integrated *in-situ/ex-situ* system encompassing abstraction, *ex-situ* bioremediation and subsequent reinjection of groundwater containing an enriched microbial culture and residual carbon supplies. This system could overcome the two major limitations of bioaugmentation outlined above, as reinjected strains may be at least partially acclimated to the aquifer environment, with use of indigenous organisms potentially more acceptable to public opinion. One example of this approach was discussed by Boussaid *et al.* (1988), who constructed three bioreactors discharging into infiltration pits each spaced 15 m from a central pumping well. A 62.9% reduction of the initial 62 mgL^{-1} as NO_3 concentration was obtained at the exit of the bioreactors and, after 30 days an overall 14.4% reduction in nitrate level at the central pumping well was achieved. Further reduction within the aquifer was confirmed by a reduction of 1.51 mgL^{-1} nitrite in bioreactor effluent to only 0.03 mgL^{-1} in the overall aquifer nitrite levels. No reference to such a system for perchlorate reduction has been noted in the published literature, with the eventual aim of many systems being potable water supply (Sakaji *et al.*, 2002; Nerenberg and Rittmann, 2004), discharge to river (Polk *et al.*, 2001) or unspecified. However, use of such a system for a bromate-contaminated aquifer is considered a promising option as, whilst the current study has shown bromate reducers are present within the aquifer, it is likely they are at a numerical and competitive disadvantage due to the slow growth rates observed during current studies. Augmentation of their numerical preponderance may reduce this disadvantage and allow development of a viable and sustainable bromate reducing community. With the exception of nitrite, which can be controlled with sufficient carbon addition, no toxic products were noted which may cause detriment to the environment.

As with the stand-alone *in-situ* option, further research would be required to ascertain efficacy of such a system. There are also potential regulatory issues, including the requirement for licences covering both abstraction and reinjection. Under the Water Resources Act 1991 (HM Government, 1991), a valid abstraction licence is required for *ex-situ* groundwater treatment. Discharge consent or authorisation under either the Groundwater Regulations 1998 or the WRA 1991 would also be necessary for re-introduction of treated water into the aquifer (Barr *et al.*, 2002). Careful aquifer monitoring would be required, especially due to the novelty of the system. However, it is not anticipated that reinjection of indigenous microbes would have significant adverse effect on the already contaminated aquifer environment. Therefore, whilst this option was not explored during the current project, it is recommended the system be considered for further investigation.

8.3.2 Potential full-scale operating conditions

Laboratory-scale chemostat studies were based upon a continuously-stirred suspended growth reactor, fed by an influent (Phases II and III) containing high levels of bromate (32 – 48 mg L^{-1} as BrO_3^-) with relatively low nitrate concentrations (average 8.49 mg L^{-1} as NO_3). Continuous running over the trial period led to a marked increase in bromate-reducing capacity, as conditions were purposely engineered to be constant and favourable to development of a bromate-reducing culture. Such conditions would be hard to reproduce at full scale, with bromate and nitrate levels within the contaminated aquifer (Table 6.1) instead more favourable to development of a denitrifying culture. During the design process for the pilot-scale bioreactor, consideration was therefore given to emulating operational conditions likely to be encountered by a full-scale system. For example neither pH nor temperature was controlled, and unspiked influent groundwater was utilised. This provided results relevant to any scaling-up process as, although some parameters may alter relative to each other, a general rule of geometric similarity can often be applied during the reactor scaling up process (Scragg, 1991).

As discussed in previous sections, an *ex-situ* fixed-film mode of operation is considered the more promising configuration for scale-up and installation at the

contaminated site. This decision has been made based on knowledge of bromate reduction characteristics gained during current studies, and also evaluation of comparable nitrate and perchlorate reduction systems. Design of a full-scale *ex-situ* bromate reducing bioreactor would be a novel process and was not an integral facet of the work described herein. However, an evaluation of points to consider can be obtained by combining results obtained at both laboratory- and pilot-scale with studies based on construction and commissioning of broadly comparable clean water remediation systems. A range of critical factors would need to be addressed and optimised during any scaling up process, including carbon source, influent bromate concentration, filter media, backwashing, pH, temperature, retention time and biomass acclimation during startup. In this section, each is outlined and discussed below in the context of full-scale *ex-situ* bioreactor operation.

8.3.2.1 Carbon source and sludge production

The decision to use glucose as carbon source during all current studies was made following discussions with relevant regulatory bodies (Environment Agency and the Drinking Water Inspectorate), as it is a non-toxic food source acceptable for water destined for drinking water quality. Other carbon sources including ethanol and methanol were rejected for use on the same grounds, despite utilisation in previous studies (i.e. Hijnen *et al.,* 1995; Hijnen *et al.,* 1999). With subsequent discussions amongst interested parties concluding development of an aquifer remediation system and not treatment for potable supply should be a priority for the study, use of alternative carbon sources may however prove more acceptable. All trials showed that glucose was capable of supporting bromate reduction, but evidence was also accumulated of fermentation within the microbial consortium prior to utilisation by any strains mediating bromate reduction. For example, 190 mg L^{-1} glucose was reduced to below 1 mg L^{-1} within 48 hours during batch trial G, with simultaneous increases in ethanol and acetate concentrations (Section 7.2.3.3). An alternative carbon source, such as a glucose fermentation product, may therefore allow selection and maintenance of a more specific bromate reducing culture.

Nitrate and perchlorate reduction trials have utilised a wide range of carbon sources, including cellulose, whey, ethanol, biogas, methanol, sucrose and acetic acid (collated by Mateju *et al.*, 1992). By contrast, full-scale plants have generally been restricted to methanol, ethanol or acetic acid. Wastewater systems commonly utilise methanol as carbon source, due to its availability, ease of application and relatively low cost (Hammer and Hammer, 2004; Viesmann and Hammer, 1985). However, both acetate (Jonsson *et al.*, 1997) and ethanol (Jonsson, 2004) have also been used successfully. Jonsson (2004) compared operating costs of three carbon sources at a wastewater treatment works treating 147 881 m^3 raw wastewater per day. Methanol was shown to be the cheapest substrate at £224.40 day^{-1}. Ethanol costs ranged from £281.56 – 410.88 day^{-1} depending on purity, with acetic acid the most expensive substrate at £612.28 day^{-1}. Over one year this difference translates to a significant difference in operating costs (£141 614) between use of methanol and acetic acid.

Potable water remediation systems also tend to utilise the same three carbon sources as wastewater techniques, with methanol (Hall, 2000), ethanol (Hatzinger *et al.*, 2000) and acetic acid (Sanders *et al.*, 2004) all employed at full-scale. A further comparative laboratory- or pilot-scale study would be necessary to ascertain the most favourable substrate for bromate reduction, as current trials suggest glucose may not be the most efficient carbon source due to its fermentable nature. In addition, as outlined by Jonsson (2004) choice of carbon source in a full-scale system is not only dependent on process efficiency, with substrate cost also necessarily a critical factor.

The current system requires use of a proportion of the applied carbon for denitrification, suggested by chemostat phase I to be approximately 40 mg L^{-1} for complete reduction of a 30 – 40 mg L^{-1} nitrate influent. Lower carbon addition was also shown during chemostat phase I to lead to build-up of nitrite (maximum 10.0 mg L^{-1} nitrite with a 30 mg L^{-1} glucose influent) via incomplete denitrification ($NO_3^- \rightarrow NO_2^-$). It has been observed that nitrite reduction is repressed under carbon-limiting conditions leading to nitrite buildup, one example being use of a submerged biological contactor with ethanol as a carbon source (Mohseni-Bandpi and Elliott, 1996). 'Low' ethanol influent concentrations resulted in 'high' nitrite effluent concentrations, although these

levels were not quantified. Attainment of a more targeted culture utilising a specific carbon source may allow bromate reduction with fewer competitive microbes, such as denitrifiers, able to use the carbon substrate. This would lead to more efficient carbon usage and a more cost-effective system, with the added advantage of a potentially reduced risk of nitrite build-up in the event of accidental carbon under-dosing.

Sludge production has not been considered in the context of current studies as, with a combined 'treat and reinject' process or a 'pump to waste' scenario (Section 8.3.1.3), surplus biomass is discharged with effluent water. However, were a bromate bioremediation process utilised for potable supplies, effluent polishing by in-line filters and/or disposal of backwash water may also be required in an *ex-situ* system. Jonsson (2004) noted that overdosing of carbon led to overproduction of sludge, disposal of which would increase operational expenditure. However, even with correct carbon dosing, waste sludge production at four full-scale denitrification plants was still 10.5 – 18.0 g per 100 g nitrate removed (Richard, 1989).

8.3.2.2 Influent bromate concentration

Although bromate influent concentration was shown to influence bromate reduction rate (Figure 8.4), contamination level within the contamination plume is relatively fixed. This would necessarily limit bromate reduction to a rate proportional to bromate concentration within the aquifer. Figure 6.1 shows that short-term fluctuations between readings of only < 100 $\mu g\ L^{-1}$ are commonly observed. Therefore, bromate aquifer contamination level would not be a major factor for determination of reduction rate in either an *in-situ* or a stand-alone *ex-situ* bioremediation system. One possibility for maximising bromate reduction rates would be to place either the entire bioreactor system or at least the abstraction borehole close (preferably < 1 km) to the source. Within this area contamination levels in a groundwater plume may be expected to be maximal, with the narrow plume width (Figure 2.2) potentially providing maximum effect on any subsequent plume growth.

8.3.2.3 *Fixed-film packing media*

Use of a fixed-film bioreactor was considered necessary given the advantages of this type of system outlined in Section 8.3.1.2, and that the majority of full-scale studies have also utilised such media-based technologies (i.e. Richard, 1989; Sanders *et al.*, 2004). The reactor trialled in current studies was therefore packed with a relatively large (63 mm) and open (96 % voidage) plastic packing media (Etapak 210). Apart from the successful use of biologically-active GAC by Kirisits and Snoeyink (1999), little information was available on the most effective media. However, a variety of other packing materials have been trialled in potable water nitrate and perchlorate bioreactors, ranging from glass beads (Logan and LaPoint, 2002), diatomaceous earth (Celite R-635; (Giblin *et al.*, 2000a) and GAC (Brown *et al.*, 2002). Sand is also commonly utilised as a packing medium, in both fixed-bed (Nurizzo and Mezzanotte, 1992; Kim and Logan, 2001) and fluidised bed reactors (Viessmann and Hammer, 1985; Twort *et al.*, 2000). Other proprietary media have been developed, such as the 'Biolite' and 'Biodagène' media used in the Nitrazur and Biodenit processes respectively (Richard, 1989). Floating media such as the polystyrene Biostyrene utilised in the wastewater denitrification Biostyr® system, are also reported in clean water systems (i.e. Sanders *et al.*, 2004; Hall, 2000). The choice of packing media determines both biofilm surface area and also propensity to clogging, with a more densely packed media such as sand (2000 $m^2 m^{-3}$ surface area) more likely to clog than an open plastic type such as Etapak 210 (230 $m^2 m^{-3}$). Some proprietary media provide a compromise between these two opposing properties, one example being Biostyrene media which has a diameter greater than sand (3 – 3.5 mm) but lower surface area (1050 – 1200 $m^2 m^{-3}$) (Borregaard, 1997).

Few comparative studies have been published on optimal packing media for anion reduction, but Min *et al.* (2004) determined a plastic media was easier to use than sand in a fixed-bed perchlorate-reducing bioreactor. Although RT required was lower with sand due to a larger surface area, sand was prone to clogging due to biofilm growth, provided less consistent perchlorate removal, and higher backpressures suggested greater pumping costs for equivalent hydraulic loading rates. Another study observed that use of Etapak 210 media increased denitrification efficiency by 27% over a sand bed (2 – 3 mm

diameter) in an autotrophic fixed-bed bioreactor (Rajapske and Scutt, 1999). A combined sand and Etapak 210 bed also reduced the need for regular backwashing, only necessary every 10 days compared with a daily requirement for sand.

Etapak 210 was utilised in the current study partially due to recommendations of the trials outlined above, with results confirming bromate reduction was feasible utilising this media. Etapak 210 is therefore an effective packing media for a bromate bioremediation system. However, with the range of fixed-film media available, careful consideration would be required to adopt the optimum trade-off between surface area and necessity for backwashing (Section 8.3.2.4), both of which would impact on treatment costs. A smaller surface area would likely lead to a requirement for larger reactors for a given reduction ability, whereas a smaller grained media increases both pumping costs due to system headloss and also system downtime caused by a necessity for frequent backwashing. Further, comparative trials would be necessary to identify whether any other commercially-available media allow superior reduction efficiency than Etapak 210.

8.3.2.4 Backwashing and mixing

Backwashing both reduces operational time and increases operating costs to an *ex-situ* bioreactor system, so a decrease in backwashing requirement would improve potential full-scale system cost-effectiveness. Excessive backwashing may also lead to biomass washout and consequent loss of performance (Viessmann and Hammer, 1985), plus disposal of the washwater leads to a 'loss' of overall influent volumes. For example, Richard (1989) reported the percentage of wash water produced at four full-scale denitrification plants to be 3 – 6%, a significant proportion of throughflow which impacts on efficiency and operating costs. The pilot-scale reactors filled with relatively large, open Etapak 210 media did not require backwashing over the entire trial period. No detrimental effect on operational efficiency was noted, highlighting an advantage of this type of plastic media over smaller sand particles (commonly ≤ 3 mm). Although Min *et al.* (2004) found weekly backwashing necessary for a smaller plastic media (3.175 cm diameter) in a perchlorate reducing bioreactor, current studies concurred with bromate-reducing BAC filter trials (Kirisits *et al.*, 2002). These BAC filters also did not require

backwashing, over the entire two-year operational run. In addition, no statistically significant effect on bromate reduction (average influent of 20 μg L^{-1}) was noted following a trial backwash, leading to the conclusion regular backwashing is not essential to maintain bromate reducing ability.

Jepson and Jansen (1993) reported that media type affected headloss characteristics of a wastewater denitrification system, when comparing a pilot-scale downflow reactor utilising an expanded slate media and the upflow Biostyr® system incorporating floating media. The Biostyr® system experienced gradual headloss over a 30-hour run due to biomass growth on the media, whilst in the downflow system this growth eventually led to a sharp rise in headloss as biomass prevented escape of hydrogen gas produced by denitrification. Therefore, an evaluation of requirements for backwashing in a full-scale system is likely to be on an individual basis, based on individual reactor configuration and filter media type.

Although not backwashed, a continuous 0.7 L min^{-1} mixing line was present on the pilot-scale bioreactor, which fed supernatant water through the entire reactor multiple times during the overall RT. For example, a 20-hour RT would include an average of approximately 23 passes through the reactor prior to effluent discharge. In addition to the increased contact between supernatant and biofilm, a mixing line also serves to dilute influent waste streams, and exerts hydraulic shearing effects on the biofilm to reduce effects of clogging (Hyman and Dupont, 2001). It has been reported that addition of a mixing pump to a fixed-film perchlorate reducing bioreactor (Giblin *et al.*, 2000a) led to a decrease in reduction efficiency, with the authors suggesting contact between biomass and supernatant to not be a limiting factor. However, Hall (2000) concluded continuous recirculation of reactor contents by an external pump was important for effective perchlorate reduction, as it provides for uniform and low contaminant concentration under all influent concentration conditions. During current studies, all pilot-scale trials were operated with a mixing line, so a direct comparison is not possible. However, efficient bromate reduction was observed with use of a mixing line, and it could be suggested its use also helped negate any requirement for backwashing by providing continuous flows past the media, thus preventing excessive biofilm development.

8.3.2.5 *Other parameters*

With a range of other operating conditions also trialled during current studies, preliminary recommendations can be made for attainment of optimum bromate reduction rates in an *ex-situ* fixed-film system. pH was not found to be a major controlling parameter, with sufficient buffering within influent water noted to provide a steady pH in the range 6.0 – 8.5 for almost all readings, with both continuous flow systems. pH manipulation is not therefore anticipated to be required within a full-scale system. The utilisation of ambient temperature within the pilot-scale reactor allowed reduction of > 85% influent bromate at retention times ≥ 40 hours. However, the temperature dependence noted (Section 8.2.5.3) showed higher temperature would increase bromate reduction rate, which may in turn reduce required RT. Conversely, a low winter temperature would be likely to limit bromate reduction within a full-scale reactor. Temperature control is not reported as being employed in any nitrate or perchlorate bioreactor system currently in operation, but may be advantageous for a temperature-dependent bromate reducing culture. Optimisation of a full-scale system would therefore be a trade-off between the additional operating costs of temperature control versus enhancement of reduction rates and therefore decreases in RT.

Retention time, along with reactor size, is a major factor in determining throughflow of contaminated water. For example, halving RT would allow a twofold increase in treated water during a given timespan. Use of pure cultures has been suggested to allow reduced retention times, with Kim and Logan (2001) achieving a perchlorate-reducing pure culture minimum RT of only 2.1 minutes for reduction of 20 mg L^{-1} perchlorate, compared with 31 minutes for a mixed culture. However, with carbon source choice suggested to be important for isolated bromate reducing strains (Section 8.2.3), further research would be required for optimisation. A more immediate solution identified from the pilot-scale trial is to allow an extended pre-acclimation period. The observation of biomass washout within reactor 1 for the initial 75 days of continuous flow operation despite a high (80-hour) RT confirmed this period to be crucial, as reactor 2 did not experience similar perturbations following a 23-day batch pre-acclimation period. In BAC trials, Kirisits and Snoeyink (1999) pre-loaded GAC

with up to 40,000 bed volumes of natural water prior to bromate addition to render the GAC biologically active. A laboratory-scale perchlorate-reducing fluidised bed reactor (Polk *et al.*, 2001) was acclimated by pumping through a synthetic feed containing 25 mg L^{-1} perchlorate contamination. Pre-acclimation of a fixed-film bioreactor may affect subsequent treatment capability by allowing development of a sustainable biofilm that is not washed out by influent flow, which effectively reverts the reactor back to a suspended growth mode of operation.

In addition to operational factors, many reactor design and engineering parameters would also need to be considered during the scaling-up process. For example, the pilot-scale reactor utilised a single influent feed line, whereas a full-scale fixed-film process would require even distribution of influent across the reactor (Polk *et al.*, 2001), to encourage plug flow conditions. There would also be a requirement for monitoring and interpretation of critical parameters such as anion concentration, pH, carbon content and temperature. Sanders *et al.* (2004) reported that a lack of regular and reliable carbon and anion analytical data, leading to insufficient carbon dosing, were major factors resulting in nitrite breakthrough during full-scale denitrification trials. The authors therefore suggested that automated in-line instruments be considered. Hultmann *et al.* (1994) commented a feedback control loop for carbon dosing was important for optimisation of a wastewater Dynasand filter, and Jonsson *et al.* (1997) noted an automated dosing system based on continuous effluent nitrate analysis was used to maintain constant denitrification conditions within their study reactor. Automation of analysis and dosing may therefore prove necessary in a full-scale bromate reducing system.

Another factor which must be given consideration is ease and clarity of system operation. Whilst a pilot-scale system is overseen by researchers with detailed knowledge of processes being investigated, this would not generally be the case during full scale operation. Reactor operation must be made as transparent as possible to avoid confusion. Finally, a full economic analysis, which is beyond the scope of the current study, would be required to quantify areas of significant operational expenditure. Major factors affecting overall cost would likely include purchase of carbon source, disposal of

sludge if necessary, and also pumping and other electrical costs. Although recommended for bromate bioremediation in this case, one major drawback of pump and treat systems relative to *in-situ* strategies is the requirement for and cost of abstracting water prior to treatment. This generally tends to lead to a higher cost treatment system (Hyman and Dupont, 2001).

8.3.3 Evaluation of system robustness

Whilst reliability of any full-scale treatment process can be maximised by good reactor design and careful consideration of operational procedures, it is impossible to eliminate system upsets entirely. Failures may be due to a variety of factors, ranging from problems in electrical, mechanical, electronic and hydraulic systems, to unforeseen disasters both natural and man-made, and also human error (Baruth, 2005). The most critical times are during 'burn-in' and 'wear-out' periods, where factors characterised by manufacturing defects and fatigue respectively are more prevalent However, failure can occur at any point during the lifetime of a process. Robustness is often evaluated at pilot-scale, prior to design and construction of the full-scale process. This ensures the technology is adequate to prevent catastrophic failure during short periods of perturbation. In the case of a bromate reducing bioreactor, system failure would be characterised by significant breakthrough of bromate, or also possibly nitrite into effluent flows.

As no large-scale bromate bioremediation process has to date been constructed, little data were available regarding system robustness. Therefore, reference has been made to comparable studies investigating nitrate and perchlorate reduction systems. One recent perchlorate reduction trial was aimed at evaluating robustness of a BAC bioremediation process (Brown *et al.*, 2005), by use of a pilot-scale column (102 mm x 4.3 m) dosing acetate and utilising an EBCT of 15 – 25 minutes. Four critical process upsets were identified as characterising robustness. These were backwashing, transient changes in anion (perchlorate and nitrate) loading, electron donor failure, and total system shutdown. The authors used evaluation of these four factors during pursuance of conditional acceptance for operation as a potable water remediation system in California,

USA. It is therefore considered investigation of these parameters to also be valid for evaluation of bromate system robustness.

8.3.3.1 Backwashing

Although backwashing was not found to be necessary for the pilot-scale bromate reduction system trialled in current studies, it was identified in Section 8.3.2.3 that remediation efficiency may be enhanced by use of media with a higher surface area. This may introduce a requirement for backwashing. Brown *et al.* (2005) reported the pilot-scale BAC filter was not affected by two trial backwashes, even when samples were taken within 15 minutes of the perturbation. As Kirisits *et al.* (2002) also did not find a single trial backwash of a laboratory-scale bromate reducing BAC system to affect bromate removal efficiency (Section 8.3.2.4) it is likely full-scale bromate bioremediation system would not be affected by backwashing. However, further pilot-scale trials would be required were backwashing identified as a necessity.

8.3.3.2 Transient changes in anion loading

Brown *et al.* (2005) investigated transient changes in both perchlorate and nitrate spiking. Perchlorate was altered in step changes from 50 μg L^{-1} to both 300 μg L^{-1} and 5 μg L^{-1}. Neither upset induced any alteration to the prior state of complete perchlorate reduction. A stepwise alteration of nitrate from 6 mg L^{-1} to 29 mg L^{-1} did however lead to perchlorate breakthrough after 19 hours, with a subsequent increase of feed acetate concentration requiring 8 days to fully remove this breakthrough. It was concluded that, whilst a perchlorate reducing culture was able to react quickly to alterations in perchlorate concentration, re-equilibration of the microbial community was required upon alteration of nitrate concentrations.

No transient perturbation of nitrate concentration on bromate reduction was completed during current trials or is available in the published literature. Kirisits and Snoeyink (1999) did report that bromate reduction slightly decreased in the presence of higher nitrate concentrations (Figure 2.3), but this was not following a transient alteration. A stepwise bromate increase was employed during current trials within

chemostat phase IIb (Section 7.2.1.2), when bromate concentration was increased from 32 mg L^{-1} to 48 mg L^{-1} on Day 105. An immediate increase in reactor 1 supernatant bromate concentration was noted (40-hour RT), but no effect was observed in reactor 2 with an 80-hour RT. The increase in reactor 1 bromate concentration was sustained until influent glucose level was increased, following which bromate concentrations dropped back to below the detection limit within 5 days, when the next sample was taken. Thus it can be concluded that bromate breakthrough was due to carbon limitation and, as observed by Brown *et al.* (2005), small transient increases in anion substrate concentration are unlikely to elicit decreases in reduction efficiency.

8.3.3.3 Electron donor failure

Electron donor failure was not specifically studied during current trials either at laboratory- or pilot-scale, but influent line blockage did occasionally lead to cessation of glucose flow for periods generally < 24 hours. The only significant period of electron donor failure was the 65-hour glucose interruption during phase B pilot-scale trials (Section 7.2.4.3). During this period, although both reactors showed signs of biomass washout and required a period of batch operation, the pre-acclimated reactor 2 recovered within 3 reactor volumes (240 hours) whereas reactor 1 required a further 240-hour period of batch operation to regain bromate reducing ability. However, on no occasion where electron donor failure was < 24 hours was a protracted decrease in bromate reduction observed, although it must be noted the sampling regime would not have highlighted transient alterations. Brown *et al.* (2003) noted a laboratory-scale BAC perchlorate reducing filter exhibited 20% and 36% breakthrough of a 50 µg L^{-1} influent after 48-hour and 72-hour acetate interruptions respectively, but a 24-hour failure did not produce similar effects, which concurs with results obtained in current studies. Brown *et al.* (2005) also observed no perchlorate breakthrough following a 24-hour interruption with the pilot-scale BAC system. It can therefore be concluded a short-term (≤ 24 hour) electron donor interruption would be unlikely to affect performance of a full-scale anion reduction system. This is an important finding as it suggests there would be sufficient time to detect and remedy a failure prior to any loss in process effectiveness.

8.3.3.4 Total system shutdown

At no point during current studies was total system shutdown undertaken. However, upon completion of trials the pilot-scale reactors were kept as a semi-batch system, only being fed with groundwater and electron donor weekly. Although not closely monitored or analysed it was noted bromate reduction still occurred even without a continuous influent supply, thus suggesting an active microbial population was maintained under these conditions. A similar stability during periods of system shutdown has also been noted with both nitrate and perchlorate reducing processes. Richard (1989) observed that daily 4 – 5 hour periods of shutdown did not affect full-scale 'Nitrazur' or 'Biodenit' denitrification plants. Brown *et al.* (2005) also did not detect any perchlorate breakthrough following recommissioning of the pilot-scale perchlorate reducing BAC column even following a long (14-day) period of total system shutdown. This agrees with current studies and suggests that, even following extended periods of shutdown, reduction capacity can be maintained within a reactor operating in 'standby' mode.

Robustness of full-scale bioreactors is now well-documented, with both denitrification (i.e. Richard, 1989) and perchlorate reducers (i.e. Polk *et al.*, 2001; Hatzinger *et al.*, 2000) system reported as operating in a consistent and effective manner over extended periods. For example, the full-scale fluidised bed reactor studied by Hatzinger *et al.* (2000) was reported to consistently remove 6 – 8 mg L^{-1} perchlorate to < 4 μg L^{-1}. Richard (1989) noted that, although biological systems require closer monitoring and more complex automation than comparable ion exchange dentrification processes, both were effective and robust and was not possible to conclude one methodology was better than the other. The evaluation of robustness carried out by Brown *et al.* (2005) on a pilot-scale perchlorate reducing BAC filter concluded the system was robust and therefore suitable for full-scale development. This view was backed by the California Department of Health Services (CHDS), who granted a conditional acceptance for use of the bioreactor process as a potable water treatment system. Current trials have suggested bromate reduction to be more sensitive to

perturbations in operational parameters such as temperature, retention time and reactor history than either nitrate or perchlorate reduction processes (Section 8.2.5). However, evaluation of robustness has shown that, although more trials are required to give a complete assessment, initial results suggest neither backwashing, transient bromate loading, electron donor failure or system shutdown would have an immediate and catastrophic effect on overall effectiveness.

8.3.4 Estimation of process effectiveness at aquifer scale

Rehabilitation of an aquifer groundwater plume is a large-scale undertaking. It is accepted that total remediation is rarely achieved by application of a single technology, and the larger the site the less likely a stand-alone process will offer a complete solution (Barr *et al.*, 2002). One overall goal of the current study was investigation and development of a bioremediation tool, but this can only operate as one aspect of an integrated aquifer rehabilitation strategy. As outlined above, siting of abstraction for a full-scale system close to the contamination source would be crucial to maximising reduction rates. Feasibility of this would be dependent on both regulatory and landowner consent in and around proposed sites.

8.3.4.1 In-situ systems

Siting of an *in-situ* system would require construction of boreholes within the contaminated area. Previous *in-situ* designs have suggested injection boreholes placed approximately 8 m apart in a 10 m radius 'daisy wheel configuration', with an extraction borehole in the centre to encourage radial flow (Godbold, 2002). A schematic is given in Figure 8.13. This would suggest an active cross-sectional area of 314 m^2, with a total active volume of approximately 2500 m^3. Linear flow within the plume may only require a half circle configuration, in which case an active volume of 1250 m^3 would be expected. However, bromate reduction would be heavily dependent on groundwater flow through the active zone. Groundwater flow patterns in the area are not well understood, and local geology may lead to complex flow regimes. Therefore, no further analysis on effectiveness of an *in-situ* system is possible without thorough investigation of hydrogeology in the affected zone.

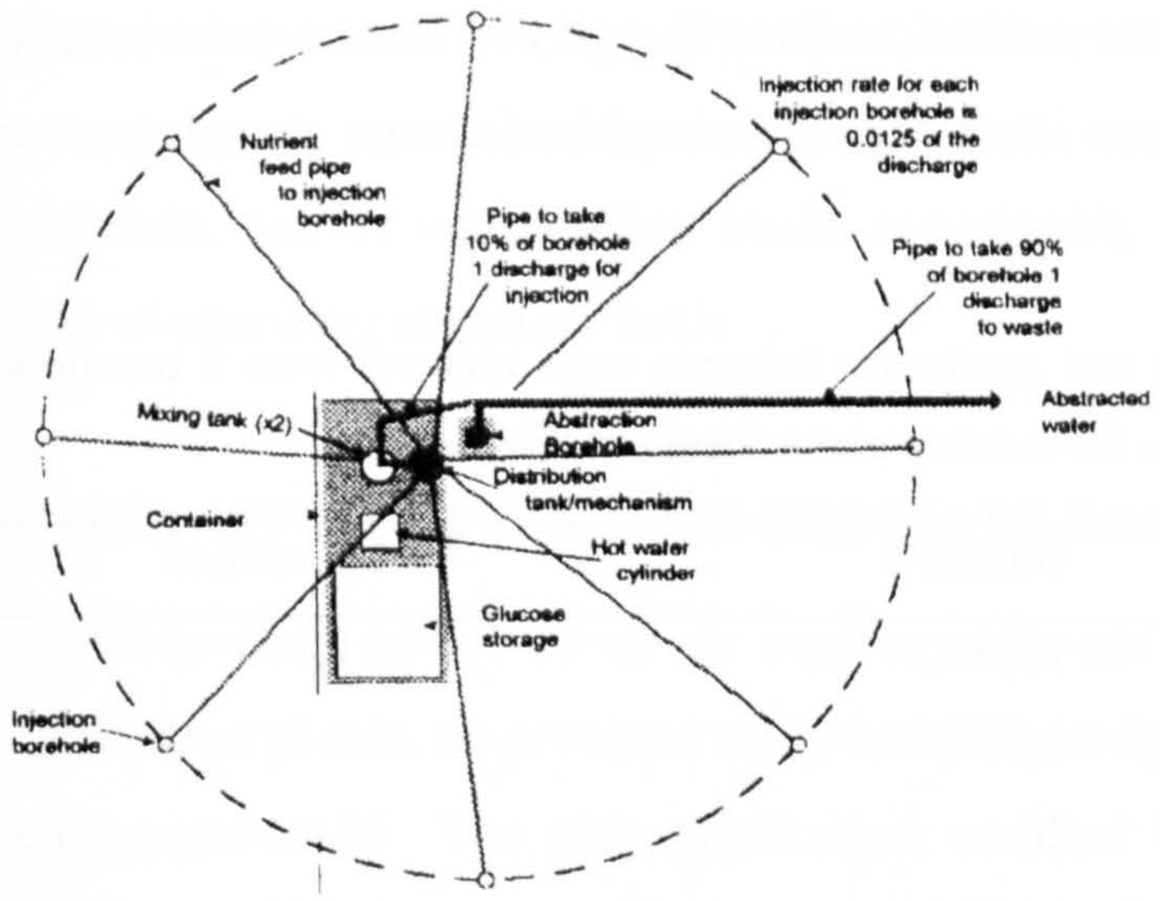


Figure 8.13 - Daisy wheel configuration for *in-situ* groundwater bioremediation (Godbold, 2002)

8.3.4.2 Ex-situ systems

Although no benchmark for scaling up bromate bioreactors is currently available, by reference to previous related studies, an estimate of remediation potential under ideal conditions can be attempted. Pilot-scale trials in the current study showed approximately 50% reduction of a 1.1 mg L^{-1} bromate influent can quickly be attained and sustained within a simple un-optimised bioreactor. This can therefore be taken as the baseline value for expected performance of a scaled-up system. Groundwater sources utilised for the study were contaminated with up to 1.4 mg L^{-1} bromate. A sustainable contamination level in excess of 2 mg L^{-1} is thought unlikely within the aquifer groundwater, so this value can be utilised as a maximum, with 100% reduction of this influent level attainable with reference to the chemostat trial. Although not achieved during the current study, it can be hypothesised complete reduction of a 2 mg L^{-1} bromate influent may be possible in a shorter RT within an optimised bioreactor system. Therefore an RT of 10 hours has been assumed for calculation of maximum bromate removal levels. A cylindrical reactor size of 1.5 m (diameter) x 6.4 m (height) was utilised by Polk *et al.* (2001) during full-scale perchlorate bioremediation trials, and this size has been used for calculation purposes. A total volume of 11,310 m^3 may therefore be expected (excluding volume reduction for packing media). Using the above figures, approximate baseline and

potential maximum bromate reduction rates (kg day^{-1}) can be calculated (Table 8.2), assuming the siting of three identical reactors placed across the plume and operating under ideal conditions.

Table 8.2 - Potential baseline and maximum bromate mass removal from a contaminated aquifer using three full-scale fixed-film bioreactors under ideal conditions

	Baseline	Maximum
Reactor volume (litres)	11,310	11,310
Bromate reduction (mg L^{-1})	0.55	2
Retention time (hours)	20	10
Mass of bromate removed by one reactor (kg day^{-1})	0.0075	0.054
Mass of bromate removed by three reactors (kg day^{-1})	0.022	0.16
Total bromate removal per year (kg yr^{-1})	8.2	59.4

Thus bromate mass removal of up to 8.2 – 59.4 kg yr^{-1} using three full-scale fixed-film bioreactors sited across the contamination plume close to the source may be expected, over a linear distance of up to 100 m assuming a 20 m reactor spacing. No accurate estimate of bromate mass within the plume is available, but unconfirmed calculations have suggested a value of > 1000 kg to not be unreasonable. Using this figure a minimum cleanup period, under ideal conditions, of approximately 17 years can be obtained. The complex hydrogeology of the area means total cleanup could never be achieved and, with siting of a plant close to the source and thus upstream of much of the plume area, a proportion of the contamination could not be reached. However, assuming continued contaminant flow from the source area, a system such as this may assist with long-term rehabilitation, reducing upstream concentrations as groundwater flow dissipates and dilutes bromate currently within the plume. In addition, were further studies able to identify and successfully culture a high-rate bromate-reducing strain, it is conceivable that RT could be further reduced. As an example, achievement of 2 mg L^{-1} reduction at a 1-hour RT would allow maximum theoretical yearly bromate removal in

the above system to rise to > 500 kg. Therefore, although reduction rates achieved at realistic aquifer bromate concentrations in current studies would not allow significant aquifer remediation, further optimisation could conceivably lead to a viable full-scale system capable of operating at aquifer scale.

8.4 OTHER APPLICATIONS FOR BROMATE BIOREMEDIATION

With bromate not perceived as an aquifer pollutant prior to discovery of the current contamination plume, no previous study had focussed on remediation of bromate-contaminated groundwater. The only application covered by published literature for bromate remediation, whether biological or otherwise, was in amelioration of potable water contamination following ozonation, as outlined in the literature review (Section 2.7). Initial work on bromate bioremediation at concentrations commonly observed following ozonation ($\leq$ 60 $\mu g\ L^{-1}$) concluded application of the process was not promising due to slow reduction rates and long retention times required for treatment to potable water guideline values (Hijnen *et al.,* 1995; Hijnen *et al.,* 1999). However, with other authors (i.e. Kirisits and Snoeyink, 1999) suggesting biological bromate removal to guideline concentrations (10 – 25 $\mu g\ L^{-1}$) was possible within an RT typical of existing GAC treatment processes (25 minutes), potential application of the current system may be possible. Bromate contamination levels below 200 $\mu g\ L^{-1}$ were not trialled in the current study but, with this concentration reduced to only 20 $\mu g\ L^{-1}$ with carbon excess (150 mg L^{-1} glucose) during initial chemostat phase I trials, reduction of low influent levels to near current UK potable water limits (10 $\mu g\ L^{-1}$) was demonstrated. Whilst current work has not provided further evidence for commercial potential in this application, use of specific bromate degrading strains in a controlled environment may allow reductions in RT to those feasible for potable water treatment. Use of an integrated process combined with filtration or ion exchange as previously described (Section 8.3.1.3) may also prove effective for ozonated water remediation, with the wider potential application providing additional financial impetus for development of halotolerant bromate reducing microbial strains.

Another possible application of bromate bioremediation has also been described. Off-gas generated by industrial incinerators is treated in gas scrubbers prior to discharge into the atmosphere. Effluent generated by this process contains a variety of inorganic compounds, with chloride, chlorate, bromate and hypochlorite all present (van Ginkel *et al.*, 2005a). Currently these wastewaters may not be treated, but increasingly stringent water quality guidelines could lead to requirements for remediation prior to discharge. Bromate influent concentrations of 100 – 400 mg L^{-1} were successfully reduced by > 90 % within this matrix in a pilot-scale autotrophic bioreactor in the presence of ≤ 1.6 g L^{-1} chlorate (van Ginkel *et al.*, 2005a). The authors concluded remediation of oxyanion-contaminated incinerator effluent by this technique to be 'very promising'. With high rate bromate reduction achieved in current studies, a heterotrophic fixed-film process could also be utilised for this application. It is possible dilution may be required to reduce high bromate concentrations and prevent potential toxic effects but, with the Department for the Environment, Transport and the Regions suggesting a requirement for 89 – 166 further incinerators in England and Wales to meet waste recovery targets in their Waste Strategy 2000 (DETR, 2000), the technique may show some promise for bioremediation of this emerging industrial waste stream.

9 CONCLUSIONS

Bromate, a 'possible human' carcinogen, is an increasingly-encountered contaminant of water both due to implementation of legislation limiting concentrations within potable water (10 – 25 μg L^{-1}) and also concurrent advances in analytical capability. As a prominent disinfection by-product, a range of techniques trialled for remediation could be collated, encompassing physical, chemical and biological methodologies. However, bromate was unknown as a groundwater contaminant until discovery of aquifer contamination forming a basis of the current study. With little relevant information available upon commencement of the project, this study has therefore encompassed a wide range of aspects of groundwater bromate contamination, including bromate effects when dosed in wastewater, investigation and development of a potential bromate bioremediation strategy, and analysis within these demanding matrices.

9.1 ANION ANALYSIS

Of the techniques trialled (Ion chromatography (IC), High Performance Liquid Chromatography (HPLC) and spectrophotometry), the combination of IC with conductivity detection (IC-CD) was most cost-effective for both groundwater and wastewater analysis in the current study. Accurate and reproducible results were obtained with bromate detection to 10 μg L^{-1}, although wastewater samples with high ionic loadings may be unsuitable due to analytical peak interference.

Techniques utilising a bromate-specific Post Column Reaction (PCR) in conjunction with both IC and HPLC techniques were accurate and free from peak interference even with high ionic loadings. These systems are ideal for bromate analysis in wastewater as well as groundwater, although capital cost is higher and other anions cannot be simultaneously analysed by the HPLC methodology.

Neither HPLC with direct UV detection nor spectrophotometry allowed reproducible bromate detection to 10 μg L^{-1}, so were not suitable for use in the current study. However, spectrophotometry could be a useful basic field technique. HPLC with direct UV detection, with careful analytical column selection, can be used for

groundwater analysis (mg L^{-1} range) using only a standard laboratory HPLC system.

9.2 BROMATE IN A WASTEWATER ENVIRONMENT

Upon dosing with bromate or bromide (≤ 200 mg L^{-1}) or elevated concentrations of both anions, no significant effect ($p < 0.05$) on overall microbial respiration rate within Membrane Bioreactor (MBR) sludge was observed during respiration inhibition trials.

Bromate dosing of a pilot-scale MBR at 2 mg L^{-1} caused a significant alteration in sludge viscosity, capillary suction time and specific cake resistance, suggesting biomass properties were subtlety altered by ingress of bromate loaded water to a sewage treatment process. However, this was not apparent at a subsequent higher (100 mg L^{-1}) bromate dosing level, with the only alterations at this concentration being a slight increase in carbohydrate and COD content within the supernatant soluble microbial product (SMP) fraction. This observation could be explained by overall alterations in ionic composition caused by potassium bromate dosing and, together with respiration inhibition data, suggested any minor fluctuations caused by ingress of bromate contamination to a wastewater treatment works would be transient and not impact greatly on microbial communities within biological treatment systems.

Gross bromate contamination in excess of that likely to be encountered within such a system (2 g L^{-1}) had a significant depressive effect on respiration of MBR biomass. However, a similar concentration did not affect MBR biomass previously acclimated to bromate dosing (≤ 100 mg L^{-1}), further confirming effects of bromate contamination on wastewater biomass to be transient.

Reversible sorption of bromate to freeze-dried MBR biomass was not observed, but instead complete reduction to bromide was noted. Analysis of sterilised samples showed the nature of this reduction in the presence of a sewage sludge biomass to be abiotic, possibly analogous to reduction on granular activated carbon (GAC).

9.3 BROMATE BIOREMEDIATION

Groundwater contaminated by bromate was shown for the first time to be effectively reduced to bromide during continuous enrichment of indigenous aquifer bacteria using addition of glucose as a carbon source. Filtration (0.45 µm pore-size) and sterilisation of the groundwater medium halted bromate reduction during batch flask trials, confirming a biological nature of reduction within a groundwater matrix under anoxic conditions.

Glucose was rapidly fermented within an inoculated batch culture system, with an initial 190 mg L^{-1} spike removed to < 1 mg L^{-1} within 48 hours. This suggested bromate reduction by direct anaerobic respiration of glucose to not be significant. Fermentation products including acetate and ethanol were produced by the fermentation process.

Initial bromate reduction rates of $\leq$ 27.8 µg L^{-1} hr^{-1} in a continuous-flow chemostat were low compared with nitrate reduction ($\leq$ 1858.7 µg L^{-1} hr^{-1}), which was efficiently reduced in all trials. However, extended use of an enrichment culture was successful in enhancing both absolute bromate reduction rates and rates relative to denitrification. Following a period of high glucose availability in the suspended-growth system, a marked and sustained enhancement of bromate reducing ability was noted, with bromate reduction rising to > 1000 µg L^{-1} hr^{-1}.

A dependence of bromate reduction rate on reactor bromate concentration was observed, with higher concentrations exhibiting enhanced reduction rates. Comparison of bromate reduction rates with previous studies suggested both initial suspended growth trials and batch flask studies exhibited bromate reduction via a 'co-metabolic' process mediated by denitrifying bacteria. However, observed high-rate bromate reduction coupled with data from a previous study suggested a shift in microbial composition occurred, allowing proliferation of putative high-rate bromate degrading strains. Subsequent trials utilising an artificial medium without nitrate or sulphate addition confirmed a high rate of bromate degradation (676.0 µg L^{-1} hr^{-1}) could be maintained with only low concentrations of these alternative electron acceptors.

Batch flask trials allowed manipulation of a range of parameters without perturbation of the continuous flow systems, but a difference in growth patterns within the two systems was noted. It is suspected conditions within batch flasks favoured denitrifying strains exhibiting bromate reduction by co-metabolism, rather than high-rate specific bromate reducing bacteria. Therefore, whilst batch flasks are useful for rapid elucidation of optimum conditions, this difference should be borne in mind.

Rapid and total nitrate removal within 4 hours of trial commencement possibly suggested an unelucidated abiotic nitrate removal mechanism within the batch flask system. Bromate reduction in the presence of nitrate during batch and continuous flow trials showed bromate could be reduced concomitantly with nitrate. Nitrite production, denoting partial denitrification, in excess of 1 mg L^{-1} was only noted under conditions of carbon limitation (continuous flow) and low (10-hour) retention times (fixed-film) in continuous flow systems. Sulphate, another potential competing electron acceptor, was only reduced following almost total removal of bromate, confirming the microbial preference for bromate over sulphate suggested by Gibbs Free energy values.

Bromate reduction was observed in the presence of influent dissolved oxygen in both suspended growth ($\leq$ 4.4 mg L^{-1}) and fixed-film (average 6.1 mg L^{-1}) systems. This may indicate a facultatively anaerobic nature of either bromate reducing isolates or a proportion of the microbial consortium.

Inhibition by high bromate concentrations, denoted by a decrease in bromate reduction ability, was noted at influent concentrations in excess of 64 – 80 mg L^{-1}. However, these concentrations are higher than would likely be encountered for most applications.

Reactor history was important in both suspended growth and fixed-film systems, with perturbations to influent quality resulting in altered bromate reducing ability, suggested to be due to alterations in microbial community composition. A slow growth rate ($\leq$ 0.025 hr^{-1}) for bromate reducing strains was demonstrated by a minimum 40-hour RT requirement for sustainable bromate reduction under suspended growth conditions.

High bromate concentrations (≤ 48 mg L^{-1}) were remediated to below 0.01 mg L^{-1} within a 40-hour retention time in a suspended growth system. Use of pilot-scale fixed-film bioreactors operating at ambient conditions with glucose as carbon source reduced an average bromate influent of 1.1 mg L^{-1} by > 90% at a comparable retention time. A 20-hour retention time led to reduced but still > 50% reduction. This trial showed for the first time that bromate groundwater contamination could be continuously remediated with a pilot-scale biological system.

Manipulation of operating parameters gave an insight into potential optimisation of a full-scale system. pH control was not required within either laboratory- or pilot-scale systems. Temperature was a rate-determining factor, with batch flask trials suggesting a temperature optimum of $\geq 44^{o}C$. A plastic packing media was successfully utilised for the fixed-film trial, with the open structure of the media also leading to no requirement for a regular backwashing cycle.

Colony isolation by plating and dilution-to-extinction procedures produced a range of potential isolates, some able to reduce bromate faster than a mixed culture, although poor growth on glucose by some isolates may be explained by a fastidious nature of these isolates. Growth rate was also slow on agar plates, with 8 weeks required for significant colony formation. Microbial characterisation was not possible during the timescale of the study. A reluctance to of many isolates to grow following sub-culture suggested agar composition was deficient or sub-optimal for continued growth.

9.4 POTENTIAL TECHNOLOGY APPLICATIONS

Based on data gained during current studies, an *ex-situ* fixed-film system was recommended for further development into a full-scale bromate bioremediation system as a tool for aquifer rehabilitation. Such a 'pump and treat' methodology would likely incur higher capital and operational costs than *in-situ* strategies, due to additional requirements for pumping and possibly also temperature regulation. Water loss from backwashing, and sludge disposal costs were also highlighted as potential drawbacks of *ex-situ* systems. However, uncertain geological conditions and typically low temperatures (~ $10^{o}C$) within the contaminated aquifer suggest an *ex-situ* approach may be the more effective

Optimisation of a potential system at full-scale was calculated to allow removal of up to 60 kg bromate per year from an aquifer under ideal conditions, with lower retention times further enhancing this figure. Due to a requirement for high influent bromate levels to maximise reduction rates, it was concluded that bioreactors should be sited as close to the contamination source as is feasible.

Combination with an *in-situ* bioaugmentation strategy would be both technologically and politically more complicated, but may provide additional *in-situ* remediation by reinjection of an acclimated bromate-reducing biota. A pump and reinject system would also potentially lower costs by removal of any requirement for effluent polishing or sludge disposal.

Other potential applications identified included treatment of potable water following ozonation processes, remediation of bromate-loaded brine generated by physical separation processes such as filtration and ion exchange, and reduction of bromate in effluent from incinerator off-gas scrubber systems.

10 FUTURE WORK

The current study has provided the first evidence for biological remediation of bromate contamination within a groundwater matrix, investigated in the context of aquifer rehabilitation. Micro- and macro-scale systems were investigated, ranging from mechanisms mediating the microbial bromate reduction process to optimisation of parameters for construction of a full-scale aquifer bioremediation tool. From this promising base, future studies should focus on more fully elucidating these systems, to optimise and improve the technology for potential application at an aquifer scale.

1) Both 'co-metabolic' and high-rate specific bromate reduction mechanisms were potentially observed during suspended growth chemostat trials. Further trials could focus on these two mechanisms, to further understand processes occurring during bromate reduction. Specific questions to address would include:

   - whether 'co-metabolic' and 'high-rate' bromate reduction were indeed separate mechanisms
   - whether bromate reduction can occur in the absence of any nitrate utilisation, or that of other anions such as chlorate
   - the identity of strains isolated during current trials
   - characterisation of these strains, including elucidation of utilisable carbon sources
   - storage of isolated strains to allow both later investigation and also reactor inoculation without requiring maintenance of a continuous chemostat culture
   - the possibility of abiotic nitrate reduction occurring within batch flasks

2) Batch flask trials, whilst able to investigate a wide range of parameters, were shown to exhibit low bromate reduction rates even with high-rate bromate reducing inoculum. It is therefore suggested a more appropriate system for optimising parameters within a high-rate bromate reducing system would be the use of a 'mini-chemostat' system. This system is being constructed as a constituent part of further studies, with a picture of such a system given in Figure 10.1.

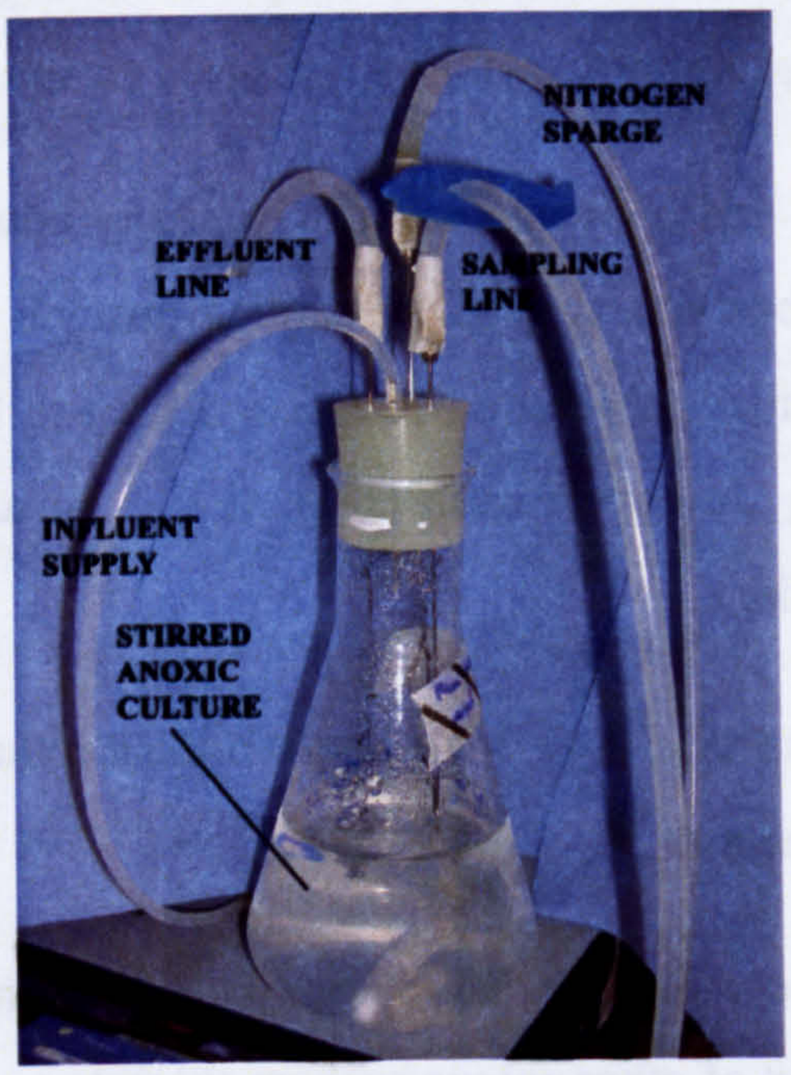


Figure 10.1 - Setup of one 'mini-chemostat' bioreactor

3) Whilst the basis of an effective *ex-situ* fixed-film system has been established, operational parameters such as temperature, carbon source, carbon dosing level, packing media, supernatant recycle and backwashing, as well as the use of an isolated specific bromate reducing microbial strain, would all require further investigation to fully optimise a full-scale bioreactor. Development of a combined *ex-situ/in-situ* bioremediation would require considerable further effort, and should be viewed as a long-term goal.

11 REFERENCES

Achilli, M., and Romele, L. (1999). Ion chromatographic determination of bromate in drinking water by post-column reaction with fuchsin. *Journal of Chromatography A,* 847(1-2), 271-277.

Achilli, M., and Romele, L. (2000). Ion chromatographic determination of bromate in drinking water by postcolumn reaction with fuchsin. *American Laboratory,* 32(17), 60-61.

Adamcikova, L., Farbulova, Z., Sevcik, P., and Kawczynski, A. (2003). Sequential oscillations in the uncatalysed bromate oscillator and their simple qualitative model. *Journal of Physical Chemistry A,* 107(4), 508-511.

Ahmad, R., Cartwright, M., and Taylor, F. (2001). *Analytical Methods for Environmental Monitoring.* Pearson Education Ltd, Cambridge, UK.

Almeida, T. (2004). *In-Sewer Transformations and the Fate of Bromate.* MSc Thesis, Cranfield University, UK.

APHA (1998). *Standard Methods for the Examination of Water and Wastewater, 20th Ed.* American Public Health Association, Washington DC, USA.

Amy, G., Douville, C., Daw, B., Sohn, J., Galey, C., Gatel, D., and Cavard, J. (2000). Bromate formation under ozonation conditions to inactivate *Cryptosporidium*. *Ozone: Science and Engineering,* 22(5), 487-499.

Amy, G., Pellegrino, J., and Liang, S. (2003). *Treatibility of perchlorate-containing water by RO, NF and UF membranes.* Project #2531, AwwaRF.

Asami, M., Aizawa, T., Morioka, T., Nishijima, W., Tabata, A., and Magara, Y. (1999). Bromate removal during transition from new granular activated carbon (GAC) to biological activated carbon (BAC). *Water Research,* 33(12), 2797-2804.

Attaway, H., and Smith, M. (1993). Reduction of perchlorate by an anaerobic enrichment culture. *Journal of Industrial Microbiology,* 12, 408-412.

Bao, M., Griffini, O., Santianni, D., Barbieri, K., Burrini, D., and Pantani, F. (1999). Removal of bromate ion from water using granular activated carbon. *Water Research,* 33(13), 2959-2970.

Barnett, D., Guevremont, R., and Purves, R. (1999). Determination of parts-per-trillion levels of chlorate, bromate, and iodate by electrospray ionization/high-field asymmetric waveform ion mobility spectrometry/mass spectrometry. *Applied Spectroscopy,* 53(11), 1367-1374.

Barr, D., Finnamore, J., Bardos, R., Weeks, J., and Nathanail, C. (2002). *Biological Methods for Assessment and Remediation of Contaminated Land: Case Studies.* Construction Industry Research and Information Association, London.

Baruth, E. (Ed). (2005). *Water Treatment and Plant Design.* McGraw-Hill, New York.

BBC (2004). *Coke recalls controversial water* (News report). http://news .bbc.co.uk/1/hi/business/3550063.htm. (www page, accessed 19 March 2004)

Bengtsson, G., and Bergwall, C. (1995). Heterotrophic denitrification potential as adaptive response in groundwater bacteria. *Microbial Ecology,* 16, 307-318.

Bentzen, G., Smith, A., Bennett, D., Webster, N., Reinholt, F., Sletholt, E., and Hobson, J. (1995). Controlled dosing of nitrate for prevention of H_2S in a sewer network and the effects on the subsequent treatment processes. *Water Science and Technology,* 31(7), 293-302.

Bernoth, L., Firth, I., McAllister, P., and Rhodes, S. (2000). Biotechnologies for remediation and pollution control in the mining industry. *Minerals and Metallurgical Processing,* 17(2), 105-111.

Bertotti, M., and Pletcher, D. (1996). The reduction of bromate at molybdenum oxide film cathodes. *Electroanalysis,* 8(12), 1105-1111.

Bolyard, M., Fair, P., and Hautman, D. (1992). Occurrence of chlorate in hypochlorite solutions used for drinking water disinfection. *Environmental Science and Technology,* 26(8), 1663-1665.

Borregaard, V. (1997). Experience witn nutrient removal in a fixed-film system at full-scale wastewater treatment plants. *Water Science and Technology*, 36(1), 129-137.

Bouland, S., Duguet, J., and Montiel, A. (2001). Hypochlorite: contribution in ions bromate. *L'Eau, l'Industrie, les Nuisances,* 244, 63-84.

Bourgine, F.,Chapman, J., Kerai, H., and Green, J. (1993). Ozone and the formation of bromate in water treatment. *Journal of the Institution of Water and Environmental Management, 7, 571-576.*

Boussaid, F., Martin, G., Morvan, J., Collin, J., Landreau, A., and Talbo, H. (1988). Denitrification *in-situ* of groundwaters with solid carbon matter. *Environmental Technology Letters*, 9, 803-816.

British Standards Institute. (1995). *Water Quality - Part 5: Biological Methods - Section 5.7: Test for Inhibition of Oxygen Consumption by Activated Sludge*. ISO8192, BSI, UK.

British Standards Institute. (2001). *Water Quality - Determination of dissolved bromate - Method by liquid chromatography of ions*. ISO 15601, BSI, UK.

Brookes, A., Judd, S., Reid, E., Germain, E., Smith, S., Alvarez, H., LeClech, P., Stephenson, T., Turra, E., and Jefferson, B. (2003). Biomass characterisation in membrane bioreactors. In: *IMSTEC '03,* University of New South Wales, 10-14 Nov. 2003.

Brown, J., Snoeyink, V., and Kirisits, M. (2002). Abiotic and biotic perchlorate removal in an activated carbon filter. *Journal of the Amercian Water Works Association,* 94(2), 70-79.

Brown, J., Snoeyink, V., Raskin, L., and Lin, R. (2003). The sensitivity of fixed-bed biological perchlorate removal to changes in operating conditions and water quality characteristics. *Water Research,* 37, 206-214.

Brown, J., Anderson, R., Min, J., Boulos, L., Prasifka, D., and Juby, G. (2005). Fixed-bed biological treatment of perchlorate-contaminated drinking water. Journal of the American Water Works Association, 97(9), 70-81.

Bruce, R., Achenbach, L., and Coates, J. (1999). Reduction of (per)chlorate by a novel organism isolated from paper mill waste. *Environmental Microbiology,* 1(4), 319-329.

Bruus, J., Nielsen, P., and Keiding, K. (1992). On the stability of activated sludge flocs with implications to dewatering. *Water Research,* 261, 597-1604.

Buchberger, W., and Ahrer, W. (1999). Combination of suppressed and non-suppressed ion chromatography with atmospheric pressure ionization mass spectrometry for the determination of anions. *Journal of Chromatography A,* 850(1-2), 99-106.

Burgess, J. (1999). *Micronutrients for Wastewater Treatment.* PhD Thesis, Cranfield University, UK.

Burton, D., and Richardson, L. (1981). *Investigation of chemistry and toxicity of ozone-produced oxidants and bromate to selected estuarine species.* US EPA report no. EPA-600/4-81-040. Office of Water and Waste Management, Florida.

Butler, R., Cartmell, E., and Godley, A. (2003). *Laboratory study of concomitant anoxic biological removal of bromate and nitrate from groundwater.* Report UC 6368, WRc plc.

Butler, R., Godley, A., and Cartmell, E. (2004). *Bromate contaminated groundwater bioremediation: Phase II enrichment of high rate bromate degrading microbes.* Report EA16702, WRc plc.

Campbell, N. (1993). *Biology, 3rd Ed.* Pearson Education, Cambridge.

Cartmell, E. (1997). *Aquifer Denitrification: An Experimental and Modelling Evaluation.* PhD thesis, Imperial College of Science, Technology and Medicine, University of London, UK.

Casella, I., and Contursi, M. (2005). Electrochemical and spectroscopic characterization of a tungsten electrode as a sensitive amperometric sensor of small inorganic ions. *Electrochimica Acta,* 50(20), 4146-4154.

Chalupa, M. (1985). Le denitrification artificielle des eaux de nappe. In: *Nitrates dans les Eaux,* Paris, 22-24 October 1985.

Chang, I-S., and Lee, C-H. (1998). Membrane filtration characteristics in membrane coupled activated sludge system - the effect of physiological states of activated sludge on membrane fouling. *Desalination,* 120, 221-233.

Chapelle, F. (2001). *Ground-Water Microbiology and Geochemistry.* John Wiley and sons, New York, USA.

Charles, L., and Pepin, D. (1998). Analysis of oxyhalides in water by ion chromatography ionspray mass spectrometry. *Journal of Chromatography A,* 804(1-2), 105-111.

Chaudhuri, S., O'Connor, S., Gustavson, R., Achenbach, L., and Coates, J. (2002). Environmental factors that control microbial perchlorate reduction. *Applied and Environmental Microbiology,* 68, 4425-4430.

Chen, X., Jeyaseelan, S., and Graham, N. (2002). Physical and chemical properties study of the activated carbon made from sewage sludge. *Waste Management,* 22(7), 755-760.

Cheng, L., and Dong, S. (2000). Electrochemical behaviour and electrocatalytic properties of ultrathin filma containing silicotungstic heteropolyanion $SiW_{12}O_4^{4-}$. *Journal of the Electrochemical Society,* 147(2), 606-612.

Christensen, T., Kjeldsen, P., Bjerg, P., Jensen, D., Christensen, J., Baun, A., Albrechtsen, H-J., and Heron, G. (2001). Biogeochemistry of landfill leachate plumes. *Applied Geochemistry,* 16(7-8), 659-718.

Clarke, J. (1998). Evaluation of censored data methods to allow statistical comparisons among very small samples with below detection limit observations. *Environmental Science and Technology,* 32(1), 1156-1164.

Coates, J., and Achenbach, L. (2004). Microbial perchlorate reduction: Rocket fuelled metabolism. *Nature Reviews Microbiology,* 2(7), 569-580.

Coates, J., Michaelidou, U., Bruce, R., O'Connor, S., Crespi, J., and Achenbach, L. (1999). Ubiquity and diversity of dissilimatory (per)chlorate-reducing bacteria. *Applied and Environmental Microbiology,* 65(12), 5234-5241.

Coca-Cola Company. (2004). Voluntary withdrawal of Dasani in UK (Company statement). 19 March 2004. www2.coca-cola.com/presscenter/viewpoints_dasani_include.html (www page, accessed 13 Aug. 2004)

Conley, J., Puzig, E., and Alleman, J. (1987). Bromine chemistry an alternative to dechlorination in cooling water and wastewater disinfection. In: *International Water Conference,* Pittburgh, USA, November 1987.

Cote, P., Buisson, H., Pound, C., and Arakaki, G. (1997). Immersed membrane activated sludge for the reuse of municipal wastewater. *Desalination,* 113(189-196).

Council of the European Communities. (1980). *Directive 80/68/EEC of the 17 December 1979 on the protection of groundwater against pollution caused by certain dangerous substances*. Directive 80/68/EEC, Official Journal of the European Communities No. L 020.

Council of the European Communities. (1991). *Council Directive 91/676/EEC of 12 December 1991 concerning the propection of waters against pollution caused by nitrates from agricultural sources*. Council Directive 91/676/EEC, Official Journal of the European Communities No. L375.

Council of the European Communities. (1998). *Council Directive on the quality of water intended for human consumption*. Council Directive 98/98/EC, Official Journal of the European Communities.

Council of the European Communities (2000). *Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for the Community Action in the Field of Water Policy*. Directive 2000/60/EC, Official Journal of the European Communities No. C327.

Cox, E., McMaster, M., Neville, S., and Bonsack, L. (2001). Successful field demostration of *in-situ* bioremediation of perchlorate in groundwater. In: *In-situ and on-situ bioremediation in the sixth international symposium,* San Diego, USA, 4-7 June 2001.

Creed, J., and Brockhoff, C. (1999). Isotope dilution analysis of bromate in drinking water matrixes by ion chromatography with inductively coupled plasma mass spectrometric detection. *Analytical Chemistry,* 71(3), 722-726.

Crittenden, J., Hand, D., Reddy, P., Arora, H., Trynoski, J., and Notthakun, S. (1989). Prediction of GAC performance using rapid small-scale column tests. In: *American Water Works Association Annual Conference Proceedings,* Los Angeles, USA.

Croll, B., and Hayes, C. (1988). Nitrate and water supplies in the United Kingdom. *Environmental Pollution,* 50(1-2), 163-167.

Crouè, J., Koudjonou, B., and Legube, B. (1996). Parameters affecting the formation of bromate ion during ozonation. *Ozone: Science and Engineering,* 18(1), 1-18.

Dahub, M., and Lee, P. (1992). Nitrate reduction by *in-situ* bio-denitrification in groundwater. *Water Science and Technology,* 26(7-8), 7-8.

Davison, W. (1995). Chemical reduction of nitrate in groundwater. In: *Proceedings of Pollutant Transport in Soils and Rocks,* London, 16 November 1995.

Delcomyn, C., Weinberg, H., and Singer, P. (2001). Use of ion chromatography with post-column reaction for the measurement of tribromide to evaluate bromate levels in drinking water. *Journal of Chromatography A,* 920(1-2), 213-219.

Depository Services Program (Canada). (1999). *Bromate*. http;//dsp.psd.pwgsc.gc.ca/collection/H48-10-1-18-1999E.pdf (www document, accessed 21 July 2002).

DETR. (2000). *Waste Strategy 2000 for England and Wales*. HMSO, London.

Diemer, J., and Heumann, K. (1997). Bromide/bromate speciation by NTI-IDMS and ICP-MS coupled with ion exchange chromatography. *Fresenius Journal of Analytical Chemistry,* 357(1), 74-79.

Divjak, B., Novic, M., and Goessler, W. (1999). Determination of bromide, bromate and other anions with ion chromatography and an inductively coupled plasma mass spectrometer as element-specific detector. *Journal of Chromatography A,* 862(1), 39-47.

Dong, S., Cheng, L., and Zhang, X. (1998). Electrochemical studies of a lanthanide heteropoly tungstate/molybdate complex in polypyrrole film electrode and its electrocatalytic reduction of bromate. *Electrochimica Acta,* 43(5-6), 563-568.

Downs, A., and Adams, C. (1975). *The Chemistry of Chlorine, Bromine, Iodine and Astatine*. Pergamon, Oxford.

Driedger, A.., Staub, E., Pinkernell, U., Marinas, B., Koster, W., and Von Gunten, U. (1991) Inactivation of *Bacillus subtilis* spores and formation of bromate during ozonation. *Water Research*, 35(12), 2950-2960.

Droste, R. (1997). *Theory and Practice of Water and Wastewater Treatment*. John Wiley, New York.

Drysdale, G., Kasan, H., and Bux, F. (1999). Denitrification by heterotrophic bacteria during activated sludge treatment. *Water SA,* 25(3), 357-362.

Dudoit, A., and Pergantis, S. (2001). Ion chromatography in series with conductivity detection and inductively coupled plasma-mass spectrometry for the determination of nine halogen, metalloid and non-metal species in drinking water. *Journal of Analytical Atomic Spectrometry,* 16(6), 575-580.

Dupois, B. (1997). The chemistry and toxicology of potassium bromate. *Cereal Foods World,* 42(3), 171-183.

DWI. (1998). *Drinking Water Inspectorate Information Letter 7/98 - 3 June 1998.* www.dwi.gov.uk/regs/infolett/1998/info798.htm (www document; accessed 4 March 2005).

DWI. (2000). *The Water Supply (Water Quality) Regulations 2000*. Report No. 3184, Drinking Water Inspectorate, UK.

Eckenfelder, W. (1965). Design procedure for biological nitrification and denitrification. *Chemical Engineering: Progressive Symposium Series,* 63(78), 230-234.

Ells, B., Barnett, D., Purves, R., and Guevremont, R. (2000). Trace level determination of perchlorate in water matrices and human urine using ESI-FAIMS-MS. *Journal of Environmental Monitoring,* 2, 393-397.

Elwaer, A., McLeod, C., and Thompson, K. (2000). On-line separation and determination of bromate in drinking waters using flow injection ICP mass spectrometry. *Analytical Chemistry,* 72(22), 5725-5730.

Environment Agency. (1997). *The determination of bromate, chlorate and chlorite in waters by ion chromatography.* Environment Agency, Bristol, UK

Environment Agency. (2001). *The Sandridge/Hatfield bromate pollution investigation: Draft Environment Agency groundwater quality report.* (Unpublished).

Environment Agency. (2002a). *Advice from Enviroment Agency to St Albans District Council.* (Unpublished).

Environment Agency. (2002b). *Contaminated Land: Part IIa of the Environmental Protection Act 1990.* Pamphlet EP-8/00-40K-E-BFPS. Environment Agency, Bristol, UK.

Environment Agency. (2002c). *Environment Agency technical advice to third parties on the pollution of controlled waters for Part IIa of the Environmental Protection Act 1990.* (Pamphlet).

European Environment Agency. (1995). *Report on 'Environment in the European Union'.* EEA, Copenhagen.

Farage, V., and Janjic, D. (1982). Uncatalysed oscillatory chemical reactions – oxidation of 1,4-cyclohexane by bromate in perchloric or orthophosphoric acid solution. *Inorganic Chemica Acta,* 65, L33-L34.

Farrell, S., Joa, J., and Pacey, G. (1995). Spectrophotometric determination of bromate ions using phenothiazines. *Analytica Chimica Acta,* 313(1-2), 121-129.

Fernandez, R., Alonso, J., and Sanz-Medel, A. (2001). Coupling of ICP-MS with ion chromatography after conductivity suppression for the determination of anions in natural and waste waters. *Journal of Analytical Atomic Spectrometry,* 16(9), 1035-1039.

Fielding, I. (2002). *Bacterial Reduction of Bromate in Natural and Laboratory Environments.* MSc Thesis, University of Leeds.

Flury, M., and Papritz, A. (1993). Bromide in the natural environment - occurrence and toxicity. *Journal of Environmental Quality,* 22(4), 747-758.

Fowler, J., and Cohen, L. (1995). *Practical Statistics for Field Biology,* John Wiley and Sons, Chichester.

Fujii, M., Oikawa, K., and Saito, H. (1984). Metabolism of potassium bromate in rats I: *In-vivo* studies. *Chemosphere,* 13, 1207-1212.

Furmage, A. (2005). *Bromate (Ref: 33/203),* Food Standards Agency, London. (Email correspondence).

Gahr, A., Huber, N., and Niessner, R. (1998). Fluorimetric determination of bromate by ion-exchange separation and post-column derivatization. *Microchimica Acta,* 129(3-4), 281-290.

Gale, I., Marks, R., Darling, W., and West, J. (1993). *Bacterial denitrification in aquifers: Evidence from the unsaturated zone and the unconfined chalk and Sherwood sandstone aquifers*. NRA Project Report 306/11/ST, NRA, Bristol, UK.

Gale, I., Marks, R., Darling, W., and West, J. (1994). *Bacterial denitrification in aquifers: Evidence from the unsaturated zone and the unconfined chalk and Sherwood sandstone aquifers*. NRA R and D Note 215, NRA, Bristol, UK.

Gander, M., Jefferson, B., and Judd, S. (2000). Membrane Bioreactors for use in small wastewater treatment plants: Membrane materials and effluent quality. *Water Science and Technology,* 41(1), 205-211.

Gauntlett, R., and Craft, D. (1979). *Biological Removal of Nitrate from River Water.* Technical Report TR98, WRc, Swindon.

Gendig, C., Domogala, G., Agnoli, F., Pagga, U., and Strotmann, U. (2003). Evaluation and further development of the activated sludge respiration inhibition test. *Chemosphere,* 52143-149.

Germain, E. (2004). *Biomass effects on membrane bioreactor operation.* PhD Thesis, Cranfield University, UK.

Giblin, T., and Frankenberger, W. (2001). Perchlorate and nitrate reductase activity in the perchlorate-respiring bacterium perclace. *Microbiological Research,* 156(4), 311-315.

Giblin, T., Herman, D., Deshusses, M., and Frankenberger, W. (2000a). Removal of perchlorate in ground water with a flow-through bioreactor. *Journal of Environmental Quality,* 29(2), 578-583.

Giblin, T., Herman, D., and Frankenberger, W. (2000b). Removal of perchlorate from ground water by hydrogen-utilizing bacteria. *Journal of Environmental Quality,* 29(4), 1057-1062.

Gisch, D. (2003). Anion exchange resins remove perchlorate from groundwater. *Water and Wastewater International,* 18(3), 10.

Godbold, P. (2002). *In-situ* denitrification: A pilot scale field trial on a chalk aquifer. In: *CIWEM National Conference - Inorganics in Focus,* London, 12 June 2002.

Gonce, N., and Voudrias, E. (1994). Removal of chlorite and chlorate ions from water using granular activated carbon. *Water Research,* 28(5), 1059-1069.

Gonzalez, R., Tao, H., Shanmugam, T., York, S., and Ingram, L. (2002). Global gene expression differences associated with changes in glycolytic flux and growth rate in *Escherichia coli* during the fermentation of glucose and xylose. *Biotechnological Progress,* 18, 6-20.

Gordon, G., and Bubnis, B. (1995). The measurement of very low level bromate ion. *Ozone: Science and Engineering,* 17(5), 551-559.

Gordon, G., Bubnis, B., Sweetin, D., and Kuo, C. (1994). A flow injection, non-ion chromatographic method for measuring low level bromate ion in ozone treated waters. *Ozone: Science and Engineering,* 16(1), 79-87.

Gordon, G., Gauw, R., Emmert, G., Walters, B., and Bubnis, B. (2002). Chemical reduction methods for bromate ion removal. *Journal of the American Water Works Association,* 94(2), 91-98.

Goslan, E. (2003). *Natural Organic Matter Character and Reactivity: Assessing Seasonal Variation in a Moorland Water*. EngD Thesis, Cranfield University, UK.

Gosselin, R., Hodge, H., Smith, R., and Gleason, M. (1976). *Clinical Toxicology of Commercial Products, 4th Ed.* Williams and Wilkins, Baltimore.

Grosvenor, T. (1999). Strategies for minimizing ozonation by-products in drinking water. *Water Engineering and Management,* 146(2), 30-33.

Gullick, R., Lechvallier, M., and Barhorst, T. (2001). Occurrence of perchlorate in drinking water sources. *Journal of the American Water Works Association,* 93(1), 66-77.

Guoy, J-L., Berge, P., and Labroue, L. (1984). *Gallionella ferruginea* as denitrification factor in waters poor in organic matter (in French). *Comptes Rendues de l'Academie des Sciences,* 298(6), 153-156.

Haigh, K. (2002). *Development of a colourmetric method for analysing bromate.* (Unpublished).

Hall, P. (2000). Perchlorate remediation at a DOD facility. In: *Proceedings of Perchlorate Treatment Technology Workshop, 5th Annual Joint Service Pollution Prevention and Hazardous Waste Management Conference and Exhibition*, Henry B Gonzales Convention Centre, San Antonio, Texas, USA, 21 – 24 August 2000

Hall, T. (1997). *Water Treatment Processes and Practices, 2nd Ed.* WRc, Swindon.

Hall, T., and Croll, B. (1993). Treatment processes for nitrate removal from water supplies. In: Burt, T., Heathwaite, A., and Trudgill, S. *Nitrate: Processes, Patterns and Management,* John Wiley and Sons, Chichester.

Hammer, M., and Hammer, M. (2004). *Water and Wastewater Technology, 5th Ed.* Pearson Educational, New Jersey

Hansen, H., Koch, C., NanckeKrogh, H., Borggaard, O., and Sorensen, J. (1996). Abiotic nitrate reduction to ammonium: Key role of green rust. *Environmental Science and Technology,* 30(6), 2053-2056.

Hatzinger, P., Greene, M., Frisch, S., Togna, M., Manning, J., and Guarini, W. (2000). Biological treatment of perchlorate-contaminated groundwater using fluidized bed reactors. In: *2nd International Conference on Remediation of Chlorinated and Recalcitrant Compounds,* Monterey, California, 22-25 May 2000.

Hautman, D., Munch, D., Frebis, C., Wagner, H., and Pepich, B. (2001). Review of the methods of the US Environmental Protection Agency for bromate determination and validation of Method 317.0 for disinfection by-product anions and low-level bromate. *Journal of Chromatography A,* 920(1-2), 221-229.

Herman, D., and Frankenberger, W. (1998). Microbial-mediated reduction of perchlorate in groundwater. *Journal of Environmental Quality,* 27(4), 750-754.

Hijnen, W., Voogt, R., Veenendaal, H., van der Jagt, H., and van der Kooij, D. (1995). Bromate reduction by denitrifying bacteria. *Applied and Environmental Microbiology,* 61(1), 239-244.

Hijnen, W., Jong, R., and van der Kooij, D. (1999). Bromate removal in a denitrifying bioreactor used in water treatment. *Water Research,* 33(4), 1049-1053.

Hiscock, K., Lloyd, J., and Lerner, D. (1991). Review of natural and artificial denitrification of groundwater. *Water Research,* 25(9), 1099-1111.

HM Government. (1990). *The Potassium Bromate (Prohibition as a Flour Improver) Regulations 1990.* Statutory Instrument 1990 No. 399, The Stationary Office, London.

HM Government. (1991). *Water Resources Act 1991.* Report c. 57, The Stationary Office, London.

HM Government. (1996). *The Protection of Water Against Agricultural Nitrate Pollution (England and Wales) Regulations 1996.* Statutory Instrument 1996 No. 888, The Stationary Office, London.

HM Government. (2000). *The Water Supply (Water Quality) Regulations 2000*. Statutory Instrument 2000 No. 3184, The Stationary Office, London.

HM Government. (2002). *The Nitrate Vulnerable Zones (Additional Legislation) (England) (No. 2) Regulations 2002*. Statutory Instrument 2002 No. 2614, The Stationary Office, London.

Hollo, J., and Czako, L. Nitrate removal from drinking water in a fluidised-bed biological denitrification bioreactor. *Acta Biotechnologia*, 7, 417-423.

Hossain, I., Longoria, R., and Joost, R. (1996). DBP formation and removal in ozonation reactions. *World Water and Environmental Engineering,* 19(2), 24-25.

Howard, K. (1985). Denitrification in a major limestone aquifer. *Journal of Hydrology,* 76(3-4), 265-280.

Huang, W., and Chen, L. (2004). Assessing the effectiveness of ozonation followed by GAC filtration in removing bromate and assimilable organic carbon. *Environmantal Technology,* 25, 403-412.

Hultman, B., Jonsson, K., and Plaza, E. (1994). Combined nitrogen and phosphorous removal in a full-scale continuous up-flow sand filter. *Water Science and Technology,* 29(10-11), 127-134.

Hutchinson, T., Hutchings, M., and Moore, K. (1997). A review of the effects of bromate on aquatic organisms and toxicity of bromate to oyster (*Crassostrea gigas*) embryos. *Ecotoxicology and Environmental Safety,* 38(3), 238-243.

Hyman, M., and Dupont, R. (2001). *Groundwater and Soil Remediation: Process Design and Cost Estimating of Proven Technologies.* ACSE Press, Reston, USA.

IARC. (1999). *Potassium Bromate (Summary of Data Reported and Evaluation), Vol. 73.* International Agency for Research on Cancer, Lyon.

Ijpelaar, G., Groenendijk, M., Hopman, R., and Kruithof, J. (2002). Advanced oxidation technologies for the degradation of pesticides in ground water and surface water. *Water Science and Technology: Water Supply,* 2(1), 129-138.

Ingrand, V., Guinamant, J., Bruchet, A., Brosse, C., Noij, T., Brandt, A., Sacher, F., McLeod, C., Elwaer, A., Croue, J., and Quevauviller, P. (2002). Determination of bromate in drinking water: development of laboratory and field methods. *Trends in Analytical Chemistry,* 21(1), 1-12.

Inoue, Y., Sakai, T., Kumagai, H., and Hanaoka, Y. (1997). High selective determination of bromate in ozonized water by using ion chromatography with postcolumn derivatization equipped with reagent preparation device. *Analytica Chimica Acta,* 346(3), 299-305.

Janda, V., Rudovsky, J., Wanner, J., and Marha, K. (1988). *In-situ* denitrification of drinking water. *Water Science and Technology,* 20, 215-219.

Jepson, S-E., and Jansen, J. (1993). Biological filters for post-denitrification. *Water Science and Technology,* 27(5-6), 369-379.

Jhanji, A., and Gould, E. (1991). Electron transfer 120: Some oxidations by bromite (BrO_2^-). *International Journal of Chemical Kinetics,* 23, 229-236.

Johnson, C., and Singer, P. (2004). Impact of a magnetic ion exchange resin on ozone demand and bromate formation during drinking water treatment. *Water Research,* 38, 3738-3750.

Jonsson, L. (2004). Denitrification rate and carbon source consumption in full-scale wastewater filtration. *Water Science and Technology,* 150(7), 105-112.

Jonsson, L., Plaza, E., and Hultman, B. (1997). Experiences of nitrogen and phosphorous removal in deep-bed filters in the Stockholm area. *Water Science and Technology*, 36(1), 183-190.

Jolles, Z. (1966). *Bromine and its Compounds*. Ernest Benn, London.

Jwo, J., and Cheng, E. (1989). Kinetic study of the initial phase of the uncatalysed oscillatory reaction of potassium bromate and gallic acid. *Journal of Physical Chemistry,* 93(6), 2388-2392.

Keiding, K., and Nielsen, P. (1997). Desorption of organic macromolecules from activated sludge: Effect of ionic composition. *Water Research,* 31(7), 1665-1672.

Kengen, S., Rikken, G., Hagen, W., van Ginkel, C., and Stams, A. (1999). Purification and characterisation of (per)chlorate reductase from the chlorate-respiring strain GR-1. *Journal of Bacteriology,* 181(21), 6706-6711.

Kim, K., and Logan, B. (2001). Microbial reduction of perchlorate in pure and mixed culture packed-bed bioreactors. *Water Research,* 35(13), 3071-3076.

Kirisits, M. (2000). *The Abiotic and Biotic Reduction of Bromate*. PhD Thesis, University of Illinois at Urbana-Champaign, USA.

Kirisits, M., and Snoeyink, V. (1999). Reduction of bromate in a BAC filter. *Journal of the American Water Works Association,* 91(8), 74-84.

Kirisits, M., Snoeyink, V., and Kruithof, J. (2000). The reduction of bromate by granular activated carbon. *Water Research,* 34(17), 4250-4260.

Kirisits, M., Snoeyink, V., Inan, H., Chee-Sanford, J., Raskin, L., and Brown, J. (2001). Water quality factors affecting bromate reduction in biologically active carbon filters. *Water Research,* 35(4), 891-900.

Kirisits, M., Snoeyink, V., Chee-Sanford, J., Daugherty, B., Brown, J., and Raskin, L. (2002). Effect of operating conditions on bromate removal efficiency in BAC filters. *Journal of the American Water Works Association,* 94(4), 182-193.

Knowles, R. (1982). Denitrification. *Microbiological Reviews,* 46(1), 43-70.

Korom, S. (1992). Natural denitrification in the saturated zone - a review. *Water Resources Research,* 28(6), 1657-1668.

Koscielna, H. (2004). Determination of bromate (V) in drinking water, a review. *Chemia Analityczna,* 49(4), 445-457.

Krasner, S., Glaze, W., Weinberg, H., Daniel, P., and Najm, I. (1993). Formation and control of bromate during ozonation of waters containing bromide. *Journal of Indian Water Works Association,* 25(2), 218-219.

Krasner, S., Yates, R., Amy, G., and Garside, J. (1996). Use of ferrous and ferric coagulants to reduce bromate and particulate levels. In: *American Water Works Association 1996 Annual Conference,* Toronto, Canada.

Kruithof, J., and Meijers, R. (1995). Bromate formation by ozonation and advanced oxidation and potential options in drinking water treatment. *Water Supply,* 13(1), 93-103.

Kruithof, J., van Paassen, J., Hijnen, W., and van Bennekom, C. (1985). Experiences with nitrate removal in the Netherlands. In: *Nitrates dans les Eaux,* Paris, 22-24 Oct. 1985.

Kurokawa, Y., Takayama, S., Konishi, Y., Hiasa, Y., Asahini, S., Takahashi, M., Maekawa, A., and Hayashi, Y. (1986). Long-term *in vivo* carcinogenicity tests of potassium bromate, sodium hypochlorite, and sodium chlorite conducted in Japan. *Environmental Health Perspectives,* 69, 221-235.

Kutom, A., Bazilinski, N., and Magana, L. (1990). Bromate intoxication: hairdressers anuria. *American Journal of Kidney Disorders,* 15(1), 84-85.

Laffray, G., Jefferson, B., Butler, R., and Cartmell, E. (2003). Impact of toxic components on the robustness of MBR operation. In: *IMSTEC '03,* University of New South Wales, 10-14 Nov. 2003.

LeClech, P. (2002). *Process Configurations and Fouling in Membrane Bio-reactors.* PhD Thesis, Cranfield University, UK.

Lefebvre, E., and Deguin, A. (1997). Compromise between bromate ion formation and pesticides degradation and/or manganese removal. *Ozone: Science and Engineering,* 19(1), 39-53.

Legube, B., Koudjonou, B., Croue, J., and Merlet, N. (1995). Influence of the presence of natural organic matter on bromate formation by ozonation. *Water Supply,* 13(1), 51-59.

Li, Y., Bu, W., Wu, L., and Sun, C. (2005). New amperometric sensor for the determination of bromate, iodate and hydrogen peroxide based on titania sol-gel matrix for immobilization of cobalt substituted Keggin-type cobalttungstate anion by vapor deposition method. *Sensors and Actuators B,* 107, 921-928.

Lillis, T., and Bissonnette, G. (2001). Detection and characterisation of filterable heterotrophic bacteria from rural groundwater supplies. *Letters in Applied Microbiology,* 32(4), 268-272.

Logan, B. (1998). A review of chlorate- and perchlorate-respiring microorganisms. *Bioremediation Journal,* 2(2), 69-79.

Logan, B. (2001). Analysis of overall perchlorate removal rates in packed-bed bioreactors. *Journal of Environmental Engineering,* 127(5), 469-471.

Logan, B., and Jiang, Q. (1990). Molecular size distribution of dissolved organic matter. *Journal of the Environmental Engineering - ASCE,* 116(6), 1046-1062.

Logan, B., and LaPoint, D. (2002). Treatment of prechlorate and nitrate-contaminated groundwater in an autotrophic, gas phase, packed-bed bioreactor. *Water Research,* 36(14), 3647-3653.

Logan, B., Bliven, A., Olsen, S., and Patnaqik, R. (1998). Growth kinetics of mixed cultures under chlorate-reducing conditions. *Journal of Environmental Engineering,* 124(10), 1008-1011.

Logan, B., Zhang, H., Mulvaney, P., Milner, M., Head, I., and Unz, R. (2001). Kinetics of perchlorate- and chlorate respiring bacteria. *Applied and Environmental Microbiology,* 67(6), 2499-2506.

Lopez-Cueto, G., Ostra, M., and Ubide, C. (2001). New way of application of the bromate-bromide mixture in kinetic analysis. *Analytica Chimica Acta,* 445, 117-126.

Losi, M., and Frankenberger, J. (1997). Reduction of selenium oxyanions by *Enterobacter cloacae* SLD1a-1: Isolation and growth of the bacterium and its expulsion of selenium particles. *Applied and Environmental Microbiology,* 63, 3079-3084.

Losi, M., Amrhein, C., and Frankenberger, W. (1994). Bioremediation of chromate contaminated groundwater by reduction and precipitation in surface soils. *Journal of Environmental Quality,* 23, 1141-1150.

Madsen, E. (1993). Groundwater microbiology; subsurface ecosystem processes. In: Ford, T. *Microbiology - An Ecological Approach,* Blackwell Scientific Publications, Boston, USA.

Magnuson, M. (1998). Determination of bromate at parts-per-trillion levels by gas chromatography mass spectrometry with negative chemical ionization. *Analytica Chimica Acta,* 377(1), 53-60.

Mallevialle, J. (1995). Impact of new guidelines for disinfection by-products on drinking water treatment. *Water Supply,* 13(1), 177-182.

Malmqvist, A., Welander, T., Moore, E., Ternstrom, A., Molin, G., and Stenstrom, I-M. (1994). *Ideonella dechloratans gen. nov.*, sp. nov., a new bacterium capable of growing anaerobically with chlorate as an electron acceptor. *Systematic and Applied Microbiology,* 17, 58-64.

Malmqvist, A., Welander, T., and Gunnarsson, L. (1991). Anaerobic growth of microorganisms with chlorate as an electron acceptor. *Applied and Environmental Microbiology*, 57(8), 2229-2232.

Manassis, M. and Constantinos, S. (2003). Conditions affecting bromate formation during ozonation of bottled water. *Ozone - Science and Engineering,* 25(3), 167-175.

Marhaba, T. (2000). Examining bromate ion removal by GAC through RSSCT and pilot-scale columns. *Environmental Engineering and Policy,* 2(2), 59-64.

Marhaba, T., and Medlar, S. (1993). Treatment/removal of bromate and bromide ions from drinking water. In: *Proceedings AWWA Water Quality Technology Conference,* Miami, USA.

Martin, M., Artola, A., Balguer, M., and Rigola, M. (2002). Towards waste minimisation in WWTP: Activated carbon from biological sludge and it's application in liquid phase adsorption. *Journal of Chemical Technology and Biotechnology,* 77(7), 825-833.

Mateju, V., Cizinska, S., Krejci, J., and Janoch, T. (1992). Biological water denitrification - a review. *Enzyme and Microbial Technology,* 14, 170-183.

Mayhew, M., and Stephenson, T. (1998). Biomass yield reduction: Is biochemical manipulation possible without affecting activated sludge process efficiency? *Water Science and Technology,* 38(8-9), 137-144.

Mazierski, J. (1994). Effect of chromium (CrVI) on the growth rate of denitrfying bacteria. *Water Research,* 28(9), 1981-1985.

McCann, B. (1993). Ozone unfriendly. *World Water and Environmental Engineering,* 23-24.

Meijers, R., and Kruithof, J. (1995). Potential treatment options for restriction of bromate formation and bromate removal. *Water Supply,* 13(1), 183-189.

Mercado, A., Libhaber, M., and Soares, M. (1988). *In*-situ biological groundwater denitrification: Concepts and preliminary field tests. Water Science and Technology, 20, 197-209.

Metcalf and Eddy Inc. (1991). *Wastewater Engineering: Treatment and Reuse, 3rd Ed.* McGraw-Hill, New York, USA.

Miller, J., and Logan, B. (2000). Sustained perchlorate degradation in an autotrophic, gas-phase, packed-bed bioreactor. *Environmental Science and Technology,* 34, 3018-3022.

Miller, J., Snoeyink, V., and Kruithof, J. (1996). The reduction of bromate by granular activated carbon in distilled and natural waters. *ACS Symposium Series: Water Disinfection and Natural Organic Matter,* 649, 251-281.

Mills, A., and Meadows, G. (1995). Heterogeneous redox catalysis: a novel route for removing bromate ions from water. *Water Research,* 29(9), 2181-2185.

Mills, A., Belghazi, A., and Rodman, D. (1995). Semiconductor photocatalysis: The removal of bromate from potable water. In: *3rd International Conference on Water and Waste Treatment,* Harrogate, UK, 13-15 Nov. 1995.

Mills, A., Belghazi, A., and Rodman, D. (1996). Bromate removal from drinking water by semiconductor photocatalysis. *Water Research,* 30(9), 1973-1978.

Min, B., Evans, P., Chu, A., and Logan, B. (2004). Perchlorate removal in sand and plastic media bioreactors. *Water Research,* 38, 47-60.

Mitrakas, M., Tzimou-Tsitouridou, R., and Keramidas, V. (2000). Interference of humic substances in the spectrophotometric determination of bromate by phenothiazines in natural waters. *International Journal of Environmental Analytical Chemistry,* 78(3-4), 343-351.

Mohseni-Bandpi, A., and Elliott, D. (1996). Nitrate removal from groundwater using an anoxic-aerobic rotating biological contactor. *Water Science and Technology,* 34(1-2), 323-330.

Mohseni-Bandpi, A., and Elliott, D. (1998). Groundwater denitrification with alternative carbon sources. *Water Science and Technology,* 38(6), 237-243.

Moreno, B., Gomez, M., Gonzalez-Lopez, J., and Hontoria, E. (2005). Inoculation of a submerged filter for biological denitrification of nitrate polluted groundwater: A comparative study. *Journal of Hazardous Materials,* 117(2-3), 141-147.

Morpeth, F., and Boxer, D. (1985). Kinetic-analysis of respiratory nitrate reductase from *Escherichia-coli*-K12. *Biochemistry,* 24(1), 40-46.

Mott Macdonald (2000a). *Bromate contamination study: Review and hydrogeological interpretation - Preliminary report.* (Unpublished).

Mott Macdonald (2000b). *Bromate contamination study: Review and Hydrological interpretation of data.* (Unpublished).

Nagoaka, H., Kono, S., Yamanishi, S., and Miya, A. (1999). Influence of organic loading rate on membrane fouling in membrane separation activated sludge process. In: P*roceedings in Membrane Technology in Environmental Management,* Tokyo, 1-4 Nov. 1999.

Nerenberg, R., and Rittmann, B. (2004). Hydrogen-based, hollow fiber membrane biofilm reactor for reduction of perchlorate and other oxidised contaminants. *Water Science and Technology,* 49(11-12), 223-230.

Nerenberg, R., Rittmann, B., and Najm, I. (2002). Perchlorate reduction in a hydrogen-based membrane biofilm reactor. *Journal of the American Water Works Association,* 94(11), 103-114.

NHS. (2001). *Independent review gives all clear on bromates.* Press Release PR39/001, National Health Service.

Nissinen, T., Miettinen, I., Martikainen, P., and Vartainen, T. (2001). Molecular size distribution of natural organic matter in raw and drinking waters. *Chemosphere,* 45(6-7), 865-873.

Noguchi, H., Nakajima, A., Watanabe, T., and Hashimoto, K. (2002). Removal of bromate ion from water using TiO_2 and alumina-laded TiO_2 photocatalysis. *Water Science and Technology,* 46(11-12), 27-31.

Noguchi, H., Nakajima, A., Watanabe, T., and Hashimoto, K. (2003). Design of a photocatalyst for bromate decomposition: surface modification of TiO_2 by pseudo boehmite. *Environmental Science and Technology,* 37(1), 153-157.

Nowak, M., and Seubert, A. (1998). Ultra-trace determination of bromate in drinking waters by means of microbore column ion chromatography and on-line coupling with inductively coupled plasma mass spectrometry. *Analytica Chimica Acta,* 359(1-2), 193-204.

Nurizzo, C., and Mezzanotte, V. (1992). Groundwater biodenitrification on sand fixed film reactor using sugars as organic carbon source. *Water Science and Technology,* 26(3-4), 826-834.

Nyman, P., Canas, B., Joe, F., and Diachenko, G. (1996). Screening method for the gas chromatographic mass spectrometric determination of microgram/litre levels of bromate in bottled water. *Food Additives and Contaminants,* 13(6), 623-631.

OECD. (2000). *OECD Guideline for the Testing of Chemicals.* OECD Guideline 106.

Parker, J., and Chilton, P. (1991). *Pore-water nitrate profiles in the chalk unsaturated zone - results of 1990 redrilling.* Technical report, Hydrogeology series WD/91/13C, British Geological Survey, Keyworth, England.

Payne, W. (1981). *Denitrification,* John Wiley, New York.

Pelczar, M., Chan, E., and Krieg, N. (1993). *Microbiology: Concepts and Applications.* McGraw-Hill, New York, USA.

Perlmutter, M., Britto, R., Cowan, J., Patel, M., Jacobs, A., Logan, B., and Craig, M. (2000). Bioremediation of perchlorate-contaminated groundwater at naval weapons reserve plant McGregor, Texas. In: *National Defense Industrial Association Proceedings, 26th Environmental Symposium and Exhibition,* Long Beach, USA.

Pinkernell, U., and von Gunten, U. (2001). Bromate minimization during ozonation: Mechanistic considerations. *Environmental Science and Technology,* 35(12), 2525-2531.

Pirt, S. (1975). *Principles of Microbe and Cell Cultivation.* Blackwell Publishing, Oxford.

Polk, J., Murray, C., Onewokae, C., Tolbert, D., Togna, A., Guarini, W., Frisch, S., and Del Vecchio, M. (2001). Case study of *ex-situ* biological treatment of perchlorate-contaminated groundwater. In: *4th Tri-services Environmental Technology Symposium,* San Diego, USA, 18-20 June 2001.

Prados-Ramirez, M., Ciba, N., and Bourbigot, M. (1995). Available techniques for reducing bromate in drinking water. *Water Supply,* 13(1), 61-70.

Prescott, L., Harley, J., and Klein, D. (1999). *Microbiology, 4th Edition Ed.* McGraw-Hill, Boston, USA.

Price, D., Iddon, S., and Wakefield, B. (1988). *Bromine Compounds: Chemistry and Applications.* Elsevier, Amsterdam.

Puckett, L., and Cowdery, T. (2002). Transport and fate of nitrate in a glacial outwash aquifer in relation to ground water age, land use practices, and redox processes. *Journal of Environmental Quality,* 31(3), 782-796.

Quastel, J. (1925). Some reactions of resting bacteria in relation to anaerobic growth. *Biochemical Journal,* 19, 304.

Quick, C. (1975). Deafness and renal failure due to potassium bromate poisoning. *Archives of Otolaryngology,* 101, 494-495.

Rajapske, J., and Scutt, J. (1999). Denitrification with natural gas and various new growth media. *Water Research,* 33(18), 3723-3734.

Richard, Y. (1989). Operating experiences of full-scale biological and ion-exchange denitrification plants in France. *Journal of the Institution of Water and Environmental Management,* 88, 154-167.

Richardson, L., Burton, D., Helz, G., and Rhoderick, J. (1981). Residual oxidant decay and bromate formation in chlorinated and ozonated sea water. *Water Research,* 15(9), 1067-1074.

Rikken, G., Kroon, A., and van Ginkel, C. (1996). Tranformation of (per)chlorate into chloride by a newly-isolated bacterium: reduction and dismutation. *Applied Microbiology and Biotechnology,* 45, 420-426.

Rio, S., Faur-Brasquet, C., Le Coq, L., Lecomte, D., and Le Cloirec, P. (2004). Preparation and characterisation of activated carbon from sewage sludge: carbonisation step. *Water Science and Technology,* 49(1), 139-146.

Ritter, W., and Scarborough, R. (1995). A review of bioremediation of contaminated soils and groundwater. *Journal of Environmental Science and Health, Part A: Environmental Science and Engineering and Toxic and Hazardous Substance Control,* A30(2), 333-357.

Rodgers, G. (1980). Evaluation of potential substrates to monitor respiratory nitrate reductase activity in soils. *Journal of Soil Science,* 31, 387-395.

Romele, L., and Achilli, M. (1998). Spectrophotometric determination of low levels of bromate in drinking water after reaction with fuchsin. *Analyst (London),* 123(2), 291-294.

Sacher, F., Matschi, A., and Brauch, H. (1995). Analysis and occurrence of bromate in raw water and drinking water. *Acta Hydrochimica et Hydrobiologica,* 23(1), 26-30.

Saez, P., and Rittmann, B. (1991). Biodegradation kinetics of 4-chlorophenol, an inhibitory co-metabolite. *Research Journal of the WPCF,* 63(6), 838.

Sakaji, R., Whittier, C., and Webster, T. (2002). *Conditional Acceptance of Biological Treatment (Fluidised Bed Reactors) for the Removal of Perchlorate during Drinking Water Production.* 2 April 2002. http://www.safedrinkingwater.com/community/Aerojetletter040222.pdf. (www document, accessed 4 Feb. 2005).

Salhi, E., and von Gunten, U. (1999). Simultaneous determination of bromide, bromate and nitrite in low ugL^{-1} levels by ion chromatography without sample pretreatment. *Water Research,* 33(15), 3239-3244.

Sanders, D., Veenstra, J., and Blair, C. (2004). Evaluation of a full-scale biological denitrification system for the treatment of drinking water. In: *Oklahoma Water 2004*, Environmental Institute, Oklahoma State University, Oklahoma, USA, 18-19 November 2004.

Schmidt, W., Bohme, U., and Brauch, H. (1995). Organo bromide compounds and their significance for drinking water treatment. *Water Supply,* 13(1), 101-116.

Scragg, A. (1991). *Bioreactors in Biotechnology: A Practical Approach.* Ellis Horwood, New York.

Schminke, G., and Seubert, A. (2000). Comparison of ion chromatographic methods based on conductivity detection, post-column-reaction and on-line-coupling IC-ICP-MS for the determination of bromate. *Fresenius Journal of Analytical Chemistry,* 366(4), 387-391.

Seubert, A., and Nowak, M. (1998). Trace analysis of bromate in drinking waters by means of on-line coupling IC-ICP-MS. *Fresenius Journal of Analytical Chemistry,* 360(7-8), 777-780.

Siddiqui, M., and Amy, G. (1994). Removal of bromate during drinking water treatment by UV irradiation. In: *IOA Regional Conference Proceedings,* Zurich, Switzerland, 31 Aug - 2 Sept 1994.

Siddiqui, M., Amy, G., Ozekin, K., Zhai, W., and Westerhoff, P. (1994a). Alternative strategies for removing bromate. *Journal of the American Water Works Association,* 86(10), 81-96.

Siddiqui, M., Amy, G., Zhai, W., Ozekin, K., Westerhoff, P., and Chandrakanth, M. (1994b). Removal of bromate after ozonation during drinking water treatment. *Water Quality: Proceedings American Water Works Association Annual Conference,* 881-995.

Siddiqui, M., Amy, G., and Cooper, W. (1996a). Bromate discharge ion removal by electric-arc and high-energy electron beam processes. *ACS Symposium Series: Water Disinfection and Natural Organic Matter,* 649, 366-382.

Siddiqui, M., Amy, G., Cooper, W., Kurucz, C., Waite, T., and Nickelsen, M. (1996b). Bromate ion removal by HEEB irradiation. *Journal of the American Water Works Association,* 88(10), 90-101.

Siddiqui, M., Amy, G., and McCollum, L. (1996c). Bromate destruction by UV irradiation and electric arc discharge. *Ozone: Science and Engineering,* 18(3), 271-290.

Siddiqui, M., Zhai, W., Amy, G., and Mysore, C. (1996d). Bromate ion removal by activated carbon. *Water Research,* 30(7), 1651-1660.

Smith, R., and Duff, J. (1988). Denitrification in a sand and gravel aquifer. *Applied and Environmental Microbiology,* 54(5), 1071-1078.

Smith, R., Miller, D., Brooks, M., Widdowson, M., and Killingstad, M. (2001). *In situ* stimulation of groundwater denitrification with formate to remediate nitrate contamination. *Environmental Science and Technology,* 35(1), 196-203.

Song, R., Minear, R., Westerhoff, P., and Amy, G. (1996a). Bromate formation and control during water ozonation. *Environmental Technology,* 17(8), 861-868.

Song, R., Minear, R., Westerhoff, P., and Amy, G. (1996b). Ozone-bromide interactions with NOM separated by XAD-8 resin and UF/RO membrane methods. In: Minear, R., and Amy, G. *Disinfection By-Products in Water Treatment,* Lewis, Boca Raton.

Song, R., Westerhoff, P., Minear, R., and Amy, G. (1997). Bromate minimization during ozonation. *Journal of the American Water Works Association,* 89(6), 69-78.

Spencer-Davies, P., and Murdoch, F. (2001). *The role of respirometry in maximising aerobic treatment plant efficiency.* Strathkelvin Instruments, Glasgow, UK.

Stanier, R., Ingraham, J., Wheelis, M., and Painter, P. (1986). *General Microbiology, 5th Ed.* MacMillan Education Ltd, Basingstoke, UK.

Stephenson, T., Judd, S., Jefferson, B., and Brindle, K. (2001). *Membrane Bioreactors for Wastewater Treatment.* IWA Publishing, London.

Teuschler, L., Gennings, C., Stiteler, W., Hertzberg, R., Colman, J., Thiyagarajah, A., Lipscomb, J., Hartley, W., and Simmons, J. (2000). A multiple-purpose design approach to the evaluation of risks from mixtures of disinfection by-products. *Drug and Chemical Toxicology,* 23(1), 307-321.

Theis, T., Zander, A., and Anderson, M. (2002). *Assessment of the electrochemical reduction of perchlorate ion.* Project #2578, AwwaRF.

Thompson, K., and Megonnell, N. (2003). Activated carbon - a media for bromate reduction. *Pollution Engineering,* 35(5), 39-44.

Thompson K., Guinamant, J., Ingrand, V., Elwaer, A., McLeod, C., Schmitz, F., De Swaef, G., and Quevauviller, P. (2000). Interlaboratory trial to determine the analytical state-of-the-art of bromate determination in drinking water. *Journal of Environmental Monitoring,* 2(5), 416-419.

Thurman, E. (1985). *Organic Geochemistry of Natural Waters.* Martinus Nijhoff, Dordrecht, Netherlands.

Twort, A., Ratnayaka, D., and Brandt, M. (2000). *Water Supply, 5th Ed.* IWA Publishing, London.

Urbansky, E. (1998). Perchlorate chemistry: Implications for analysis and remediation. *Bioremediation Journal,* 2(2), 81-95.

Urbansky, E. (2002). Perchlorate as an environmental contaminant. *Environmental Science and Pollution Research,* 9(3), 187-192.

US EPA. (1991). *R.E.D Facts: Potassium Bromide.* Report 738-F-91-105, US Environmental Protection Agency.

US EPA. (1993). *R.E.D. Facts: Inorganic Halides.* Report EPA-738-F-93-015, US Environmental Protection Agency.

US EPA. (1997). *Determination of Inorganic Anions in Drinking Water by Ion Chromatography, Method 300.1, Revision 1.0.* US Environmental Protection Agency, Cincinnati, Ohio, USA.

US EPA. (1999). *Alternative Disinfectants and Oxidants Guidance Manual.* Report EPA 815-R-99-014, US Environmental Protection Agency.

US EPA. (2001). *Toxicological Review of Bromate.* Report EPA/635/R-01/002, Environmental Protection Agency, Washington, DC.

US EPA. (2002). *Determination of Inorganic Oxyhalide Disinfection By-products in Drinking Water using Ion Chromatography Incorporating the Addition of a Suppressor Acidified Postcolumn Reagent for Trace Bromate Analysis, Method 326.1, Revision 1.0.* US EPA, Cincinnatti, Ohio.

US EPA. (2003). *National Primary Drinking Water Regulations: Stage 2 Disinfectants and Disinfection Byproducts Rule; National Primary and Secondary Drinking Water Regulations: Approval of Analytical Methods for Chemical Contaminants.* http://www.epa.gov/fedrgstr/EPA-WATER/2003/August/Day-18/w18149a.htm (www document, accessed 17 Sept. 2005).

Valsecchi, S., Isernia, A., Polesello, S., and Cavalli, S. (1999). Ion chromatography determination of trace level bromate by large volume injection with conductivity and spectrophotometric detection after post column derivatisation. *Journal of Chromatography A,* 864(2), 263-270.

van der Hoek, J., van der Ven, P., and Klapwijk, A. (1988). Combined ion exchange/biological denitrification for nitrate removal from ground water under different process conditions. *Water Research,* 22, 679-684.

van der Jagt, H., Noij, T., and Ooms, P. (1995). Analysis and identification of bromate in water by ion chromatography and multiple detection at the low-ppb level. *Water Supply,* 13(1), 21-27.

van Ginkel, C., Plugge, C., and Stroo, C. (1995). Reduction of chlorate with various energy substrates and inocula under anaerobic conditions. *Chemosphere,* 31(9), 4057-4066.

van Ginkel, C., Middelhuis, B., Spijk, F., and Abma, W. (2005a). Cometabolic reduction of bromate by a mixed culture of microorganisms using hydrogen gas in a gas-lift bioreactor. *Journal of Industrial Microbiology and Biotechnology,* 32(1), 1-6.

van Ginkel, C., van Haperen, A., and van der Togt, B. (2005b). Reduction of bromate to bromide coupled to acetate oxidation by anaerobic mixed microbial cultures. *Water Research,* 39, 59-64.

van Wijk, D., and Hutchinson, T. (1995). The ecotoxicity of chlorate to aquatic organisms: A review. *Ecotoxicology and Enviromental Safety,* 32, 244-253.

van Wijk, D., Kroon, S., and Garttener-Arends, I. (1998). Toxicity of chlorate and chlorite to selected species of algae, bacteria and fungi. *Ecotoxicology and Environmental Safety,* 40, 206-211.

Velizarov, S., Crespo, J., and Reis, M. (2004). Removal of inorganic anions from drinking water supplies by membrane bio/processes. *Reviews in Environmental Science and Biotechnology,* 3, 261-380.

Versteegh, J., Neele, J., Cleven, R., Smeenk, J., and Westra, R. (1993). *Bromide and bromate in drinking and surface water.* RIVM Report 734301001 (In Dutch), Bilthoven, Holland.

Vesilind, P. (Ed.). (2003). *Wastewater Treatment Plant Design.* IWA Publishing, London.

Viessman, W., and Hammer, M. (1985). *Water Supply and Pollution Control, 4th Ed.* HarpurCollins, London.

Vigneswaran, S., and Visvanathan, C. (1995). *Water Treatment Processes: Simple Options.* CRC Press, Boca Raton.

Vivendi Water (2002). *Concentration and Dilution of Bromate in the River Colne and Ellenbrook during Pumping to Waste of the Hatfield Source.* (Unpublished).

Vogel, J., Talma, A., and Heaton, T. (1981). Gaseous nitrogen as evidence for denitrification in groundwater. *Journal of Hydrology,* 50(1/3), 191-200.

von Gunten, U. (2003). Ozonation of drinking water: Part II. Disinfection and by-product formation in presence of bromide, iodide or chloride. *Water Research,* 37(7), 1469-1487.

von Gunten, U., and Hoigne, J. (1994). Bromate formation during ozonation of bromide-containing waters: interaction of ozone and hydroxyl radical reactions. *Environmental Science and Technology,* 28(7), 1234-1242.

von Gunten, U., and Hoigne, J. (1996). Ozonation of bromide-containing waters: Bromate formation through ozone and hydroxyl radicals. In: Minear, R., and Amy, G. *Disinfection by-products in Water Treatment,* Lewis, Boca Raton.

von Gunten, U., Hoigne, J., and Bruchet, A. (1995). Bromate formation during ozonation of bromide-containing waters. *Water Supply,* 13(1), 45-50.

Wagner, H., Pepich, B., Hautman, D., and Munch, D. (2000). Eliminating the chlorite interference in US Environmental Protection Agency Method 317.0 permits analysis of trace bromate levels in all drinking water matrices. *Journal of Chromatography A,* 882(1-2), 309-319.

Waite, T., and Truesdale, V. (2003). Iodate reduction by *Isochrysis galbana* is relatively insensitive to de-activation of nitrate reductase activity - are phytoplankton really responsible for iodate reduction in seawater? *Marine Chemistry,* 81(3-4), 137-148.

Wallace, W., Beshear, S., Williams, D., Hospadar, S., and Owens, M. (1998). Perchlorate reduction by a mixed culture in an upflow anaerobic fixed bed reactor. *Industrial and Microbiological Biotechnology,* 20, 126-131.

Walters, B., Gordon, G., and Bubnis, B. (1997). An ion chromatographic method for measuring less than 5 μg per litre bromate ion in drinking water. *Analytical Chemistry,* 69(20), 4275-4277.

Wang, B., Cheng, L., and Dong, S. (2001). Construction of a heteropolyanion-modified electrode by a two-step sol-gel method and its electro-catalytic applications. *Journal of Electroanalytical Chemistry,* 516(1-2), 17-22.

Wang, S., and Du, D. (2003). Preparation and electrochemical properties of Keggin-type phosphomolybdic anions in electrostatically linked L-cysteine self-assembled monolayers. *Sensors and Actuators B - Chemical,* 94(3), 282-289.

Watson, C. (1994). *Oficial and Standardised Methods of Analysis, 3rd Ed.* Royal Society of Chemistry, Cambridge.

Weinberg, H., Delcomyn, C., and Unnam, V. (2003). Bromate in chlorinated drinking waters: Occurrence and implications for future regulation. *Environmental Science and Technology,* 37(14), 3104-3110.

Westerhoff, P. (2003). Reduction of nitrate, bromate, and chlorate by zero-valent iron (Fe^0). *Journal of Environmental Engineering,* 129, 10-16.

Westerhoff, P., Siddiqui, M., Zhai, W., Ozekin, K., and Amy, G. (1994). Bromate reduction: Another benefit of GAC. In: *American Water Works Association 1994 Engineering Design Conference Proceedings,* Cincinatti, USA, 20-23 March 1994.

Westerhoff, P., Song, R., Amy, G., and Minear, R. (1998). NOM's role in bromine and bromate formation during ozonation. *Journal of the American Water Works Association,* 90(2), 82-94.

Williams, D., Romero, P., and Eagon, R. (1978). Denitrifying *Pseudomonas aeruginosa* - Some parameters of growth and active transport. *Applied and Environmental Microbiology,* 36(2), 257-263.

Wolterink, A., Jonker, A., Kengen, S., and Stams, A. (2002). *Pseudomonas chloritidismutans sp nov.*, a nondenitrifying, chlorate-reducing bacterium. *International Journal of Systematic and Evolutionary Microbiology,* 52(6), 2183-2190.

Wolterink, A., Schilz, E., Hagedoorn, P., Hagen, W., Kengen, S., and Stams, A. (2003). Characterisation of the chlorate reductase from *Pseudomonas chloritidismutans*. *Journal of Bacteriology,* 185, 3210-3213.

World Health Organisation. (1993). *Bromate*. http://www.who.int/water_sanitation_health/GDWQ/chemicals/bromatefull.htm (www document, Accessed 5 August 2002).

World Health Organisation. (1996). *Guidelines for Drinking-water Quality, 2nd Ed.* WHO, Geneva.

World Health Organisation. (2000). *Disinfectants and Disinfectant By-products*. Environmental Health Criteria 216, WHO, Geneva.

World Health Organisation. (2005). *Bromate in Drinking Water: Background Document for Development of WHO Guidelines for Drinking Water Quality*. WHO/SDE/WSH/05.08/78, WHO, Geneva.

Xu, J., Song, Y., Min, B., Steinberg, L., and Logan, B. (2003). Microbial degradation of perchlorate: Principles and applications. *Environmental Engineering Science,* 20(5), 405-422.

Xu, J., Trimple, J., Steinberg, L., and Logan, B. (2004). Chlorate and nitrate reduction pathways are separately induced in the perchlorate-respiring bacterium *Dechlorosoma* sp KJ and the chlorate-respiring organism *Pseudomonas* sp PDA. *Water Research,* 38(3), 673-680.

Yamada, H. (1993). By-products of ozonation of low bromide waters and reduction of the by-products by activated carbon. In: *Proceedings of the 11th International Ozone Association Conference,* San Franscisco, USA.

Yang, M., Uesugi, K., and Myoga, H. (2000). Study on by-products of ozonation during ammonia removal under the existence of bromide: Factors affecting formation and removal of the by-products. *Ozone: Science and Engineering,* 22(1), 23-29.

Yang, W., Vollertsen, J., and Hvitved-Jacobsen, T. (2004). Anoxic control of odour and corrosion from sewer networks. *Water Science and Technology,* 50(4), 341-349.

Young, J. (1999). Oxygen uptake rate as a monitoring and control parameter for activated sludge processes. In: *WEF/Indiana Water Pollution Control Association (IAWPCA)/Purdue University Industrial Wastes Technical Conference,* Hyatt Regency, Indianapolis, USA, 27-30 June 1999.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). *Salmonella* mutagenicity tests. V. Results from the testing of 311 chemicals. *Environmental and Molecular Mutagenesis,* 19 (Suppl. 21), 2-141.

Zhang, X., Bishop, P., and Kinkle, B. (1999). Comparison of extraction methods in quantifying extracellular polymers in biofilms. *Water Science and Technology,* 39(7), 211-218.

12 APPENDICES

APPENDIX A – CARBON ANALYSIS METHODOLOGIES

Carbon	Test kit	Range of test	Procedure
Glucose	Merck 'Reflectoquant' glucose test (1.16720)	1 – 100 mg L^{-1} glucose	Using stored test information in the RQFlex reflectometer, simultaneously start counter and place test strip in sample for 15 seconds. Shake off excess sample from strip. When prompted (after 55 seconds) insert strip in RQFlex adapter and read off result
Lactic acid (Lactate)	Merck 'Reflectoquant' lactic acid test (1.16127)	1.0 – 60.0 mg L^{-1} lactic acid	Using stored test information in the RQFlex reflectometer, simultaneously start counter and place test strip in sample for 2 seconds. Absorb excess sample on strip onto a paper towel. 10 seconds prior to end of test (300 seconds), insert strip in RQFlex adapter and read off result.
Ethanol	Merck 'Reflectoquant' alcohol test (1.16130)	20 – 200 mg L^{-1} ethanol	Place 1 tablet of supplied reagent A-1 into test vial and add 0.5 mL sample. Swirl until tablet dissolves. Using stored test information in the RQFlex reflectometer, start counter for 240 seconds waiting time. At end of waiting time add 10 mL distilled water to test vial and mix. Restart RQFlex counter and simultaneously place test strip in sample for 2 seconds. Absorb excess sample on strip onto a paper towel. At end of reaction time (300 seconds) insert strip in RQFlex adapter and read off result.
Acetic acid (acetate)	R-biopharm Acetic acid test (10 148 261 035)	30 – 150 mg L^{-1} acetic acid	Pipette into a 1 cm light path (4 mL) cuvette 1 mL supplied solution 1, 0.2 mL solution 2 and 0.1 mL sample or RO water (for blank). Mix and read absorbance (A_0) on a spectrophotometer at 340 nm. Add 0.01 mL solution 3, mix, wait 3 minutes and read absorbance (A_1). Add 0.02 mL solution 4, mix, wait 15-20 minutes and read absorbance (A_2). Use equations A1 – A3 to calculate acetic acid concentration.

$$\Delta A=\left[\left(A_2-A_0\right)_{sample}-\frac{\left(A_1-A_0\right)^2{}_{sample}}{\left(A_2-A_0\right)_{sample}}\right]-\left[\left(A_2-A_0\right)_{blank}-\frac{\left(A_1-A_0\right)^2{}_{blank}}{\left(A_2-A_0\right)_{blank}}\right]$$

Equation A1

$$c=\frac{V\times MW}{\varepsilon\times d\times v\times 1000}\times\Delta A$$

Equation A2

where: c = concentration (g L^{-1})
V = final volume (mL)
v = sample volume (mL)
MW = molecular weight ($gmol^{-1}$)
d = light path (cm)
ε = extinction coefficient of nicotinamide-adenine dinuceotide (NADH) at 340 nm

It follows for acetic acid that:

$$c_{acetic\ acid}\left(gL^{-1}\right)=\frac{3.23\times 60.05}{6.4\times 1.0\times 0.1\times 1000}\times\Delta A=\frac{1.94}{6.3}\times\Delta A$$

Equation A3

APPENDIX B – BROMATE REDUCTION RATE CALCULATIONS

Bromate (mg L^{-1}) Influent	Effluent	Type of reactor*	Retention/ Experimental	C_{lm} (ug L^{-1})	Rate (ug $L^{-1}hr^{-1}$)	Study reference (Current trials) / Reference (other trials)	
1.4	0.9	S	20 hr	1012.4	27.8	Phase I	
0.9	0.005	S	80 hr	174.8	11.4		
1.8	0.005	S	80 hr	305.5	22.5		
4.1	0.9	S	80 hr	2117.8	41.4		
4.7	1.2	S	80 hr	2607.7	42.5	Phase IIa	
16.1	11.5	S	80 hr	13654.0	83.8		Laboratory-scale chemostat
30.7	26.9	S	80 hr	28728.1	52.3		
69.8	58.2	S	80 hr	63815.4	240.5		
130.8	120.7	S	80 hr	125647.5	149.9		
32.9	29.5	S	40 hr	31144.3	84.7	Phase IIb	
32.9	0.005	S	40 hr	3738.5	1086.8		
33.2	6.1	S	40 hr	16014.3	677.0	Phase III (Artificial medium)	
7.9	3.2	B	16 days	5213.9	12.1	Batch trial F (chemostat inoculation)	
9.4	6.1	B	8 days	7612.6	17.1	Batch trial K (chemostat inoculation)	
22.9	0.005	B	16 days	2711.9	59.4	Phase A	
1.1	0.1	F	80 hr	471.2	12.4		
1.2	0.1	F	60 hr	447.4	17.4		
1.2	0.2	F	40 hr	493.4	25.1	Phase Bii	Pilot-scale bioremediation
1.5	0.7	F	20 hr	1075.1	37.5		
0.9	0.8	F	10 hr	847.7	10.4		
1.0	0.005	B	> 50 days	187.8	0.7	(Hijnen *et al.*, 1995)	
5.0	0.005	B	> 150 days	723.1	1.4		
0.02	0.005	F	18 min	9.0	16.8		
0.04	0.02	F	18 min	26.8	49.8	(Hijnen *et al.*, 1999)	
0.04	0.02	F	26 min	29.7	41.4		
0.02	0.003	F	25 min	9.1	43.2	(Kirisits and Snoeyink, 1999)	
0.02	0.001	F	49 min	6.2	24.6		
294.4	0.001	S	48 hr	23378.6	6132.0	(van Ginkel *et al.*, 2005)	

n/d - No data; * Batch (B), suspended growth (S) or fixed film reactor (F)

APPENDIX C – PUBLICATION LIST

Butler, R., Godley, A., Lytton, L., and Cartmell, E. (2005). Bromate Environmental Contamination: Review of Impact and Possible Treatment. *Critical Reviews in Environmental Science and Technology* 35: 193-217. (Paper given in Appendix D)

Butler, R., Lytton, L., Godley, AR., Tothill, I., and Cartmell, E. (2005) Bromate analysis in groundwater and wastewater samples. *Journal of Environmental Monitoring*, 7(10), 999-1006. (Paper given in Appendix D)

Butler, R., Godley, AR., Lake, R., Lytton, L., and Cartmell, E. Reduction of bromate in groundwater with an *ex-situ* suspended growth bioreactor. (Presentation for IWA World Water Congress, Marrakech, Morocco, 19-22 September 2004). *Water Science and Technology*, 52(9), 265-273. (Paper given in Appendix D)

Butler, R., Ehrenberg, S., Godley, AR., Lake, R., Lytton, L., and Cartmell, E. Remediation of bromate-contaminated groundwater in an *ex-situ* fixed-film bioreactor. *Science of the Total Environment* (In press, published online 7 February 2006). (Paper given in Appendix D)

Butler, R., Cartmell, E., Godley, AR., and Lytton, L. Remediation of bromate contaminated groundwater. Presented at and in proceedings for Groundwater Quality 2004 conference, University of Waterloo, Canada, 19-22 July 2004. (Paper given in Appendix D)

Butler, RM., Cartmell, E., Godley, AR., Lake, R., and Lytton, L. (2004). Remediation of bromate contaminated groundwater. In: Lens, P., and Stuetz, R. *Young Researchers 2004*. Water and Environmental Management Series. IWA Publishing, London. ISBN 1-843395-05-3. Presentation at IWA Young Researchers conference, University of Wageningen, Netherlands, 22-24 April 2004

Laffray, G., Jefferson, B., Butler, R., and Cartmell, E. (2003). Impact of toxic components on the robustness of MBR operation. Presented as poster/conference by Dr Jefferson at IMSTEC '03, 10-14 Nov. 2003, University of New South Wales, Australia.

Poster presentation: UK IWA Young Researchers conference, University of Newcastle-upon-Tyne, 2-3 April 2003

Poster presentation: What's new in Groundwater conference, University of Birmingham, 13 May 2003

Poster presentation: FIRST Faraday annual conference, PERA, Melton Mowbray, 26 September 2003

APPENDIX D – COPIES OF PEER-REVIEWED PAPERS

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Bromate Environmental Contamination: Review of Impact and Possible Treatment

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Contamination of drinking water with bromate (BrO_3^-) at levels ranging from 0.4 to 60 μg L^{-1} may be found following ozonation of water containing background bromide (Br^-). Based on rodent studies, bromate is classified as a "possible human" carcinogen, and drinking water standards of 10–25 μg L^{-1} are now implemented in many countries. Bromate is highly soluble, stable in water, and difficult to remove using conventional treatment technologies. This has led to investigations into novel removal techniques, but many have not developed beyond laboratory trials. Analytical advances have recently led to detection of bromate contamination within both rivers and groundwater, which has provided an additional requirement for bromate remediation. This review summarizes bromate environmental characteristics and the regulatory situation, and outlines bromate remediation processes, including filtration, ultraviolet irradiation, catalysis, chemical reduction, activated carbon, and biodegradation. These techniques are evaluated for developmental progress in a potable water system and also for potential application within the natural water environment.

KEY WORDS: bioremediation, bromate, groundwater, oxyanion, remediation, toxicology

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I. INTRODUCTION

Bromate (BrO_3^-) is classified by the International Agency for Research on Cancer (IARC), a part of the World Health Organization (WHO), as a Group 2B or "possible human" carcinogen.[37] This classification has led authorities, including those in the European Union (EU) and the United States, to implement maximum bromate levels in drinking water. EU directive 98/83/EC (Drinking Water Directive), adopted November 1998, specifies a maximum bromate concentration of 25 $\mu g\ L^{-1}$ by 2003 and 10 $\mu g\ L^{-1}$ by 2008.[58] The U.S. Environmental Protection Agency (EPA) Stage 1 Disinfectants/Disinfection By-Products rule, also signed in 1998, set a maximum bromate level of 10 $\mu g\ L^{-1}$.[41] In the United Kingdom, the Water Supply (Water Quality) Regulations 2000 specified a maximum bromate level within drinking water of 10 $\mu g\ L^{-1}$, which came into force in December 2003.[16]

Contamination by bromate is commonly associated with disinfection by-product (DBP) formation during the treatment by ozonation of potable water containing background bromide concentrations. Bromide (Br^-) is not regulated and is found naturally within most water systems. Concentrations in freshwater are around 15–200 $\mu g\ L^{-1}$,[18] with slightly higher levels in groundwater, especially in regions with saltwater intrusion, dissolution from sedimentary rock, domestic or industrial effluent, and road runoff.[36] The average bromide concentration within natural waters in the United States is almost 100 $\mu g\ L^{-1}$.[83]

Treatment processes where bromate can form as a DBP also include ozonation followed by hypochlorination and/or chloramination, and hypochlorination alone. In a study of 40 U.S. water treatment plants, hypochlorite feedstocks were found to contain 141–326 mg bromate/pg free chlorine formed by oxidation of naturally occurring bromide during manufacture, which contributed up to 3 $\mu g\ L^{-1}$ bromate in treated water.[95] Bromate can be formed directly in drinking water via a molecular ozone pathway by oxidation of bromide to hypobromite (Eqs. 1 and 2) and indirectly via a free radical pathway, the predominant mechanism of formation depending on parameters including organic carbon, bromide content, and pH of the source water.[83] Following ozonation, bromate concentrations in potable waters generally range from 0.4 to 60 $\mu g\ L^{-1}$.[40,81]

$$O_3 + Br^- \rightarrow O_2 + BrO^- \quad (1)$$

$$2O_3 + BrO^- \rightarrow 2O_2 + BrO_3^- \quad (2)$$

In contrast to bromide, bromate is not reported as occurring naturally in surface waters[36] and is not normally present in aquifers.[92,98] However, following recent advances in analytical techniques, bromate has been detected in the surface water environment, possibly as a result of industrial oxidation/disinfection processes, with one study of 36 river samples detecting

bromate at levels from 4 to 8 $\mu g\ L^{-1}$.[46] In addition, bromate contamination has now been detected within an aquifer in the United Kingdom. Historical spillage from a chemical production plant has led to groundwater contamination in a chalk aquifer, with bromate levels in areas of the plume exceeding 2 $mg\,L^{-1}$.

Research work to date has focused on limiting impact within potable water supplies by understanding bromate formation mechanisms during disinfection processes, and developing posttreatment removal techniques. However, limited information on environmental impact and possible groundwater treatment methodologies has been published. This review covers these areas.

II. BROMATE TOXICOLOGY

The bromate anion has been designated a class 2B carcinogen based on extrapolation from rodent studies. Evidence has been noted of tumor induction in rats and mice,[47] with a dose of 0.38–2.1 $mg\,kg^{-1}$ per day for 100 wk estimated to result in a 10% increase in cancer risk.[92] The acute toxicity of bromate to rats is reported as an LD_{50} value (internal dose required to cause 50% test population mortality) between 136 and 4955 $mg\,kg^{-1}$.[98] Corresponding human toxicity data are limited to acute accidental poisoning cases, where symptoms may include severe gastrointestinal irritation, depression of the central nervous system, and renal failure.[92] Oral lethal doses from accidental poisoning have been reported as 5–50 $mg\,kg^{-1}$ body weight.[13] Once ingested, bromate may be rapidly absorbed from the gastrointestinal tract and reduced to bromide within body tissues[19] and possibly also to hydrobromic acid within the stomach.[48] Other reports however, suggest that bromate is "surprisingly stable within the body."[25] Excretion of ingested bromate is mainly in urine as bromate and bromide.[19]

Commercial applications of bromate have included use in flour, fish paste products, cheese, beer, gold extraction and wool production processes, and in permanent hair wave neutralizing solutions.[38,92,98] In addition, hypobromous acid is used as a biocide and has been shown to cause 90% lethality within a 4-min contact time to *Pseudomonas* bacteria at a concentration of 1.2 $mg\,L^{-1}$ at pH 8.2.[17] However, many of these uses, such as in breadmaking, have now been reduced or discontinued due to health concerns.[15]

Bromate ecotoxicity has been investigated, with one literature review of water-borne organisms (including the crustacean *Daphnia magna*, the flatworm *Polycelis nigra*, and juveniles of various fish species) concluding that lethal concentrations (LC_{50}) ranged from 31 $mg\,L^{-1}$ for newly hatched striped bass (*Morone saxatilis*) larvae to 2258 $mg\,L^{-1}$ for *P. nigra*. Therefore, a precautionary ecotoxicity exposure safety value of 3.0 $mg\,L^{-1}$ bromate in natural water sources was suggested, allowing for a 10-fold safety factor in the most sensitive species.[36] Fish eggs exposed to bromate developed chronic,

pathological disorders, especially to the brain and spine,[6] and a study on marine phytoplankton found 13.6 mg L^{-1} bromate increased cell division in at least 2 of the 4 species investigated.[36] Therefore, although the current regulatory situation is largely precautionary, with a 25-μg L^{-1} limit providing an estimated excess human lifetime cancer risk of 7×10^{-5},[98] little is known about the long-term effects of human bromate exposure or the behavior of bromate within the natural environment.

III. ENVIRONMENTAL CHARACTERISTICS

The bromate ion exists as a number of salts, the most industrially important being potassium ($KBrO_3$) and sodium bromates ($NaBrO_3$). Both are white crystalline solids that dissolve readily in water ($KBrO_3$ solubility at 25°C is 75 g L^{-1}),[13] a characteristic of the Group I, II, and III salts with the exception of mercuric and barium bromates.[38] Once in solution, bromate is highly stable at room temperature, does not volatilize and is not removed by boiling.[13] In addition, although bromate is thermodynamically a powerful oxidant suggested to degrade abiotically within air-dried soil by up to 64% in 14 days under aerobic and anaerobic conditions,[75] bromate reaction rates do not actually appear significant in a natural context.[51] The high solubility of bromate salts would facilitate contamination of water bodies following any industrial spillage, and low chemical reduction rates suggest it is conservative while in surface and groundwaters, acting in an analogous manner to bromide, which has been extensively used as a tracer in aquifers due to its unreactive nature.

IV. NATURAL BIODEGRADATION

Oxygen is generally the preferred bacterial respiratory electron acceptor due to a high energy yield and is utilized under aerobic conditions. Under anoxic conditions or at low oxygen concentrations many microbes, including facultative anaerobes, are able to use alternative respiratory electron acceptors such as nitrate (during denitrification). If nitrate becomes limiting, then other, lower energy yielding electron acceptors, including iron, manganese, and sulfate may be utilized by species able to metabolize them. To give an understanding of the anticipated order of utilization, the major redox reactions occurring in the subterranean environment can be categorized in order of Gibbs free energy changes (kilojoules per mole organic matter) of a standard reaction [$\Delta G_o(W)$] with the model organic compound (CH_2O) at pH 7 (Eqs. 3–8).[9]

Aerobic respiration: $CH_2O + O_2 \rightarrow H_2O + CO_2$

$$\Delta G_o(W) = -501.6 \text{ kJ mol}^{-1} \quad (3)$$

$$\text{Denitrification: } 5CH_2O + 4NO_3^- + 4H^+ \rightarrow 7H_2O + 5CO_2 + 2N_2$$

$$\Delta G_o(W) = -476.5 \text{ kJ mol}^{-1} \quad (4)$$

$$\text{Bromate reduction: } 6CH_2O + 4BrO_3^- \rightarrow 6H_2O + 6CO_2 + 4Br^-$$

$$\Delta G_o(W) = -453 \text{ kJ mol}^{-1} \quad (5)$$

$$\text{Iron reduction: } CH_2O + 4Fe(OH)_3 + 8H^+ \rightarrow 11H_2O + CO_2 + 4Fe^{2+}$$

$$\Delta G_o(W) = -117.0 \text{ kJ mol}^{-1} \quad (6)$$

$$\text{Sulfate reduction: } 2CH_2O + SO_4^{2-} + H^+ \rightarrow 2H_2O + 2CO_2 + HS^-$$

$$\Delta G_o(W) = -104.5 \text{ kJ mol}^{-1} \quad (7)$$

$$\text{Methanogenesis: } 2CH_2O \rightarrow CH_3COOH \rightarrow CH_4 + CO_2$$

$$\Delta G_o(W) = -92.0 \text{ kJ mol}^{-1} \quad (8)$$

Other electron acceptors, including chlorate,[49] perchlorate,[29,91] selenate,[53] chromate,[52] iodate,[94] and bromate,[31] have also been studied. Biological bromate reduction proceeds according to Eq. 5 and, based on thermodynamics, would be expected to occur in preference to all but denitrification and aerobic respiration. This was shown in practice by Hijnen et al.[30,31] who attempted to induce bromate reduction in denitrifying bacterial species. It was noted that when supplemented with ethanol in a denitrifying bioreactor, bromate reduction to bromide did occur. However, the reduction rate of 0.6–0.8 $\mu g\,L^{-1}\,min^{-1}$ from an initial concentration of 25–35 $\mu g\,L^{-1}$ at 12°C was over 100 times lower than the equivalent rate for denitrification,[30] and both oxygen and nitrate were found to be inhibitory.[31] It was suggested that bromate is a substrate for nitrate reductase and that this mechanism produced the observed bromate reduction.

Biological bromate reduction processes have therefore been demonstrated as a viable, if slow, laboratory bromate removal method and have also been observed to occur within a biological activated carbon (BAC) filter.[40] In groundwater, it is possible that biological bromate reduction may occur in anaerobic areas of an aquifer. However, it is unclear whether this would be a significant process as, although redox zones within aquifers have been shown to overlap, degradation patterns are extremely site-specific.[44] An example of spatial variation in the aquifer denitrification processs is caused by preferential oxygen utilization as the electron acceptor, typically leading to higher nitrate concentrations near the surface.[70,72] Availability of electron donor in the form of organic carbon (OC) is another key issue in determining potential for biological reduction of any electron acceptor, as aquifers are generally carbon limited, with the majority of groundwaters suggested to have a dissolved organic carbon content (DOC) below 2 $mg\,L^{-1}$.[89] This has been shown in practice, with Smith and Duff [84] and Vogel et al.[93] both concluding carbon availability was the major factor limiting denitrification

within aquifers in the United States and Kalahari desert, respectively. Interaction with surface water or vegetation can resupply an aquifer with OC,[44] but this will only occur in specific areas and will accentuate any spatial variations. These variations in aquifer structure and OC content may lead to small pockets of bromate reduction, but there has been little investigation into patterns of natural biological bromate reduction in a generally oxic and organic matter-limiting environment.

V. ANALOGOUS ENVIRONMENTAL CONTAMINANTS

The environmental behavior and biological reduction of bromate, while still poorly understood, is likely to be analogous to other oxyanions. Two well-studied examples are nitrate, which is a significant contaminant through use in nitrogenous fertilizers and subsequent leaching from the soil, and perchlorate, which is manufactured for use as an oxidiser in solid rocket propellant.

Nitrate (NO_3^-) is the most common groundwater contaminant[44] and is found as a diffuse pollutant in many agricultural areas. It is not uncommon for the nitrate residence time within various aquifer types to exceed 50 years, with one example of 50–70 years maximum residence time in a glacial outwash aquifer in Minnesota.[70] Therefore, nitrate can generally be thought of as conservative within a groundwater system. However, natural bacterial denitrification does occur given the requirements of suitable bacterial strains, an electron donor, and anaerobic conditions or restricted oxygen availability.[44] Nitrate reduction is carried out by a wide range of prokaryotic microorganisms, including typically aerobic heterotrophs such as *Pseudomonas* species, which only utilize nitrate when oxygen becomes limiting. Even so, despite the widespread occurrence of denitrifying organisms, the role of denitrification in aquifers is extremely difficult to evaluate or generalize, with some authors stating it is not significant and dilution effects can account for observed concentration reductions,[34] while others suggest that although degradation rates are small (in the region of 0.005–0.047 mmol NO_3 yr^{-1}), they can be significant over time.[70]

Perchlorate (ClO_4^-) salts are widely used in industry, primarily as ammonium perchlorate in solid rocket fuel, but also in the manufacture of car air bags, explosives, and pyrotechnics. Therefore, perchlorate contamination is typically a localized problem from point sources, with many affected sites found near military-related facilities.[27] Perchlorate is nonlabile, and chemical perchlorate reduction is slow despite the species' strength as an oxidizing agent. Perchlorate is extremely soluble[91] and does not appear to adsorb onto or react with the bulk aquifer matrix.[39] It was also not known as an environmental contaminant until as recently as 1996,[10] when a combination of toxicology research and analytical advances led to discovery of widespread perchlorate contamination in the United States. One

example is found in the state of California, where at least 38 drinking water wells were found to exceed an 18-$\mu g\,L^{-1}$ state advisory limit, a limit that has now been further reduced to 4 $\mu g\,L^{-1}$.[21,50] Perchlorate analytical technologies can now detect concentrations of around 4 $\mu g\,L^{-1}$, U.S. advisory limits between 4 and 32 $\mu g\,L^{-1}$ have been introduced in some states, and a recent U.S. EPA draft reference dose of 0.03 $\mu g\,kg^{-1}$ body weight may lead to implementation of a federal drinking water limit of 1 $\mu g\,L^{-1}$.[50,64,103]

Information on perchlorate and the related oxyanion chlorate (a compound used in weed control, as a paper bleaching agent and in water disinfection[49]) in groundwater systems is limited, but relevant literature is now growing. It is thought that perchlorate and chlorate [together known as (per)chlorate] are stable and mobile in aqueous systems and can migrate substantial distances within aquifers.[27] Ion-exchange systems are among technologies being trialed for (per)chlorate treatment, along with reverse osmosis, nanofiltration, granular/biological activated carbon, electrochemical reduction, and a novel use of autotrophic microorganisms within a membrane bioreactor environment.[1,5,22,27,64,87,90,91] Therefore, as outlined earlier, there are similarities between the environmental characteristics of bromate and those of both nitrate and (per)chlorate, and these could be used in the elucidation of bromate behavior and development of treatment technologies.

VI. MECHANISMS OF BROMATE BIODEGRADATION

The mechanism of biological bromate reduction is poorly understood, and there has been little research to date into this area. However, the biological denitrification process has been widely studied since 1882, when the term "denitrification" was coined by U. Gayon and G. Dupetit.[44] Many of the mechanisms occurring during bacterial denitrification have been elucidated, and reviews have been published focusing on this subject.[32,57] In addition, the recent issues surrounding groundwater (per)chlorate contamination have led to studies into bacterial reduction of these compounds, which, combined with the extensive denitrification literature, may provide insights into bromate reduction pathways.

The range of bacteria known to possess an ability to denitrify encompasses at least 27 genera, which were collated and listed by Mateju et al.[57] Denitrification can be carried out by heterotrophic bacteria, which require OC as an electron donor, and also by autotrophs, which derive these requirements from inorganic sources such as hydrogen or reduced sulfur compounds. The majority of known denitrifiers are heterotrophic,[44] with a wide variety of organic compounds, including methanol, ethanol, glucose, acetate, formic acid, molasses, and whey, reported as being utilized during the process.[57] The denitrification pathway they follow is typically shown as

a series of reducing steps, commencing with nitrate (oxidation state +5) and completed with the evolution of dinitrogen gas (oxidation state 0).[67]

$$\underset{\text{nitrate}}{NO_3^-} \rightarrow \underset{\text{nitrite}}{NO_2^-} \rightarrow \underset{\text{nitric oxide}}{NO} \rightarrow \underset{\text{nitrous oxide}}{N_2O} \rightarrow \underset{\text{dinitrogen}}{N_2} \quad (9)$$

The reduction of nitrate is catalyzed by a discrete enzyme system at each individual step, with some denitrifiers able to complete the entire pathway but many only capable of carrying out one or two steps.[32] The initial step of nitrate reduction to nitrite is mediated by membrane-bound nitrate reductases, with nitrite reductases catalyzing reduction of nitrite to either nitric oxide or nitrous oxide, and the less-well-characterized nitric oxide and nitrous oxide reductases completing the pathway to dinitrogen.[57] Thus the overall denitrifying microflora may be thought of as a group of complementary bacterial strains, only together able to totally convert nitrate to dinitrogen gas.

It has been known since the 1920s that some of these nitrate-reducing strains can also reduce chlorate to chlorite.[71] The mechanism was thought to be a competitive interaction between nitrate and chlorate as a terminal electron acceptor for nitrate reductase, with bacterial growth and energy conservation not a consequence of this chlorate utilization.[11,64] However, it is now known not all denitrifying bacteria are capable of chlorate utilization,[29] and specific chlorate reductases have since been isolated from denitrifying species.[49] In addition, recent studies have shown certain species have the ability to use (per)chlorate as a terminal electron acceptor for anaerobic respiration and to conserve energy for growth from this reduction pathway. Reduction of (per)chlorate to chlorite is mediated by (per)chlorate reductases, and a second reaction disproportionates chlorite utilizing a highly conserved chlorite dismutase enzyme[100] in a reaction suggested to be a detoxification mechanism to remove toxic chlorite.[29] The end product is therefore chloride in a pathway first elucidated in 1996 by Rikken et al.[73]

$$\underset{\text{perchlorate}}{ClO_4^-} \rightarrow \underset{\text{chlorate}}{ClO_3^-} \rightarrow \underset{\text{chlorite}}{ClO_2^-} \rightarrow \underset{\text{chloride}}{Cl^-} \quad (10)$$

Until recently, only six bacterial species capable of anaerobic respiration linked to (per)chlorate reduction had been identified, but it is now thought to be a much more ubiquitous process,[11] with the (per)chlorate degradation mechanism in certain species shown to be mediated by specific perchlorate and chlorate reduction pathways.[11,100] One recent paper by Xu et al.,[102] discussing the relationship between chlorate, perchlorate, and nitrate reduction, concluded that generalizations among (per)chlorate-reducing bacterial strains were often inappropriate. It had previously been shown in strains Perclace and *Dechlorosoma suillum* that a single enzyme was responsible for both perchlorate and nitrate reduction.[8,20] However, it

was found *Dechlorosomas* sp. KJ contained separate perchlorate and nitrate pathways, and *Pseudomonas* sp. PDA was able to mediate the complete chlorate degradation pathway but unable to reduce either perchlorate or nitrate.[102] Another recently identified chlorate-reducing strain, *Pseudomonas chloritidismutans* strain AW-1, has also been shown as incapable of perchlorate or nitrate reduction.[99,100] Xu et al. (2003) suggested that, while all perchlorate reducing bacteria are also able to reduce chlorate, not all chlorate reducers can degrade perchlorate.[101] Thus, previously observed variable effects of nitrate concentration on (per)chlorate reduction[49] may be a consequence of different reduction pathways, some utilizing the nitrate reduction system and some completely independent.

There is no direct evidence for a specific bromate reduction pathway,[31] and it has been concluded by most authors that biological bromate reduction is a side reaction of the nitrate reduction pathway.[31,40] For example, it was shown that *Escherichia coli* nitrate reductase catalyzes the reduction of bromate.[62] However, these conclusions are made in the absence of experimental evidence, and the recent (per)chlorate studies suggest oxyanion reduction pathways are more complex and interlinked than previously known. Lack of naturally occurring bromate (and therefore evolutionary selection pressures) was cited as a reason for lack of a specific bromate pathway,[31] but perchlorate contamination is also of recent anthropogenic origin.[11] In addition, it has recently been demonstrated by Wolterink et al.[99,100] that, while unable to reduce nitrate, *P. chloritidismutans* strain AW-1 is capable of bromate reduction, although this reaction could not be utilized for growth. Therefore, it is quite possible a separate bromate reduction pathway does exist independently of the denitrification system, and this may help to explain the bromate reduction observed by Kirisits and Snoeyink[40] in the presence of nitrate. However, more work is required before any conclusions can be made on the exact mechanism of biological bromate reduction.

VII. WATER TREATMENT AND REMEDIATION

Bromate removal methods have been researched since the early 1990s,[59] with the vast majority of research into remediation focusing on developing postozonation methodologies for potable water supplies. This has led to development of a number of techniques, many currently still in the laboratory evaluation and development stages. These different approaches are detailed next, and are also summarized and compared in Table 1.

1. Physical and Electrical Techniques

Filtration uses membranes to separate higher molecular weight compounds from bulk water. Marhaba and Medlar[56] tested nanofiltration at 40 and

TABLE 1. Developmental Stage, Performance, and Assessment of Potential for Full-Scale Implementation of Techniques Trialed for Bromate Removal

	Operating conditions					Bromate removal					
Technique	Laboratory/ Pilot scale	Batch/ Continuous flow	Throughflow	Bromate range tested	Water type[a]	Efficiency (percentage bromate removed)	Waste stream	Efficiency reduction by interference	Potential cost of implementation	References	Notes
Physical											
Nanofiltration/ Hyperfiltration	Pilot	Continuous	n/d	5–285 $\mu g L^{-1}$	Real	Averages 89% & 97% for 10 min runs	***	*	***	Marhaba and Medlar, 1993	
Nanofiltration	Laboratory	Batch	n/a	300 $\mu g L^{-1}$	Real	77% (20 L batch volume)	***	*	***	Prados-Ramirez et al., 1995	
UV	Laboratory	Continuous	Approx 4–30 L min^{-1}	50–100 $\mu g L^{-1}$	Real	50–100%	*	***	***	Siddiqui and Amy, 1994	
UV	Pilot	Continuous	Approx 70–640 L min^{-1}	15–50 $\mu g L^{-1}$	Real	5–46%	*	***	***	Siddiqui et al., 1996[c]	
Arc discharge	Laboratory	Continuous	7.6 L min^{-1}	100 $\mu g L^{-1}$	Real	12–45%	*	n/d	***	Siddiqui et al., 1996a	
HEEB	Laboratory	Batch	n/a	100–320 $\mu g L^{-1}$	Real	Up to 100%	*	***	***	Siddiqui et al., 1996b	
HEEB	Pilot	Continuous	Approx 380 L min^{-1}	180–700 $\mu g L^{-1}$	Real	Around 40%	*	***	***	Siddiqui et al., 1996b	
Photocatalytic decomposition	Laboratory	Continuous	0.5 L min^{-1}	50–75 $\mu g L^{-1}$	Real	50% with 15–43 min contact time	*	**	***	Mills et al., 1995; Mills et al., 1996	
Photocatalytic decomposition	Laboratory	Batch	n/a	200 $\mu g L^{-1}$	Artificial	Up to 100% after 1 hour (pH 5)	*	n/d	***	Noguchi et al., 2002	Long reaction time required
Chemical											
Coagulants	Laboratory	Batch	n/a	n/d	Real	5% (Alum) & 20% (Ferric chloride)	**	n/d	n/d	Siddiqui et al., 1994a	Concluded treatment not worth pursuing
Coagulants	Laboratory	Batch	n/a	n/d	Artificial	4.8% (Alum)	**	n/d	n/d	Hossain et al., 1996	
Reducing agent (Ferrous)	Laboratory	Batch	n/a	25–80 $\mu g L^{-1}$	Real	Up to 90% in 30 min	**	**	**	Siddiqui et al., 1994b	

Reducing agent (Sulphite)	Laboratory	Batch	n/a	7.6 mg L^{-1}	Artificial	Up to 63% at pH 9.5	##	n/d	##	Prados-Ramirez et al., 1995	High bromate concentration trialled
Reducing agent (Ferrous)	Laboratory	Batch	n/a	100 $\mu g L^{-1}$	Artificial	100% in 2 min (pH 8)	##	##	##	Gordon et al., 2002	
Reducing agent (Sulphite)	Laboratory	Batch	n/a	100 $\mu g L^{-1}$	Artificial	Up to 99% in 4 days (pH 7)	##	##	##	Gordon et al., 2002	Reaction time considered unrealistic
Reducing agent (Ferrous)	Pilot	Continuous	2.75 hour total contact time	15 $\mu g L^{-1}$	Real	Up to 70% (≤41% after 30 min flocculation)	##	##	##	Krasner et al., 1996	
Zero-valent iron (Fe^0)	Laboratory	Continuous	5–20 min EBCT[b] for 2–4 bed volumes	10 mM	Artificial	Up to 90% with EBCT of 20 min	##	n/d	n/d	Westerhoff, 2003	
Biological											
Denitrifiers	Laboratory	Batch	n/a	1 mg L^{-1}	Artificial	Up to 99% in 23 days	#	n/d	#	Hijnen et al., 1995	High bromate concentration trialled
Denitrifiers	Pilot	Continuous	250 L min^{-1}; 18–36 min contact time	15 + 35 $\mu g L^{-1}$	Real	Up to 71%	#	##	#	Hijnen et al., 1999	
HFMBfR	Pilot	Continuous	1.5 L min^{-1}	n/d	Real	Up to 95%	#	##	##	Nerenberg et al., 2002; Nerenberg and Rittman, 2004	Not trialled for bromate reduction (perchlorate/nitrate only)
BAC	Laboratory	Continuous	25–50 min EBCT[b]	10–11 $\mu g L^{-1}$	Real	Up to 86% for 30 days	#	#/##	#/##	Kirisits Snoeyink, 1999	Cost low where GAC already installed
BAC	Laboratory	Continuous	20 min EBCT[b]	20 $\mu g L^{-1}$	Real	40% for 10 days during sustained run	#	#/##	#/##	Kirisits et al., 2001	
BAC	Laboratory	Continuous	20 min EBCT[b]	10–50 $\mu g L^{-1}$	Real	37–41% continuous, up to 74% peak	#	#/##	#/##	Kirisits et al., 2002	Sustained run (>11 months)

(Continued on next page)

TABLE 1. Developmental Stage, Performance, and Assessment of Potential for Full-Scale Implementation of Techniques Trialed for Bromate Removal. *(Continued)*

	Operating conditions					Bromate removal					
Technique	Laboratory/ Pilot scale	Batch/ Continuous flow	Throughflow	Bromate range tested	Water type[a]	Efficiency (percentage bromate removed)	Waste stream	Efficiency reduction by interference	Potential cost of implementation	References	Notes
Activated carbon											
PAC	Laboratory	Batch	n/a	25–100 $\mu g L^{-1}$	Real	5–99% over 24 h	**	**	n/d	Siddiqui et al., 1994a; Westerhoff et al., 1994	GAC considered more effective and worth pursuing
GAC	Pilot	Continuous	Approx 1.3 L min^{-1}	3–290 $\mu g L^{-1}$	Real	Up to 100% for 32 h	*	n/d	*/**	Marhaba and Medlar, 1993	
GAC	Laboratory	Continuous	10–15 EBCT[b] equivalent[c]	50 $\mu g L^{-1}$	Real	Up to 100% for at least 1000 bed volumes	*	***	*/**	Siddiqui et al., 1994a; Siddiqui et al., 1994b	
GAC	Laboratory	Continuous	Approx 11–38 L min^{-1} equivalent[c]; 4–10 min EBCT[b] equivalent[c]	50 $\mu g L^{-1}$	Real	Up to 100% for around 1000 bed volumes	*	n/d	*/**	Westerhoff et al., 1994	50–80% breakthrough after 4000 bed volumes
GAC	Laboratory	Continuous	0.0125 L min^{-1}; 10 min EBCT[b] equivalent[c]	9 mg L^{-1}	Artificial	67% after 122 h	*	n/d	*/**	Prados-Ramirez et al., 1995	High bromate concentration trialled, with short run times
GAC	Laboratory	Continuous	20 min EBCT[b]	n/d	Artificial	No removal observed	n/a	n/a	*/**	Meijers and Kruithof, 1995; Kruithof and Meijers, 1995	
GAC	Laboratory	Continuous	0.17 L min^{-1} equivalent[c]; 5–10 min EBCT[b] equivalent[c]	25–100 $\mu g L^{-1}$	Real	30–40% for 60 days equivalent	*	***	*/**	Siddiqui et al., 1996d	

GAC	Pilot	Continuous	0.32 L min^{-1}	50 $\mu g L^{-1}$	Real	>60% for 50 days	#	##	#/##	Asami et al., 1999	Efficiency reduction noted as GAC colonised to BAC
GAC	Pilot	Continuous	0.51 L min^{-1}; 20 min EBCT[b]	Up to 320 $\mu g L^{-1}$	Real	Average 75% for 3 months	#	###	#/##	Bao et al., 1999	Suggested BAC may be more suitable in long-term & GAC type critical
GAC	Pilot	Continuous	3.7 L min^{-1}; 10 min EBCT[b]	50 $\mu g L^{-1}$	Real	Average 50% over 1 month	#	n/d	#/##	Marhaba, 2000	Suggested GAC may be cost-effective
GAC	Laboratory	Continuous	2 min EBCT[b]	9–10 $\mu g L^{-1}$	Real	Above 50% for 1000–41000 bed vols (dependent on water source)	#	###	#/##	Kirisits et al., 2000	Suggested BAC may be more suitable in long-term
GAC	Pilot	Continuous	0.63 L min^{-1}; 15 min EBCT[b]	17–47 $\mu g L^{-1}$ (1 month), then 94–163 $\mu g L^{-1}$	Real	100% continuously for one month, then declining to ~5% after 11 months	#	###	#/##	Huang and Chen, 2004	

Key:

Waste stream

#—Little residual other than bromide (and low-level microbial biomass) produced by treatment; ##—Treated water includes chemical or carbon residues; ###—Concentrated ionic waste stream produced which requires treatment prior to disposal.

Interference (Examples include pH, organic matter, oxygen, nitrate, competing anions)

#—Little efficiency reduction noted under real conditions; ##—Moderate efficiency reduction noted under real conditions; ###—High efficiency reduction noted under real conditions.

Potential cost of implementation (based on comments within publications)

#—Low potential capital and/or operating costs; ##—Medium potential capital and/or operating costs; ###—High potential capital and/or operating costs.

[a]'Real' describes a natural water matrix, 'Artificial' describes a distilled water sample spiked with bromate.

[b]Empty bed contact time.

[c]Trial tested an RSSCT (Rapid Small-Scale Column Test) configuration; data given for theoretical scale-up to pilot scale.

n/a—Not applicable.

n/d—No data.

75 psi, and hyperfiltration at 115 psi. From an initial bromate level of up to 285 $\mu g\,L^{-1}$, 75–100% removal was obtained for nanofiltration, and an average of 97% for hyperfiltration was achieved. It was concluded that nanofiltration was more cost-effective due to lower water pressures, later confirmed by Prados-Ramirez et al.[68] Reverse osmosis[68] and ion-exchange processes have also been suggested, although little published research has been carried out to date. However, there are major disadvantages related to these physical techniques. The resultant water stream is effectively deionized and requires reionization, and a concentrated waste stream is produced, which would need remediation prior to disposal. Finally the cost for bromate treatment alone is high, and it is unlikely that membrane filtration would be cost-effective without significant process integration.[54]

Ultraviolet (UV) irradiation (wavelength 100 to 400 nm) is widely used for water disinfection.[14] The wavelengths 180–300 nm also provide enough energy for bromate decomposition to bromite (BrO_2^-) and subsequently bromide.[80] A preliminary study using batch reactors and 50–100 $\mu g\,L^{-1}$ bromate concluded that irradiation by a low-pressure mercury lamp (180–254 nm output) led to 3–38% removal.[82] A 50 times more powerful 200–300 nm medium pressure lamp was also investigated[77] and, in conjunction with continuous-flow reactors, both these and low-pressure lamps[80] were shown to have advantages, in reduced contact time (5–20 s as opposed to 30 s) and energy efficiency, respectively. However, efficiency is heavily dependent on wavelength and pressure,[26] and the high-power, low-wavelength lamps required to significantly improve reduction rates above those already achieved may prove not to be cost-effective.

Photocatalytic decomposition utilizes UV irradiation with a titanium dioxide (TiO_2) catalyst, generating an electron–hole pair (e^-h) capable of reducing bromate to bromide at the semiconductor surface. TiO_2 is widely used in photocatalysis, being biologically and chemically inert, photoactive, stable, and inexpensive.[60] Batch and continuous-flow trials with 254 nm UV radiation and platinized TiO_2 used as a dispersion (batch) or coating (continuous flow) concluded that bromate reduction was enhanced over UV irradiation alone by 4–5 and 4.2 times, respectively, with overall disinfection also enhanced.[60] Long reaction times (15.2 min and 43 min, respectively) and ionic competition meant the technique was impractical,[61] and, although recent studies have enhanced rates by manipulating pH and surface charge,[65] significant further improvement is required prior to commercial use.[66]

Arc discharges are created by discharging a capacitor at 10–50 kV, 10–100 A, and a pulse rate between 50 and 100 pulses per second across the water flow. This produces an intense, localized high-temperature plasma, which rapidly expands and contracts, causing shock waves and a burst of UV radiation.[78] The UV irradiation reduces bromate, although free radicals and hydrogen atoms produced by the plasma mediate the major reduction processes. *High-energy electron beams* (HEEB) produce ionizing radiation,

which induces formation of reducing species, including aqueous electrons, hydrogen atoms, and hydroxyl radicals.[79] Arc discharges and HEEB were found to provide destruction efficiencies of 12–45% and 70%, respectively, from an initial 100 $\mu g\,L^{-1}$ bromate concentration. Destruction efficiency was reduced by electron scavengers (HEEB), water temperature (arc discharge), and DOC (both techniques).[78,79] Arc discharge uses less energy than UV irradiation, but is less efficient.[78] HEEB is an effective technique, but high capital costs and presence of contaminants such as nitrate that increase dosage required currently make it economically unfeasible.

2. Chemical Techniques

Coagulants including alum (Al^{3+}) and ferric chloride (Fe^{3+}) were evaluated for adsorption ability, but bromate removal rates were only 5% and 20%, respectively, and it was concluded that coagulating agents were unable to significantly reduce bromate concentrations in natural waters.[33,81]

Reducing agents include sulfur compounds such as thiosulfate, sulfite, and sulfur dioxide. Sulfite addition induced a bromate removal rate of 16–63% at pH 5–9.5 and an initial bromate concentration of 7.6 $mg\,L^{-1}$. The removal rate increased with pH, with a neutral pH leading to approximately 50% removal.[68] A subsequent sulfite trial concluded that although 99% bromate removal could be achieved with initial contamination levels of 0.1 $mg\,L^{-1}$, reaction time under drinking-water conditions was up to 4 days at pH 7.[24] The authors concluded that ferrous iron (Fe^{2+}) was the more promising reducing agent. Fe^{2+} reduces bromate to bromide and oxidizes into the ferric form (Fe^{3+}), with any remaining Fe^{2+} oxidised by dissolved ozone or oxygen (Eqs. 11–13).[81]

$$BrO_3^- + 6Fe^{2+} + 6H^+ \Leftrightarrow Br^- + 6Fe^{3+} + 3H_2O \quad (11)$$

$$4Fe^{2+} + O_2 + 4H^+ \Rightarrow 4Fe^{3+} + 2H_2O \quad (12)$$

$$Fe^{3+} + 3HCO_3^- \Rightarrow Fe(OH)_{3(s)} + 3CO_2 \quad (13)$$

Studies using natural water showed bromate reduction occurs within 10 min, and equilibrium was reached after 15 min.[81,82] Reduction was pH dependent, with higher rates at lower pH and dissolved oxygen (DO) levels. Results indicated bromate reduction was possible within a flocculation basin with a hydraulic residence time of 30 min.[82] Ferrous chloride ($FeCl_2$) addition was initially shown to produce unacceptable turbidity, so ferric chloride ($FeCl_3$) was also added to improve effluent turbidity. Reduction rates at various Fe^{2+} concentrations in 30-min and 2-h contact time flocculation basins varied from 4 to 41% and from 36 to 70%, respectively, with temperature confirmed as a rate-determining factor.[45] Hossain et al.[33] achieved a "significant reduction" of bromate with 15 $mg\,L^{-1}$ $Fecl_2$, and a study of use of "pickling

Fe" (a by-product of the steel industry) led to removal of 100 $\mu g\,L^{-1}$ bromate by 30 $mg\,L^{-1}$ (as Fe^{2+}) in only 2 min.[24] Pickling Fe is produced when concentrated hydrochloric acid is used to clean steel sheets during manufacture, and contains principally Fe^{2+}, with a high chloride content and low Fe^{3+} levels. The residual Fe^{3+} was useful as a flocculant, although no indication was given of other contaminants, which may preclude use in a drinking water situation. A methodology for removal of residual iron was proposed by Krasner et al.[45] utilizing pH control to oxidize residual ferrous iron prior to removal in filters. However, despite effectiveness of the system, the issue of cost-effectively removing residual iron from the water stream has not been adequately addressed; given a UK limit of 200 $\mu g\,L^{-1}$ Fe in drinking water,[16] this problem must be resolved prior to development of a viable full-scale technique.

Zero-valent iron (Fe^0) was first proposed as a remediation methodology for nitrate in the early 1990s. Westerhoff recently investigated reduction of nitrate, bromate and chlorate using seven sources of Fe^0 at laboratory scale in batch and column tests.[96] Bromate was reduced at a faster rate than either nitrate or chlorate, with approximately 90% of 10 m*M* bromate reduced to bromide with an empty-bed contact time of 20 min during column trials, compared with only 10% for nitrate. Fe^0 source was determined as a critical factor in overall reduction rate, but the mechanisms leading to these differences were not further investigated. However, use of spiked ultrapure water in the study and the lack of other competing groundwater constituents mean it is hard to relate these results to a groundwater situation. Other factors, including longevity of effectiveness, formation of by-products such as dissolved iron species, and rise in pH due to proton consumption, must also be considered prior to trialing this technique at a larger scale.[96]

3. Biological Techniques

Biological remediation methods are used to degrade many organic compounds,[74] and some inorganic species, including manganese,[4] nitrate,[85] and perchlorate.[101] Denitrification is one of the most studied systems, and has been investigated in a wide range of pilot- and full-scale remediation trials, encompassing both in situ and ex situ processes. Reviews covering many of these can be found in Hiscock et al. (1991) and Mateju et al. (1992)[32,57] Recent examples of in situ denitrification applications include pilot trials of a system injecting formate as an electron donor, which removed 80–100% nitrogen up to 15 m from the injection point,[85] and a comparable process injecting glucose.[23] Ex situ biological drinking-water denitrification has been developed using many different unit processes, including fluidized-bed reactors, packed-bed reactors, biofilters, and even denitrifying bacteria immobilized in alginate beads or a polymer matrix.[57] Ex situ perchlorate reduction has also

been widely investigated at laboratory and pilot scale in recent years,[101] with data on one full-scale bioreactor reported. Perchlorate feed concentrations of approximately 8 $mg\,L^{-1}$ were reduced to below the practical quantification limit (<4 $\mu g\,L^{-1}$) for more than 8 months in an ethanol-fed fluidized bed reactor containing granular activated carbon (GAC) as the fluidization media.[28]

Bromate bioremediation has not been widely studied to date, but the work by Hijnen et al. has confirmed bromate can be degraded to bromide by denitrifying bacteria.[30,31] Although reduction rates were initially reported as being slower than those for denitrification, more recent studies have suggested both rates may be of the same order of magnitude at comparable initial concentrations.[30] Investigations into ex situ bromate remediation using BAC have indicated reduction occurs within a mixed bacterial population in the presence of both oxygen and nitrate.[40,42] In addition, trials using carbon augmentation of indigenous aquifer bacterial populations under continuous-flow suspended growth conditions have now led to bromate reduction to below the limit of detection (<5 $\mu g\,L^{-1}$) from a 48-$mg\,L^{-1}$ influent, with 40- and 80-h residence times.[7] Taken together, these findings have increased the probability of successful process development, and it is possible that, with further investigation, in situ and ex situ biological techniques could both be utilized for bromate remediation.

Membrane bioreactors combining membrane technology with biological reactors have been used commercially in wastewater treatment for over 20 years.[86] Membrane bioreactors have also been used in denitrification systems in various configurations.[64] However, the development of hollow-fiber membrane biofilm reactors (HFMBfR) for biological nitrate and perchlorate reduction also shows promise for bromate removal.[63] The HFMBfR uses hollow membrane fibers, through which the supplied pressurized hydrogen gas diffuses, encouraging development of an autotrophic biofilm utilizing and thus reducing oxidized contaminants. A pilot-scale system currently operating for nitrate and perchlorate reduction is proving cost-effective, and preliminary results suggest 95% bromate removal can also be achieved.[63]

4. Activated Carbon Techniques

Activated carbon in granular (GAC) or powdered (PAC) form has a large internal surface area (600–1600 $m^2\,g^{-1}$ for most GAC) amenable to adsorption, and can be produced from almost any carbonaceous material (i.e., wood, coal, coconut shells) by anoxic carbonization.[14] Activated carbon previously proved effective in removing inorganic disinfection by-products such as chlorite and chlorate by adsorption followed by chemical (abiotic) reduction,[83] and a preliminary study on bromate removal at concentrations up to 300 $\mu g\,L^{-1}$ achieved almost 100% reduction on GAC for at least 32 h.[56] Using both batch reactors with PAC, and GAC-filled continuous-flow rapid small-scale

column tests (RSSCT) to simulate a pilot plant,[12] PAC was found to be successful but slow, with 63–99% reduction over 12–24 h time frames.[97] GAC induced the higher rate of 0.79 mg bromate removed per gram carbon for more than 7 days. However DO, DOC, and competing anions including nitrate, sulfate, and chloride have all been shown to reduce efficiency in both processes.[3,43,81,82] It was concluded GAC was preferable for further studies, either as a full-length column or as a filter cap.[97]

Prados-Ramirez et al.[68] reported fresh coconut carbon could remove 9 mg L^{-1} bromate from distilled water for 122 h, leading to only 33% breakthrough. However, in the presence of organic contamination, little bromate adsorption was noted. Other investigations concluded GAC-facilitated bromate removal was only possible at impractically low pH levels,[46,59] and that reduction levels would not exceed 20%.[76] Subsequent studies have suggested GAC can mediate significant bromate reduction for limited time periods, with GAC columns removing an average of 30–40% and 50% bromate from natural waters for 2 months and 1 month, respectively.[55,83] At pH 3 almost total bromate removal was achieved, although this decreased to 38% at pH 5.5.[103]

Marhaba[55] suggested that observed decreases in reduction capacity of GAC could be attributed to biological or biofilm growth, although no possible mechanism was outlined and the system was run under ambient oxygen conditions, which may encourage fouling by aerobic microorganisms. In a separate trial, fresh GAC was observed for 2–10 months, over which time bromate reduction levels decreased from 1.5 mg bromate per gram carbon to effectively zero due to bacterial colonization. It was therefore concluded BAC was ineffective at bromate reduction.[2] However, 86% bromate removal was subsequently observed during a BAC filtration trial,[40] using GAC as a matrix for microbial growth and biological reduction under controlled oxygen conditions (2–8 mg L^{-1}). The report also suggested effective bromate removal could be achieved by conversion of existing commercial GAC filters. More recent work has shown BAC filters can continuously remove 37–41% bromate from an initial bromate concentration of 20 μg L^{-1} for over 1 year under realistic BAC operating conditions, including influent DO and nitrate concentrations of 2 mg L^{-1} and 0.3–5 mg L^{-1}, respectively.[41] Therefore, while adsorption and abiotic reduction on GAC active sites have the potential to provide almost complete bromate removal in the short term under favorable conditions, GAC type and source water composition are critical in the longer term.[35,88] It has also been noted that reduction efficiency can decline rapidly, with one pilot plant receiving up to 163 μg L^{-1} bromate showing rapidly declining removal after 3 months,[35] and Kirisits et al.[43] suggesting an operational lifetime of only 30 days with a 10-μg L^{-1} bromate influent. BAC, by contrast, has shown to provide continuous biological reduction over a period of months; if it is possible to extrapolate the rate to higher bromate concentrations, this could significantly reduce bromate in highly contaminated groundwater.

VIII. SUMMARY

Bromate contamination has become a growing problem over the past decade, due to a combination of increased use of disinfection by ozonation, improved analytical capabilities, and tightening of drinking water limits following evidence of bromate carcinogenicity. Remediation technologies have improved significantly, but few pilot or full-scale trials have currently been completed. In addition, many studies have used distilled or tap water and bromate concentrations in the range 10–100 $\mu g L^{-1}$. While this is a reasonable approximation of postozonation conditions, differences will exist when applied to contaminated groundwater with bromate concentrations up to three orders of magnitude higher. However, the issue of groundwater bromate contamination must be addressed, especially where extraction for human consumption occurs, as conventional water treatment processes are not designed to remove bromate to the new regulatory standards.

Using techniques already investigated for bromate removal as a basis, the most developed technologies are chemical removal by ferrous iron and ex situ treatment by BAC. Both have been undertaken at pilot scale and have shown some promise under commercially relevant conditions. In addition, both techniques can utilize modified but preexisting processes and equipment, with ferrous iron treatment requiring mixing, clarification, and filtration tanks and BAC making use of existing GAC columns. However, in both cases further development is required prior to full-scale use, and detailed assessments would be required on a case-by-case basis as to the cost-effectiveness at a particular site. Where a contaminated aquifer can be identified, in situ biological removal appears a promising technology. Successful development of this technique could provide a remediation process effective in degrading bromate to bromide across the entire width of a contamination plume, thus both protecting the aquifer and extraction points downstream. In addition, knowledge of biological bromate reduction could be transferred to an ex situ methodology, analogous to existing fixed-film potable water processes. While bromate is not currently known as a significant environmental contaminant, production of bromine compounds has historically been high, peaking at 403,000 tonnes in 1979.[69] Therefore, it is possible the recent introductions of parts per billion drinking water standards may uncover further contaminated sites within potable water supplies, leading to increased requirements for remediation of bromate within groundwater.

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REFERENCES

[1] Amy, G., Pellegrino, J., and Liang, S., Treatibility of perchlorate-containing water by RO, NF and UF membranes, *Project #2531*, AwwaRF, Denver, CO, 2003.

[2] Asami, M., Aizawa, T., Morioka, T., Nishijima, W., Tabata, A., and Magara, Y., Bromate removal during transition from new granular activated carbon (GAC) to biological activated carbon (BAC), *Water Res.* 33, 2797, 1999.

[3] Bao, M., Griffini, O., Santianni, D., Barbieri, K., Burrini, D., and Pantani, F., Removal of bromate ion from water using granular activated carbon, *Water Res.* 33, 2959, 1999.

[4] Bernoth, L., Firth, I., McAllister, P., and Rhodes, S., Biotechnologies for remediation and pollution control in the mining industry, *Miner. Metall. Process.* 17, 105, 2000.

[5] Brown, J., Snoeyink, V., and Kirisits, M., Abiotic and biotic perchlorate removal in an activated carbon filter, *J. Am. Water Works Assoc.* 94, 70, 2002.

[6] Burton, D., and Richardson, L., Investigation of chemistry and toxicity of ozone-produced oxidants and bromate to selected estuarine species, *US EPA report EPA-600/4-81-040*, Office of Water and Waste Management, FL, 1981.

[7] Butler, R., Cartmell, E., Godley, A., and Lytton, L., Remediation of bromate contaminated groundwater, Paper presented at *Groundwater 2004*, University of Waterloo, Waterloo, Ontario, Canada, 19–22 July 2004.

[8] Chaudhuri, S., O'Connor, S., Gustavson, R., Achenbach, L., and Coates, J., Environmental factors that control microbial perchlorate reduction, *Appl. Environ. Micro.* 68, 4425, 2002.

[9] Christensen, T., Kjeldsen, P., Bjerg, P., Jensen, D., Christensen, J., Baun, A., Albrechtsen, H.-J., and Heron, G., Biogeochemistry of landfill leachate plumes, *Appl. Geochem.* 16, 659, 2001.

[10] Coates, J., and Achenbach, L., Microbial perchlorate reduction: Rocket fuelled metabolism, *Nature Rev. Micro.* 2, 569, 2004.

[11] Coates, J., Michaelidou, U., Bruce, R., O'Connor, S., Crespi, J., and Achenbach, L., Ubiquity and diversity of dissimilatory (per)chlorate-reducing bacteria, *Appl. Environ. Micro.* 65, 5234, 1999.

[12] Crittenden, J., Hand, D., Reddy, P., Arora, H., Trynoski, J., and Notthakun, S., Prediction of GAC performance using rapid small-scale column tests, *American Water Works Association Annual Conference Proceedings*, Los Angeles, 1989.

[13] Depository Services Program (Canada), Citing Internet resources (www document) http://dsp.psd.pwgsc.gc.ca/collection/H48-10-1-18-1999E.pdf (accessed 17/3/04), 1999.

[14] Droste, R., *Theory and Practice of Water and Wastewater Treatment*, John Wiley, New York, 1997.

[15] Dupois, B., The chemistry and toxicology of potassium bromate, *Cereal Foods World* 42, 171, 1997.

[16] DWI, The Water Supply (Water Quality) Regulations 2000, *No. 3184*, Drinking Water Inspectorate, 2000.

[17] Fellers, B., Block, E., and Conley, J., Bromine replaces chlorine in cooling water treatment, *Power* 132, 15, 1988.

[18] Flury, M., and Papritz, A., Bromide in the natural environment—Occurrence and toxicity, *J. Environ. Qual.* 22, 747, 1993.
[19] Fujii, M., Oikawa, K., and Saito, H., Metabolism of potassium bromate in rats I: *In-vivo* studies, *Chemosphere* 13, 1207, 1984.
[20] Giblin, T., and Frankenberger, W., Perchlorate and nitrate reductase activity in the perchlorate-respiring bacterium perclace, *Micro. Res.* 156, 311, 2001.
[21] Giblin, T., Herman, D., Deshusses, M., and Frankenberger, W. J., Removal of perchlorate in ground water with a flow-through bioreactor, *J. Environ. Qual.* 29, 578, 2000.
[22] Gisch, D., Anion exchange resins remove perchlorate from groundwater, *Water Wastewater Int.* 18, 10, 2003.
[23] Godbold, P., *In-situ* denitrification: A pilot scale field trial on a chalk aquifer, Paper presented at *CIWEM National Conference—Inorganics in Focus*, London, 2002.
[24] Gordon, G., Gauw, R., Emmert, G., Walters, B., and Bubnis, B., Chemical reduction methods for bromate ion removal, *J. Am. Water Works Assoc.* 94, 91, 2002.
[25] Gosselin, R., Hodge, H., Smith, R., and Gleason, M., *Clinical Toxicology of Commercial Products*, Williams & Wilkins, Baltimore, MD, 1976.
[26] Grosvenor, T., Strategies for minimizing ozonation by-products in drinking water, *Water Eng. Manage.* 146, 30, 1999.
[27] Gullick, R., Lechevallier, M., and Barhorst, T., Occurrence of perchlorate in drinking water sources, *J. Am. Water Works Assoc.* 93, 66, 2001.
[28] Hatzinger, P., Greene, M., Frisch, S., Togna, M., Manning, J., and Guarini, W., Biological treatment of perchlorate-contaminated groundwater using fluidized bed reactors, *2nd International Conference on Remediation of Chlorinated and Recalcitrant Compounds*, Monterey, CA, 22–25 May 2000.
[29] Herman, D., and Frankenberger, W., Microbial-mediated reduction of perchlorate in groundwater, *J. Environ. Qual.* 27, 750, 1998.
[30] Hijnen, W., Jong, R., and van der Kooij, D., Bromate removal in a denitrifying bioreactor used in water treatment, *Water Res.* 33, 1049, 1999.
[31] Hijnen, W., Voogt, R., Veenendaal, H., van der Jagt, H., and van der Kooij, D., Bromate reduction by denitrifying bacteria, *App. Environ. Micro.* 61, 239, 1995.
[32] Hiscock, K., Lloyd, J., and Lerner, D., Review of natural and artificial denitrification of groundwater, *Water Res.* 25, 1099, 1991.
[33] Hossain, I., Longoria, R., and Joost, R., DBP formation and removal in ozonation reactions, *World Water Environ. Eng.* 19, 24, 1996.
[34] Howard, K., Denitrification in a major limestone aquifer, *J. Hydrol.* 76, 265, 1985.
[35] Huang, W., and Chen, L., Assessing the effectiveness of ozonation followed by GAC filtration in removing bromate and assimilable organic carbon, *Environ. Technol.* 25, 403, 2004.
[36] Hutchinson, T., Hutchings, M., and Moore, K., A review of the effects of bromate on aquatic organisms and toxicity of bromate to oyster (*Crassostrea gigas*) embryos, *Ecotox. Environ. Safety* 38, 238, 1997.
[37] IARC, Potassium Bromate (Summary of Data Reported and Evaluation), IARC, Lyon, 1999.

[38] Jolles, Z., *Bromine and Its Compounds*, Ernest Benn, London, 1966.
[39] Kim, K., and Logan, B., Microbial reduction of perchlorate in pure and mixed culture packed-bed bioreactors, *Water Res.* 35, 3071, 2001.
[40] Kirisits, M., and Snoeyink, V., Reduction of bromate in a BAC filter, *J. Am. Water Works Assoc.* 91, 74, 1999.
[41] Kirisits, M., Snoeyink, V., Chee-Sanford, J., Daugherty, B., Brown, J., and Raskin, L., Effect of operating conditions on bromate removal efficiency in BAC filters, *J. Am. Water Works Assoc.* 94, 182, 2002.
[42] Kirisits, M., Snoeyink, V., Inan, H., Chee-Sanford, J., Raskin, L., and Brown, J., Water quality factors affecting bromate reduction in biologically active carbon filters, *Water Res.* 35, 891, 2001.
[43] Kirisits, M., Snoeyink, V., and Kruithof, J., The reduction of bromate by granular activated carbon, *Water Res.* 34, 4250, 2000.
[44] Korom, S., Natural denitrification in the saturated zone—A review, *Water Resources Res.* 28, 1657, 1992.
[45] Krasner, S., Yates, R., Amy, G., and Garside, J., Use of ferrous and ferric coagulants to reduce bromate and particulate levels, *American Water Works Association 1996 Annual Conference*, Toronto, 1996.
[46] Kruithof, J., and Meijers, R., Bromate formation by ozonation and advanced oxidation and potential options in drinking water treatment, *Water Supply* 13, 93, 1995.
[47] Kurokawa, Y., Takayama, S., Konishi, Y., Hiasa, Y., Asahini, S., Takahashi, M., Maekawa, A., and Hayashi, Y., Long-term *in vivo* carcinogenicity tests of potassium bromate, sodium hypochlorite, and sodium chlorite conducted in Japan, *Environ. Health Perspect.* 69, 221, 1986.
[48] Kutom, A., Bazilinski, N., and Magana, L., Bromate intoxication: hairdressers anuria, *Am. J. Kidney Disorders* 15, 84, 1990.
[49] Logan, B., A review of chlorate- and perchlorate-respiring microorganisms, *Bioremed. J.* 2, 69, 1998.
[50] Logan, B., and LaPoint, D., Treatment of prechlorate and nitrate-contaminated groundwater in an autotrophic, gas phase, packed-bed bioreactor, *Water Res.* 36, 3647, 2002.
[51] Lopez-Cueto, G., Ostra, M., and Ubide, C., New way of application of the bromate-bromide mixture in kinetic analysis, *Anal. Chim. Acta* 445, 117, 2001.
[52] Losi, M., Amrhein, C., and Frankenberger, W., Bioremediation of chromate contaminated groundwater by reduction and precipitation in surface soils, *J. Environ. Qual.* 23, 1141, 1994.
[53] Losi, M., and Frankenberger, J., Reduction of selenium oxyanions by *Enterobacter cloacae* SLD1a-1: Isolation and growth of the bacterium and its expulsion of selenium particles, *Appl. Environ. Micro.* 63, 3079, 1997.
[54] Mallevialle, J., Impact of new guidelines for disinfection by-products on drinking water treatment, *Water Supply* 13, 177, 1995.
[55] Marhaba, T., Examining bromate ion removal by GAC through RSSCT and pilot-scale columns, *Environ. Eng. Policy* 2, 59, 2000.
[56] Marhaba, T., and Medlar, S., Treatment/removal of bromate and bromide ions from drinking water, *Proceedings AWWA Water Quality Technology Conference*, Miami, FL, 1993.

[57] Mateju, V., Cizinska, S., Krejci, J., and Janoch, T., Biological water denitrification—A review, *Enzyme Micro. Technol.* 14, 170, 1992.
[58] McCann, B., By-product blues, *Water 21*, July 15, 1999.
[59] Meijers, R., and Kruithof, J., Potential treatment options for restriction of bromate formation and bromate removal, *Water Supply* 13, 183, 1995.
[60] Mills, A., Belghazi, A., and Rodman, D., Semiconductor photocatalysis: The removal of bromate from potable water, *3rd International Conference on Water and Waste Treatment*, Harrogate, UK, 1995.
[61] Mills, A., Belghazi, A., and Rodman, D., Bromate removal from drinking water by semiconductor photocatalysis, *Water Res.* 30, 1973, 1996.
[62] Morpeth, F., and Boxer, D., Kinetic-analysis of respiratory nitrate reductase from *Escherichia coli* K12, *Biochemistry* 24, 40, 1985.
[63] Nerenberg, R., and Rittmann, B., Reduction of oxidised water contaminants with a hydrogen-based, hollow-fiber membrane biofilm reactor, *Water Sci. Technol.*, In press, 2004.
[64] Nerenberg, R., and Rittmann, B., Hydrogen-based, hollow fiber membrane biofilm reactor for reduction of perchlorate and other oxidised contaminants. *Water Sci. Technol.* 49, 11–12, 2004.
[65] Noguchi, H., Nakajima, A., Watanabe, T., and Hashimoto, K., Removal of bromate ion from water using TiO_2 and alumina-loaded TiO_2 photocatalysis, *Water Sci. Technol.* 46, 27, 2002.
[66] Noguchi, H., Nakajima, A., Watanabe, T., and Hashimoto, K., Design of a photocatalyst for bromate decomposition: surface modification of TiO_2 by pseudo boehmite, *Environ. Sci. Technol.* 37, 153, 2003.
[67] Payne, W., *Denitrification*, John Wiley, New York, 1981.
[68] Prados-Ramirez, M., Ciba, N., and Bourbigot, M., Available techniques for reducing bromate in drinking water, *Water Supply* 13, 61, 1995.
[69] Price, D., Iddon, S., and Wakefield, B., *Bromine Compounds: Chemistry and Applications*, Elsevier, Amsterdam, 1988.
[70] Puckett, L. and Cowdery, T., Transport and fate of nitrate in a glacial outwash aquifer in relation to ground water age, land use practices, and redox processes, *J. Environ. Qual.* 31, 782, 2002.
[71] Quastel, J., Some reactions of resting bacteria in relation to anaerobic growth, *Biochem. J.* 19, 304, 1925.
[72] Ray, C., and Jain, R., Nitrate pollution of wells: Pollution prevention policy options, *Environ. Eng. Policy* 1, 165, 1999.
[73] Rikken, G., Kroon, A., and van Ginkel, C., Transformation of (per)chlorate into chloride by a newly-isolated bacterium: Reduction and dismutation, *Appl. Micro. Biotechnol.* 45, 420, 1996.
[74] Ritter, W., and Scarborough, R., A review of bioremediation of contaminated soils and groundwater, *Journal Environ. Sci. Health A Environ. Sce. Eng. Toxic Hazard. Subst. Control* A30, 333, 1995.
[75] Rodgers, G., Evaluation of potential substrates to monitor respiratory nitrate reductase activity in soils, *J. Soil Sci.* 31, 387, 1980.
[76] Schmidt, W., Bohme, U., and Brauch, H., Organo bromide compounds and their significance for drinking water treatment, *Water Supply* 13, 101, 1995.

[77] Siddiqui, M., and Amy, G., Removal of bromate during drinking water treatment by UV irradiation, *IOA Regional Conference, Proceedings*, Zurich, Switzerland, 1994.
[78] Siddiqui, M., Amy, G., and Cooper, W., Bromate discharge ion removal by electric-arc and high-energy electron beam processes, *ACS Symp. Ser. Water Disinfect. Natural Organic Matter* 649, 366, 1996a.
[79] Siddiqui, M., Amy, G., Cooper, W., Kurucz, C., Waite, T., and Nickelsen, M., Bromate ion removal by HEEB irradiation, *J. Am. Water Works. Assoc.* 88, 90, 1996b.
[80] Siddiqui, M., Amy, G., and McCollum, L., Bromate destruction by UV irradiation and electric arc discharge, *Ozone Sci. Eng.* 18, 271, 1996c.
[81] Siddiqui, M., Amy, G., Ozekin, K., Zhai, W., and Westerhoff, P., Alternative strategies for removing bromate, *J. Am. Water Works. Assoc.* 86, 81, 1994a.
[82] Siddiqui, M., Amy, G., Zhai, W., Ozekin, K., Westerhoff, P., and Chandrakanth, M., Removal of bromate after ozonation during drinking water treatment, *Water Quality: Proceedings American Water Works Association Annual Conference*, 881, 1994b.
[83] Siddiqui, M., Zhai, W., Amy, G., and Mysore, C., Bromate ion removal by activated carbon, *Water Res.* 30, 1651, 1996d.
[84] Smith, R., and Duff, J., Denitrification in a sand and gravel aquifer, *Appl. Environ. Micro.* 54, 1071, 1988.
[85] Smith, R., Miller, D., Brooks, M., Widdowson, M., and Killingstad, M., *In situ* stimulation of groundwater denitrification with formate to remediate nitrate contamination, *Environ. Sci. Technol.* 35, 196, 2001.
[86] Stephenson, T., Judd, S., Jefferson, B., and Brindle, K., *Membrane Bioreactors for Wastewater Treatment*, IWA Publishing, London, 2000.
[87] Theis, T., Zander, A., and Anderson, M., Assessment of the electrochemical reduction of perchlorate ion, *Project #2578*, AwwaRF, Denver, CO, 2002.
[88] Thompson, K., and Megonnell, N., Activated carbon—A media for bromate reduction, *Pollut. Eng.* 35, 39, 2003.
[89] Thurman, E., *Organic Geochemistry of Natural Waters*, Martinus Nijhoff, Dordrecht, the Netherlands, 1985.
[90] Urbansky, E., Perchlorate chemistry: Implications for analysis and remediation, *Bioremed. J.* 2, 81, 1998.
[91] Urbansky, E., Perchlorate as an environmental contaminant, *Environ. Sci. Pollut. Res.* 9, 187, 2002.
[92] U.S. Environmental Protection Agency, Toxicological review of bromate, *EPA/635/R-01/002*, Environmental Protection Agency, Washington, DC, 2001.
[93] Vogel, J., Talma, A., and Heaton, T., Gaseous nitrogen as evidence for denitrification in groundwater, *J. Hydrol.* 50, 191, 1981.
[94] Waite, T., and Truesdale, V., Iodate reduction by *Isochrysis galbana* is relatively insensitive to de-activation of nitrate reductase activity—Are phytoplankton really responsible for iodate reduction in seawater?, *Mar. Chem.* 81, 137, 2003.
[95] Weinberg, H., Delcomyn, C., and Unnam, V., Bromate in chlorinated drinking waters: Occurrence and implications for future regulation, *Environ. Sci. Technol.* 37, 3104, 2003.

[96] Westerhoff, P., Reduction of nitrate, bromate, and chlorate by zero-valent iron (Fe^0), *J. Environ. Eng.* 129, 10, 2003.

[97] Westerhoff, P., Siddiqui, M., Zhai, W., Ozekin, K., and Amy, G., Bromate reduction: Another benefit of GAC, *American Water Works Association 1994 Engineering Design Conference, Proceedings*, Cincinnati, OH, 1994.

[98] WHO, *Guidelines for Drinking-Water Quality*, vol. 2, World Health Organization, Geneva, 1996.

[99] Wolterink, A., Jonker, A., Kengen, S., and Stams, A., *Pseudomonas chloritidismutans* sp nov., a nondenitrifying, chlorate-reducing bacterium, *Int. J. System. Evol. Micro.* 52, 2183, 2002.

[100] Wolterink, A., Schilz, E., Hagedoorn, P., Hagen, W., Kengen, S., and Stams, A., Characterisation of the chlorate reductase from *Pseudomonas chloritidismutans*, *J. Bacteriol.* 185, 3210, 2003.

[101] Xu, J., Song, Y., Min, B., Steinberg, L., and Logan, B., Microbial degradation of perchlorate: Principles and applications, *Environ. Eng. Sci.* 20, 405, 2003.

[102] Xu, J., Trimble, J., Steinberg, L., and Logan, B., Chlorate and nitrate reduction pathways are separately induced in the perchlorate-respiring bacterium *Dechlorosoma* sp KJ and the chlorate-respiring organism *Pseudomonas* sp PDA, *Water Res.* 38, 673, 2004.

[103] Yang, M., Uesugi, K., and Myoga, H., Study on by-products of ozonation during ammonia removal under the existence of bromide: Factors affecting formation and removal of the by-products, *Ozone Sci. Eng.* 22, 23, 2000.

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Bromate analysis in groundwater and wastewater samples

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Bromate (BrO_3^-) is a disinfection by-product formed during ozonation of potable water supplies containing bromide (Br^-). Bromate has been classed by the World Health Organisation as a 'possible human carcinogen', leading to implementation of 10–25 μg L^{-1} (as BrO_3^-) drinking water limits in legislative areas including the United States and European Union. Techniques have been developed for bromate analysis at and below regulatory limits, with Ion Chromatography (IC) coupled with conductivity detection (IC-CD), post-column reaction and ultra-violet (UV) detection (IC-PCR), or inductively coupled plasma-mass spectrometry detection (IC-ICPMS) in widespread use. The recent discovery of bromate groundwater contamination in a UK aquifer has led to a requirement for analysis of bromate in a groundwater matrix, for environmental monitoring and development of remediation strategies. The possibility of bromate-contaminated water discharge into sewage treatment processes, whether accidental or as a pump-and-treat strategy, also required bromate analysis of wastewater sources. This paper summarises techniques currently available for trace bromate analysis in potable water systems and details studies to identify a methodology for routine analysis of groundwater and wastewater samples. Strategies compared were high performance liquid chromatography (HPLC) with direct UV or PCR/UV detection, IC-CD, IC-PCR, and a simple spectrophotometric technique. IC-CD was the most cost-effective solution for simultaneous analysis of bromate and bromide within groundwater samples, having a 5 μg L^{-1} detection limit of both anions with limited interference from closely-eluting species. Wastewater samples were successfully analysed for bromate only using HPLC with PCR/UV detection, with detection limits below 20 μg L^{-1} (as BrO_3^-) and low interference. HPLC with direct UV detection was unsuitable for bromate analysis within the concentration range 50–5000 μg L^{-1} which was required for this project, but column choice was shown to be a major factor in determining limits of detection. Spectrophotometry could not reproducibly determine bromate concentration, although the technique showed promise as a quick field method for high-level groundwater bromate analysis.

Introduction

Bromate (BrO_3^-) is an oxyanion of bromine which can be found in potable water following treatment processes including hypochlorination, where it is a contaminant of hypochlorite feedstocks, and also ozonation of water containing bromide where it is formed as a disinfection by-product (DBP).[1] Bromide (Br^-), unregulated in the United Kingdom (UK), is a component of natural water sources with fresh water concentrations within the range 15–200 μg L^{-1} as Br^-.[2] Following ozonation processes, bromate (given as concentration of BrO_3^- throughout the text) may be found in treated water within the range 0.4-60 μg L^{-1}.[3,4] Bromate contamination of potable supplies has been an issue since the mid-1980's, when animal studies suggested a link between low-level long-term exposure and tumour formation.[5] This prompted the World Health Organisation (WHO) to classify bromate as group 2B or a 'possible human' carcinogen.[6] Concentrations of bromate suggested to cause an excess lifetime cancer risk of 10^{-5} were low, calculated as a drinking water concentration of 3 μg L^{-1}, but the recommended maximum drinking water limit was initially set at 25 μg L^{-1}. This was mainly due to limitations in bromate analysis, which was effectively restricted to use of relatively crude titrimetric methods. Over the past two decades there has been extensive literature published on bromate analysis.[7–9] A range of techniques have been developed for detection down to ng L^{-1} concentrations, with the aim directed towards detecting bromate as a DBP from ozonation of potable water.

Ion chromatography (IC) techniques

Most techniques currently available for bromate analysis use IC as the underlying separation mechanism. There are three main IC technologies used for bromate detection. 'Traditional' IC is coupled with conductivity detection (IC-CD) and provides the basis of formally-approved bromate analytical methodologies in areas such as the United States (US) and UK.[10–12] Two other variants with lower method reporting levels (MRLs) have subsequently been developed for bromate analysis—use of a post-column reaction (PCR) whereby a bromate-reactive compound is injected post-column into the eluent stream and the spectrally active product analysed spectrophotometrically; and incorporation of an on-line inductively coupled plasma-mass spectrometer (ICP-MS) into the eluent stream.

IC-CD. The use of IC-CD for bromate analysis was officially recognised in 1989 with publication of US Environmental Protection Agency (US EPA) method 300.0, later amended in 1993 as Revision 2.1.[13] Subsequent refinements to the technique have generally been related to sample volume, column capacity and sample pre-treatment. Pre-treatment can involve sample pre-concentration[8] and/or removal of interfering anions, of which the most important is chloride due to close elution with bromate. One pre-treatment method is by use of commercially-available silver (for halide removal),

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barium (for sulfate removal) and hydrogen (for metal and carbonate removal) cartridges.[11,14,15] High capacity anion exchange columns are now utilised along with large sample volumes, generally 200–1000 μL, which can more efficiently resolve trace bromate signals whilst reducing or eliminating the need for pre-treatment and pre-concentration. US EPA method 300.1 is based on such a system, using the high-capacity Dionex AS9-HC column to give a method detection limit (MDL) of 1.4 μg L^{-1} and MRL of 5 μg L^{-1}.[13]

IC-PCR. To overcome the limitations of IC-CD a post-column step can be added. This extra step is specific to bromate and results in analysis being less prone to interference, thus reducing the requirement for sample pre-treatment. **Hydrobromic acid** reacts with bromate to form tribromide (Br_3^-), which can be detected at 268 nm within a range of 0.5–10 000 μg L^{-1} [16] or to a lower limit of 0.05 μg L^{-1} [17] with no sample pre-treatment. **Chlorpromazine** is a phenothiazine derivative, oxidised by bromate under acidic conditions, forming a spectrally-active species. Detection within a 0.5–100 μg L^{-1} range was noted without sample pre-treatment.[18] **Sulfonaphtholazoresorcinol** reacts with bromate to give a decrease in fluorescence at 585 nm, with a calibration range of 0.28–15 μg L^{-1} bromate suggested by Gahr *et al.*[19] **Fuschin** can react with bromate in acidic medium, forming a strongly-absorbing product at 530 nm. A range of 0.1–100 μg L^{-1} was noted by Achilli and Romele[20,21] using a standard carbonate/bicarbonate eluent, with Valsecchi *et al.*[22] trialling a tetraborate eluent and sample size up to 1.5 ml with no pre-treatment to improve resolution. **Iodide** in acidic solution (KI-$(HN_4)_6Mo_7O_{24}$) reacts with bromate to form I_3^-, which can be detected at 352 nm. Salhi and von Gunten[23] used iodide with a carbonate eluent and 500 μL sample loop, giving a 0.1 μg L^{-1} detection limit with little interference from matrix anions. US EPA method 326.0 is based on the iodide reaction, and utilises a carbonate eluent and 225 μL sample volume to give bromate detection limits of 0.17 μg L.[24] ***σ*-Dianisidine** reacts with bromate to form a product that can be detected at 450 nm. A methodology incorporating this technique has also been published by the US EPA, and designated method 317.0. With a carbonate eluent and 225 μL sample volume, method 317.0 can provide bromate detection limits of 0.12 μg L^{-1},[13] although pre-treatment may be necessary for some water types to remove chlorite.[25]

IC-ICPMS. The IC-ICPMS technique has been developed for detection of very low (sub-ug L^{-1}) bromate concentrations without pre-treatment. Nowak and Seubert utilised a high-capacity column with large sample volume (>500 μL) and an ammonium nitrate eluent to give a detection limit of 50–65 ng L^{-1}.[26,27] An ammonium nitrate eluent was used by Creed and Brockhoff[28] and Schminke and Seubert,[7] with US EPA method 321.8 also using this eluent along with large sample volumes (500–580 μL) and no pre-treatment to give an MDL of 0.3 μg L^{-1}.[13] An ammonium carbonate eluent was used by Divjak *et al.*[29] with a small sample size (50 μL), giving low detection limits (0.67 μg L^{-1}) and short analysis times (4 min). Carbonate/bicarbonate eluents were not used initially with IC-ICPMS due to column clogging by sodium and potassium salts[30] but, with suppression, these eluents can be utilised to give detection limits on 'real' water samples of 1 ng L^{-1} using 500 μL[30] or 100 μL[31] samples with no pre-treatment.

Advanced IC-based techniques. Other related technologies are also being developed which claim advantages in detection ability or freedom from interference. Electrospray ion chromatography-tandem mass spectrometry (IC-MS/MS) uses IC with a methanol/ammonium sulfate eluent to give a detection range of 0.05–10 μg L^{-1} bromate,[32] although extensive sample preparation was required. Ion chromatography with atmospheric pressure ionisation mass spectrometry (IC-APIMS)[33] is a development of IC-ICPMS, and utilises a sodium-based eluent for suppressed IC, or an ammonium citrate eluent in non-suppressed mode. Sample pre-treatment was necessary for 'real' water samples, with detection limits of 0.5 μg L^{-1} and 40 μg L^{-1}, respectively.[33]

Other techniques

Non IC-based methodologies have also been investigated and developed. These mainly fall into two categories: techniques using mass spectrometry (MS) detection; and relatively cheap and simple spectrophotometric methodologies. Methods based on electrochemical techniques have also been investigated recently for bromate analysis.

Mass spectrometry-based techniques. Gas chromatography-mass spectrometry (GC-MS) is an established analytical methodology, and has been utilised for bottled water bromate analysis. Nyman *et al.*[34] converted bromate into a chlorobromostyrene derivative, which was then identified and quantified by GC-MS. Concentrations of 1 μg L^{-1} were detected in bottled water, although potential interferences were noted which may preclude use on a more demanding matrix. Gas chromatography-mass spectrometry with negative chemical ionisation (GC-NCIMS) reduces bromate to bromine, followed by reaction with organic material to form volatile, detectable species. The technique is sensitive and rapid, but exhibited strong interference in chlorinated waters.[35] Negative thermal ionisation-isotope dilution mass spectrometry (NTI-IDMS) was found by Diemer and Heumann[36] to be a very accurate method, but time-consuming sample preparation may preclude its use for routine analytical purposes. Electrospray ionisation-high field asymmetric waveform ion mobility spectrometry-mass spectrometry (ESI-FAIMS-MS) is capable of analysing bromate at ng L^{-1} concentrations with minimal interference,[37] and has been shown to detect the related contaminant perchlorate in wastewater and river water samples at sub-μg L^{-1} concentrations.[38] However, no further work has been published on bromate analysis using this technique. ICPMS coupled with flow injection (FI-ICPMS) has also shown some promise, with low detection limits (0.13 μg L^{-1}) and short analysis times (10 minutes per sample) in 'real' samples.[39]

Spectrophotometry techniques. These methodologies utilise similar reactions to IC-PCR, but with direct spectrophotometric detection. Phenothiazones produce a coloured species following reaction with bromate under acid conditions. Chlorpromazine is the most widely trialled phenothiazone. Gordon and co-workers[40,41] concluded chlorpromazine was accurate over a 1–40 μg L^{-1} range, although there was interference from some metal ions. Triflouperazine, another phenothiazone, can be detected at 504 nm, with a detection limit of 0.67 μg L^{-1} and range of 1–700 μg L^{-1}.[42] However, it has been noted spectrophotometric methods using phenothiazones are suspectible to interference by humic substances found in natural water samples.[43] Reduced fuschin can be reacted with bromine, produced from bromate by reaction with metabisulfate. The red product is detected at 530 nm, within a range of 1–40 μg L^{-1}. Pre-treatment is required to remove heavy metals, but the method is then straightforward.[44] Methylene blue can also be reacted with bromate under acidic conditions, with analysis at 745 nm. The method produced a detection range of 4–50 μg L^{-1} and was largely free of interference, although hypochlorite had to be removed by hydroxylamine hydrochloride addition. Although not sensitive enough to analyse low bromate levels

(<10 μg L^{-1}), the authors concluded this technique did show promise as a simple field method.[45]

Electrochemical techniques. Electrochemical techniques utilizing potentiometry have been applied for the detection of bromide, such as the use of ion selective electrodes (ISEs).[46–48] These electrodes are capable of selectively measuring the concentration of a particular ionic species. The performance of an ISE is based on the selective passage of charged species from one phase to another leading to the creation of a potential difference. The use of these types of electrodes involves dipping the electrodes in a test solution and reading the signal from the voltmeter. ISEs are reasonably sensitive, with the analytical capability of commercially-available electrodes for bromide being within the range 40 μg L^{-1}–1 g L^{-1}.[49] ISEs perform better in clean solutions without interferences. The main disadvantages are that the limit of detection can be high and selectivity poor in some environmental samples.

Very limited reports have been found in the literature for bromate detection using electrochemical methods. The use of a glassy carbon electrode modified with a sol–gel thin containing heteropolyanion was found to exhibit high electrocatalytic response for bromate reduction.[50] A linear relationship was found between the catalytic current taken at −0.4 volts and bromate concentration in 0.5 M H_2SO_4. However, this electrode failed to actively catalyse bromate at pH 4.6 since the reduction process needs proton participation.[51] A composite

Table 1 Operating conditions of techniques studied

		High performance liquid chromatography			Ion chromatography	
	Spectrophotometry	Direct UV detection	Direct UV detection	Post-column reaction	IC-CD	IC-PCR
Method number	1	2	3	4	5	6
Equipment	Jenway 6505 spectrophotometer	Shimadzu vp-series system	Shimadzu vp-series system	Shimadzu vp-series system with Metrohm post-column reactor unit	Dionex ICS-2500 system	Dionex DX-600 system
Column	N/a	Hamilton PRP-X100 (4.1 × 150 mm) + guard column	Hamilton PRP-X110S (4.1 × 150 mm) + guard column	Metrohm MetroSupp-5	Dionex AS9-HC 4 mm id (Dionex AG9-HC 4 mm id guard column)	Dionex AS9-HC 2 mm id (Dionex AG9-HC 2 mm id guard column)
Based on methodology	Ref. 55	Application note 318 (Developed by Hamilton Company, Reno, NV, USA)	Application note 356 (Developed by Hamilton Company, Reno, NV, USA)	Application work AW CH6-0777-032003 (Developed by Metrohm UK, Buckingham, England)	Application note 81 (Developed by Dionex Corp., Sunnyvale, CA, USA)	Application note 149 (Developed by Dionex Corp., Sunnyvale, CA, USA)
Eluent	N/a	1.7 mM sodium bicarbonate + 1.9 mM sodium carbonate + 0.1 mM sodium thiocyanate	1.7 mM sodium bicarbonate + 1.9 mM sodium carbonate +0.1 mM sodium thiocyanate	100 mM sulfuric acid + 45 μM ammonium molybdate tetrahydrate	9 mM sodium carbonate	6.75 mM sodium carbonate
Eluent flow rate	N/a	2 mL min^{-1}	2 mL min^{-1}	0.7 mL min^{-1}	1 mL min^{-1}	0.38 mL min^{-1}
Injection (sample) volume	41.2 ml	50 μL (1.5 ml vials)	50 μL (1.5 ml vials)	200 μL (1.5 ml vials)	250 μL (5 ml filtercap vials)	200 μL
Post-column reagent	N/a	N/a	N/a	0.26 M potassium iodide	N/a	0.26 M potassium iodide (acidified) at 0.4 mL min^{-1}
Other reagents	250 μL methylene blue (200 mg L^{-1}) + 8.6 ml hydrochloric acid (37%)	N/a	N/a	N/a	N/a	N/a
Method notes (method 1)/ alterations to published methodology (methods 2–6)	Mix reagents and 40 ml sample in 50 ml volumetric flask, complete to 50 ml with sample; wait 10 min and measure using 4 cm cuvettes	Absorbance at 220 nm instead of conductivity detection	Absorbance at 220 nm instead of conductivity detection	Metrohm MetroSupp-5 column instead of Phenomenex Star Ion A300 HC		Autosuppression external water mode at 38 mA; AMMS III suppressor; PCR heater temperature 40 °C
Anions studied	Bromate	Bromate; bromide	Bromate; bromide	Bromate	Bromate; bromide	Bromate
Detection	Absorbance at 745 nm	Absorbance at 220 nm (Shimadzu SPD-10A vp)	Absorbance at 220 nm (Shimadzu SPD-10A vp)	Absorbance at 352 nm (Shimadzu SPD-10A vp)	Conductivity (Dionex ED50)	Absorbance at 352 nm (Dionex AD25)

film containing heteropolyanion fabricated on a modified gold electrode was also investigated.[52] This electrode showed good catalytic activity for the reduction of bromate in acidic solutions. More recently a tungsten oxide film was used as an amperometric sensor for the analytical determination of bromate, chlorite and nitrite ions. Detection limits evaluated using cyclic voltammetry were high, being approximately 8, 27 and 32 g L^{-1}, respectively.[53] Other amperometric sensors based on a titania sol–gel matrix have also been investigated for the determination of bromate, iodate and hydrogen peroxide. These sensors gave a linear range from 1.6–350 mg L^{-1} and a detection limit of 0.4 mg L^{-1} for bromate in acidic aqueous solutions.[54] However, most of the sensors reported for bromate analysis are still in the development stage.

The current project is based around a groundwater bromate contamination incident in the UK, in which a 'significant' quantity of bromate contamination was released into a chalk aquifer over an undefined but possibly extended period of time. Abstraction for potable supplies in the area has been affected, with some abstracted water significantly exceeding the recently-introduced UK regulatory limit of 10 μg L^{-1} and consequently not permitted for use in supply. Studies into potential remediation techniques, including a biological reduction methodology are currently ongoing. Fate and environmental impact of bromate-contaminated water discharge into sewage treatment processes are also being investigated, both as a potential pump-and-treat disposal methodology and also for the possibility of accidental discharge to sewer. Cost-effective but accurate analysis of relevant anions within both a groundwater and wastewater matrix was essential for the project, but only sparse literature was available for bromate analysis specifically on untreated groundwater or surface water sources. No published literature dealing with bromate analysis within wastewater or other 'dirty' environmental matrices was available. Detection of anions, notably bromate at concentrations in the range 10–100 000 μg L^{-1}, but also including bromide, nitrate, nitrite and sulfate, was therefore defined as a priority for investigation. A comparative study was undertaken to investigate a range of the methodologies available for bromate and bromide analysis, and compare their efficacy in providing accurate results from real water matrices. This is, to our knowledge, the first paper to examine and compare analytical techniques for this emerging environmental contaminant exclusively within untreated water and wastewater samples.

Experimental

Six techniques were examined for bromate analysis in groundwater and wastewater samples. Equipment, methodologies and operating conditions investigated are given in Table 1. Chromatography methods (methods 2–6) can all be carried out on standard IC or HPLC (high performance liquid chromatography) systems with only minor modifications. Where possible, manufacturers' or suppliers' recommended conditions were used, with any deviations given in Table 1. In addition, a spectrophotomentric technique using methylene blue (method 1) was investigated as a simple laboratory or field based method. Two clean water matrices were investigated; standard solutions containing anions of interest (AnalaR grade potassium bromate or a proprietory IC anion standard solution (Fisher Scientific, Loughborough, UK) in Milli-Q (18.2 MΩ) water); and a real bromate contaminated groundwater source collected from two areas within the contaminated aquifer. Properties of the two groundwater samples used (GW-A and GW-B) are given in Table 2. Wastewater samples were collected from a pilot-scale Membrane BioReactor (MBR), using the sludge biomass as a matrix for spiking with AnalaR-grade potassium bromate (given as BrO_3^-) and potassium bromide (given as Br^-) solutions. Wastewater samples were filtered using a 0.2 μm syringe-tip filter (Fisher Scientific, Loughborough, UK) prior to analysis. No further pre-preparation, concentration or preservation was carried out on any groundwater or wastewater samples, which were stored in a cold room at 6 °C prior to analysis. No dilutions were made with the exceptions of the groundwater samples for methods 1 and 6, in both cases to bring results within the calibration range. Standard solutions and eluent were made up shortly before analysis from stock solutions, and eluent was degassed by sonication immediately prior to use.

Anion concentrations trialled with each system were based on the recommended ranges of the technique, with a lower bromate detection limit ascertained for each method. The

Table 2 Selected properties of groundwater samples[a]

	Groundwater A (GW-A)	Groundwater B (GW-B)
pH	6.99	7.04
Total organic carbon/mg L^{-1}	1.9	1.5
Total dissolved solids/mg L^{-1}	443	406
Bromate as BrO_3/μg L^{-1}	1380.5	225.7
Bromide as Br/μg L^{-1}	3973	644
Total oxidised nitrogen as NO_3/mg L^{-1}	41	30
Nitrite as NO_2/mg L^{-1}	0.01	0.011
Sulfate as SO_4/mg L^{-1}	36	24
Chloride as Cl/mg L^{-1}	32	26
Total phosphorous as P/μg L^{-1}	62	51

[a] Results of analysis on groundwater samples analysed 22nd January 2003. Bromate was analysed using IC-PCR (method 6), with other anions by IC-CD (method 5).

Table 3 Bromate concentration range studied and bromate/bromide detection in standard and groundwater samples

		Spectrophotometry	High performance liquid chromatography			Ion chromatography	
		Method 1	Method 2	Method 3	Method 4	Method 5	Method 6
Bromate range studied/μg L^{-1}		10–50	1000–100 000	1–20 000	1–100 000	1–2000	0.5–50
Bromate detection limit/μg L^{-1}		10	5000	50	10	5	0.5
Simultaneous bromide detection?		No	Yes	Yes	No	Yes	No[a]
Sample run time/min		N/a	12	15	15	25	25
Bromate elution time/min		N/a	5.7	3.3	10.6	6.5	5.7
Bromide elution time/min		N/a	7.7	3.8	N/a	12.8	N/a
R-squared calibration values	Bromate	0.6809	0.9985	0.9985;[b] 0.9984[c]	0.9995	0.9993	0.9996
	Bromide	N/a	0.9933	0.9923;[b] 0.9752[c]	N/a	0.9963	N/a

[a] Simultaneous detection possible with addition of conductivity detector. [b] Higher calibration range. [c] Lower calibration range; n/a—not applicable.

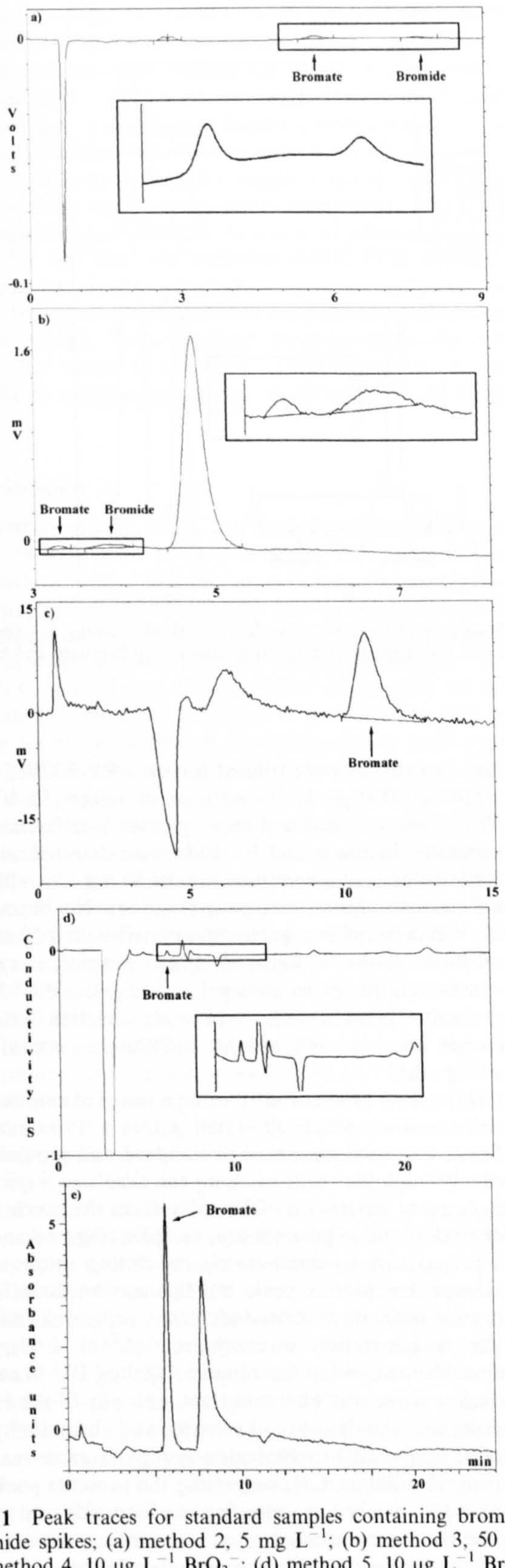

Fig. 1 Peak traces for standard samples containing bromate and bromide spikes; (a) method 2, 5 mg L^{-1}; (b) method 3, 50 μg L^{-1}; (c) method 4, 10 μg L^{-1} BrO_3^-; (d) method 5, 10 μg L^{-1} BrO_3^-; (e) method 6, 10 μg L^{-1} BrO_3^-. Enlarged section of trace containing bromate and bromide peaks given in boxed inserts where necessary.

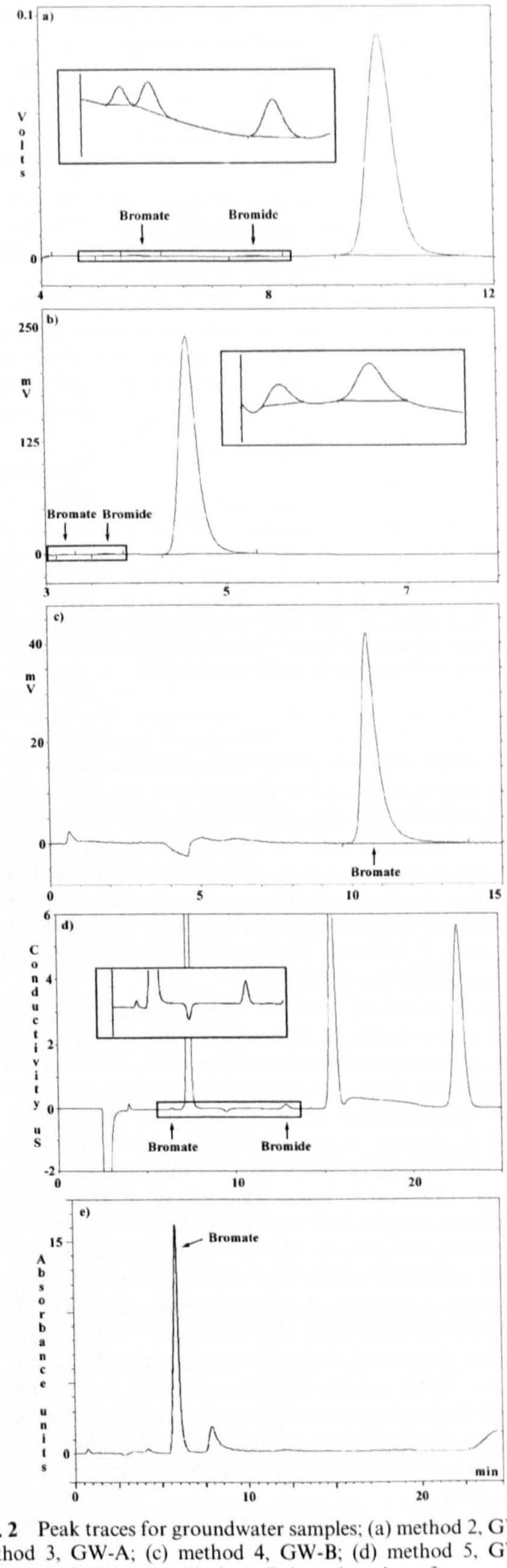

Fig. 2 Peak traces for groundwater samples; (a) method 2, GW-A; (b) method 3, GW-A; (c) method 4, GW-B; (d) method 5, GW-B; (e) method 6, GW-B (10× dilution). Enlarged section of trace containing bromate and bromide peaks given in boxed inserts where necessary.

bromate detection limit was assumed for the purposes of this trial to be the lowest concentration trialled where a discernable peak, or change in absorbance for spectrophotometry, was reproducibly obtained. The range of bromate concentrations studied in each case is given in Table 3. In each case a calibration line was obtained for the bromate standard concentrations studied, with an indication of linearity given by the *R*-squared value. Bromide detection was also studied, with a calibration to give *R*-squared values carried out where possible (methods 2, 3 and 5 only), although bromide detection limits were not ascertained during the trial.

Results and discussion

All the techniques trialled were able to detect bromate within standard solutions and an undiluted groundwater matrix in the

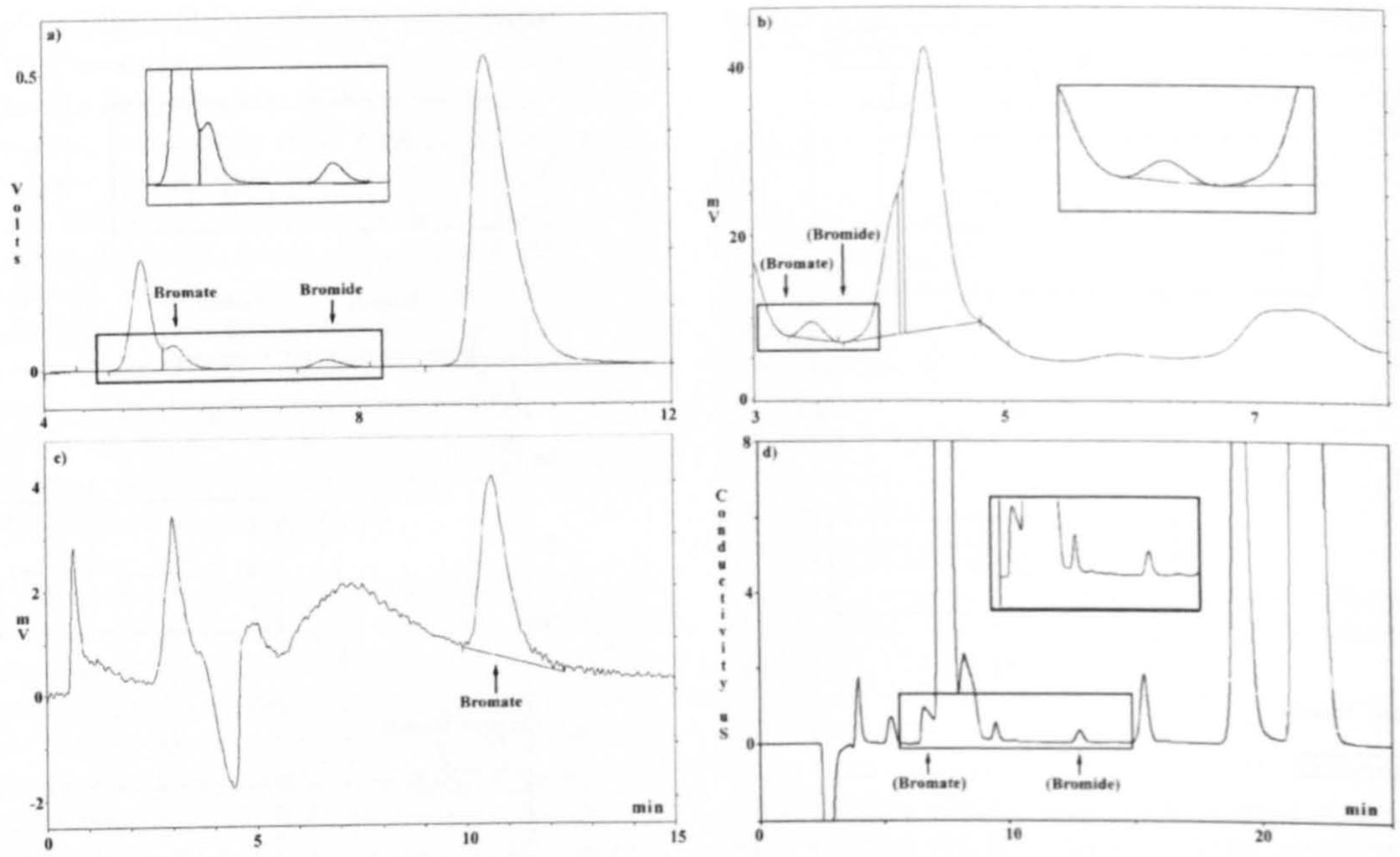

Fig. 3 Peak traces for wastewater samples containing bromate and bromide spikes; (a) method 2, 50 mg L^{-1}; (b) method 3, 20 mg L^{-1} (no peaks visible); (c) method 4, 20 μg L^{-1} BrO_3^-; (d) method 5, 20 μg L^{-1} (peak interference). Enlarged section of trace containing bromate and bromide peaks given in boxed inserts where necessary.

mg L^{-1} range, with only HPLC using the Hamilton PRP-X100 column unable to detect bromate at lower (μg L^{-1}) concentrations. Results obtained for each technique are given in Table 3. Example chromatograms for standard samples are given in Fig. 1 and chromatograms for groundwater samples in Fig. 2. Wastewater samples provided a much more demanding matrix, with extensive interference observed using spectrophotometry and direct UV detection. Example chromatograms for wastewater samples are given in Fig. 3.

Spectrophotometry (method 1) was trialled using bromate concentrations in the 10–50 μg L^{-1} range, but was prone to sampling error upon duplication, even using standard samples. An *R*-squared value of only 0.6809 was obtained for this method. Groundwater samples were also investigated and, for GW-B (diluted 10 times to come within the calibration range), an average of 201.7 μg L^{-1} was obtained (standard deviation = 58.3 μg L^{-1}, $n = 3$). Analysis of uncontaminated groundwater samples indicated the technique was not affected by interfering compounds. However, wastewater samples were prone to extensive interference and therefore no calibration was possible. A previous study on bromate analysis in groundwater using the methylene blue technique[55] obtained a more acceptable *R*-squared calibration value of 0.8978, but suggested that use of a more accurate method in tandem was also necessary for quality control purposes. The study concluded a linear relationship with absorbance within the range 10–150 μg L^{-1} would be obtainable, but that higher concentrations would require dilution.

HPLC techniques using direct UV detection (methods 2 and 3) were able to simultaneously detect both bromate and bromide, and peaks were well defined in standard and groundwater samples with elution times under 10 minutes. However, the limitations of the PRP-X100 column were apparent, with a bromate detection limit of only 5 mg L^{-1} (Figs. 1a and 2a). The PRP-X110S column was able to detect bromate up to two orders of magnitude lower, with a detection limit of 50 μg L^{-1} (Figs. 1b and 2b). Other peaks were apparent in groundwater samples, most notably chloride, but did not interfere with either the bromate or bromide peaks. *R*-squared values showed good linear correlation (>0.97 in all cases) within the calibration ranges used, for both bromate and bromide (Table 3). PRP-X100 calibration was carried out in the range 5–100 mg L^{-1}, and two ranges were trialled for the PRP-X110S, with a higher (500–20 000 μg L^{-1}) and a lower range (50–1000 μg L^{-1}). Wastewater samples showed greater interference with both methods. Bromate and bromide were detected with the PRP-X100 column at a concentration of 50 mg L^{-1}, although bromate peak resolution was poor (Fig. 3a). No bromate or bromide was detected in a wastewater matrix with a 20 mg L^{-1} spike of both anions. Analysis of spiked wastewater samples with concentrations up to 20 mg L^{-1} using the PRP-X110S column resulted in no bromate or bromide detection. Extensive interference was observed around the elution time of both anions (Fig. 3b).

IC-CD (method 5) was trialled using a range of anions in the standard solutions, which all eluted within a 25 minute run time. Peaks were well separated in standard and groundwater samples, although the relatively long run time was required to ensure adequate separation of bromate from the much larger chloride peak found in groundwater samples (Figs. 1d and 2d). Sample preparation to eliminate closely-eluting anions would have reduced the chloride peak, but this was not found to be necessary for bromate and bromide peaks to be well resolved. Good linear correlation was achieved ($R^2 > 0.99$), with bromate calibrated within the range 5–2000 μg L^{-1} (Table 3). Less baseline noise was observed than with any of the HPLC methodologies. Analysis of bromate and bromide-spiked wastewater samples led to anomalously high bromate readings in the range 2–1000 μg L^{-1}, suggesting the bromate peak was being partially masked by interference. Bromide concentrations were also anomalously high at low concentrations (20 μg L^{-1}), although this effect was not noted at higher concentrations ($\geq$ 500 μg L^{-1}). The chromatogram obtained with a 20 μg L^{-1} bromate and bromide spike is given in Fig. 3d. It is unlikely that, without sample pre-treatment, wastewater samples could be successfully analysed for trace bromate concentrations using IC-CD.

Post-column reaction methods were trialled using both HPLC (method 4) and IC (method 6) systems. Due to the specificity of the applications only bromate detection was possible. With the HPLC methodology, well-resolved peaks were obtained using standard and groundwater samples, with a detection limit of 10 μg L^{-1}. Interference was minimal even in samples with high ionic loadings, although some baseline noise

was evident (Figs. 1c and 2c). Good bromate peak resolution was also obtained with wastewater samples, specificity of the post-column reaction eliminating much of the interference obtained with other methods (Fig. 3c). IC-PCR trials on standard and groundwater samples were carried out by an external laboratory (Veolia Water Laboratories, Staines, Middlesex, England). Although a range of anions can be analysed on the system, which incorporates conductivity and UV detection capabilities, only PCR detection of bromate was investigated for this trial. As with the HPLC-PCR system, good detection capabilities of standard and groundwater samples (Figs. 1e and 2e) with minimal interference and a detection limit of 0.5 μg L^{-1} was obtained. An *R*-squared value of 0.9996 within the range 10–50 μg L^{-1} was achieved. It was not possible to analyse wastewater samples by IC-PCR for this trial.

Conclusions

Spectrophotometry using the methylene blue technique is a cheap method able to give a rough indication of bromate concentrations in groundwater matrices, for example during an initial field study, but inherent inconsistencies preclude it for use where accurate and reproducible data is required. This method would not be of use for wastewater studies.

HPLC with direct UV detection is capable of accurate bromate and bromide detection in groundwater, but column choice is critical. Although producing similar peak traces, the PRP-X100 column was unable to detect anions at concentrations required for the study, whereas the PRP-X110S column was successful. Neither system was able to satisfactorily analyse wastewater samples. However, where an HPLC system is already installed, bromate/bromide analysis to drinking water regulatory limits is not required and samples do not have high concentrations of interfering compounds, direct UV detection using a column such as the PRP-X110S would be a cost-effective solution for groundwater analysis.

IC-CD using the Dionex AS-9HC column produces comparable results to the HPLC systems, but use of a high-capacity column and large injection loop (250 μL) leads to increased resolution at lower concentrations, and allows analysis of samples at the 10 μg L^{-1} bromate regulatory limit. The method, similar to US EPA standard method 300.1, is the technique of choice for groundwater analysis if simultaneous detection of a range of anions is required using a standard conductivity-based IC as, apart from the analytical and guard columns, no additional equipment is required.

HPLC and IC using PCR are both specific to bromate and, as such are less prone to interference from other compounds. If bromate is the only compound to be analysed, then these methods will both provide detection in groundwater to concentrations below regulatory limits. PCR-based techniques are also the method of choice for samples with high ionic loadings, such as wastewater samples. HPLC-PCR was able to detect bromate at 20 μg L^{-1} and, although not tested in this trial it is likely IC-PCR would have similar capabilities. HPLC-PCR is the cheaper option, only requiring a post-column reactor and pump in addition to a standard HPLC system. However, the eluent used in this application precludes simultaneous detection of other anions, which can be achieved using an IC system with post-column capability and the additional equipment required to carry out conductivity as well as absorbance detection.

Detection of bromate in groundwater and wastewater samples is possible by a range of methodologies, and cost, application, sample composition and accuracy required are all factors in method selection. Bromate is not currently regarded as a significant environmental pollutant, but implementation of bromate drinking water standards is leading to commencement of routine monitoring in countries such as the UK. This increased vigilance may lead to discovery of further areas of bromate contamination, with the ability to monitor in a variety of water matrices becoming essential to subsequent containment and remediation strategies.

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References

1 N. Ashbolt, *Toxicology*, 2004, **198**, 255.
2 M. Flury and A. Papritz, *J. Environ. Qual.*, 1993, **22**, 747.
3 M. Siddiqui, G. Amy, K. Ozekin, W. Zhai and P. Westerhoff, *J. Am. Water Works Assoc.*, 1994, **86**, 81.
4 M. Kirisits and V. Snoeyink, *J. Am. Water Works Assoc.*, 1999, **91**, 74.
5 Y. Kurokawa, S. Takayama, Y. Konishi, Y. Hiasa, S. Asahini, M. Takahashi, A. Maekawa and Y. Hayashi, *Environ. Health Perspect.*, 1986, **69**, 221.
6 World Health Organisation, *Guidelines for Drinking Water Quality*, World Health Organisation, Geneva, 1993, vol. 2.
7 G. Schminke and A. Seubert, *Fresenius' J. Anal. Chem.*, 2000, **366**, 387.
8 K. C. Thompson, J. Guinamant, V. Ingrand, A. Elwaer, C. McLeod, F. Schmitz, G. De Swaef and P. Quevauviller, *J. Environ. Monit.*, 2000, **2**, 416.
9 H. Koscielna, *Chem. Anal. (Warsaw)*, 2004, **49**, 445.
10 US Environmental Protection Agency, *Determination of Inorganic Anions in Drinking Water by Ion Chromatography*, method 300.1, revision 1.0, US EPA, Cincinnati, OH, 1997.
11 Environment Agency, *The Determination of Bromate, Chlorate, and Chlorite in Waters by Ion Chromatography*, Environment Agency, Bristol, UK, 1997.
12 British Standards Institute, *Water Quality-Determination of Dissolved Bromate-Method by Liquid Chromatography of Ions*, ISO 15601, British Standards Institute, London, 2001.
13 D. Hautman, D. Munch, C. Frebis, H. Wagner and B. Pepich, *J. Chromatogr. A*, 2001, **920**, 221.
14 F. Sacher, A. Matschi and H. Brauch, *Acta Hydrochim. Hydrobiol.*, 1995, **23**, 26.
15 H. van der Jagt, T. Noij and P. Ooms, *Water Supply*, 1995, **13**, 21.
16 Y. Inoue, T. Sakai, H. Kumagai and Y. Hanaoka, *Anal. Chim. Acta*, 1997, **346**, 299.
17 C. Delcomyn, H. Weinberg and P. Singer, *J. Chromatogr. A*, 2001, **920**, 213.
18 B. Walters, G. Gordon and B. Bubnis, *Anal. Chem.*, 1997, **69**, 4275.
19 A. Gahr, N. Huber and R. Niessner, *Microchim. Acta*, 1998, **129**, 281.
20 M. Achilli and L. Romele, *J. Chromatogr. A*, 1999, **847**, 271.
21 M. Achilli and L. Romele, *Am. Lab.*, 2000, **32**, 60.
22 S. Valsecchi, A. Isernia, S. Polesello and S. Cavalli, *J. Chromatogr. A*, 1999, **864**, 263.
23 E. Salhi and U. von Gunten, *Water Res.*, 1999, **33**, 3239.
24 US Environmental Protection Agency, *Determination of Inorganic Oxyhalide Disinfection By-products in Drinking Water using Ion Chromatography Incorporating the Addition of a Suppressor Acidified Postcolumn Reagent for Trace Bromate Analysis*, method 326.1, revision 1.0, US EPA, Cincinnatti, OH, 2002.
25 H. Wagner, B. Pepich, D. Hautman and D. Munch, *J. Chromatogr. A*, 2000, **884**, 201.
26 A. Seubert and M. Nowak, *Fresenius' J. Anal. Chem.*, 1998, **360**, 777.
27 M. Nowak and A. Seubert, *Anal. Chim. Acta*, 1998, **359**, 193.
28 J. Creed and C. Brockhoff, *Anal. Chem.*, 1999, **71**, 722.
29 B. Divjak, M. Novic and W. Goessler, *J. Chromatogr. A*, 1999, **862**, 39.
30 R. Fernandez, J. Alonso and A. Sanz-Medel, *J. Anal. At. Spectrom.*, 2001, **16**, 1035.
31 A. Dudoit and S. Pergantis, *J. Anal. At. Spectrom.*, 2001, **16**, 575.

32 L. Charles and D. Pepin, *J. Chromatogr. A*, 1998, **804**, 105.
33 W. Buchberger and W. Ahrer, *J. Chromatogr. A*, 1999, **850**, 99.
34 P. Nyman, B. Canas, F. Joe and G. Diachenko, *Food Addit. Contam.*, 1996, **13**, 623.
35 M. Magnuson, *Anal. Chim. Acta*, 1998, **377**, 53.
36 J. Diemer and K. Heumann, *Fresenius' J. Anal. Chem.*, 1997, **357**, 74.
37 D. Barnett, R. Guevremont and R. Purves, *Appl. Spectrosc.*, 1999, **53**, 1367.
38 B. Ells, D. Barnett, R. Purves and R. Guevremont, *J. Environ. Monit.*, 2000, **2**, 393.
39 A. Elwaer, C. McLeod and K. Thompson, *Anal. Chem.*, 2000, **72**, 5725.
40 G. Gordon, B. Bubnis, D. Sweetin and C. Kuo, *Ozone: Sci. Eng.*, 1994, **16**, 79.
41 G. Gordon and B. Bubnis, *Ozone: Sci. Eng.*, 1995, **17**, 551.
42 S. Farrell, J. Joa and G. Pacey, *Anal. Chim. Acta*, 1995, **313**, 121.
43 M. Mitrakas, R. Tzimou-Tsitouridou and V. Keramidas, *Int. J. Environ. Anal. Chem.*, 2000, **78**, 343.
44 L. Romele and M. Achilli, *Analyst (London)*, 1998, **123**, 291.
45 V. Ingrand, J. Guinamant, A. Bruchet, C. Brosse, T. Noij, A. Brandt, F. Sacher, C. McLeod, A. Elwaer, J. Croue and P. Quevauviller, *Trends Anal. Chem.*, 2002, **21**, 1.
46 V. Farage and D. Janjic, *Inorgan. Chim. Acta*, 1982, **65**, L33.
47 J. Jwo and E. Cheng, *J. Phys. Chem.*, 1989, **93**, 2388.
48 L. Adamcikova, Z. Farbulova, P. Sevcik and A. Kawczynski, *J. Phys. Chem. A*, 2003, **107**, 508.
49 R. Ahmad, M. Cartwright and F. Taylor, *Analytical Methods for Environmental Monitoring*, Pearson Education Ltd, Harlow, UK, 2001.
50 B. Wang, L. Cheng and S. Dong, *J. Electroanal. Chem.*, 2001, **516**, 17.
51 L. Cheng and S. Dong, *J. Electrochem. Soc.*, 2000, **147**, 606.
52 S. Wang and D. Du, *Sens. Actuators, B*, 2003, **94**, 282.
53 I. Casella and M. Contursi, *Electrochim. Acta*, 2005, **50**, 4146.
54 Y. Li, W. Bu, L. Wu and C. Sun, *Sens. Actuators, B*, 2005, **107**, 921.
55 K. Haigh, unpublished work, Veolia Water Laboratories, Staines, UK.

Reduction of bromate in groundwater with an *ex situ* suspended growth bioreactor

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Abstract A potential remediation technique for groundwater contaminated by bromate has been investigated, utilising biological bromate reduction to bromide by augmentation of indigenous microbial populations. This technique, involving addition of a carbon source to contaminated groundwater, is being developed as an *ex-situ* methodology analogous to commercial denitrification systems, but may also have *in-situ* applications. Trials have focussed on a laboratory-scale anaerobic suspended growth chemostat system, investigating glucose addition to real groundwater supplies. Steady states for a range of glucose and bromate concentrations demonstrated bromate reduction up to 700 $\mu g\,l^{-1}$ (50% of 1400 $\mu g\,l^{-1}$ influent) with glucose excess (above 52 $mg\,l^{-1}$), but specific reduction rates (up to 2.83 μmol Br.g dry $wt^{-1}\,hr^{-1}$ for 1400 $\mu g\,l^{-1}$ bromate influent) were low compared to denitrification (up to 305 μmol N g dry $wt^{-1}\,hr^{-1}$). More recent enrichment trials have demonstrated reduction of 32 $mg\,l^{-1}$ bromate within a 40 hour residence time with specific reduction rates of up to 160.48 μmol Br.g dry $wt^{-1}\,hr^{-1}$, suggesting the presence of high rate bromate reducing bacterial strains.

Keywords Bromate; bioremediation; chemostat; *ex situ*; groundwater

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Introduction

Bromate (BrO_3^-) is classed by the International Agency for Research on Cancer as a Group 2B or 'possible human carcinogen' (IARC, 1999), due to evidence of tumour induction in rats and mice (Kurokawa *et al.*, 1986). This classification has resulted in legislation implementing maximum bromate levels in drinking water in an increasing number of countries, including EU directive 98/83/EC (Drinking Water Directive) specifying 25 $\mu g\,l^{-1}$ by 2003 and 10 $\mu g\,l^{-1}$ by 2008 (McCann, 1999) and the US Environmental Protection Agency a limit of 10 $\mu g\,l^{-1}$ (Kirisits *et al.*, 2000). In the UK, the Water Supply (Water Quality) Regulations 2000 set a maximum bromate level within drinking water of 10 $\mu g\,l^{-1}$, which came into force on 25 December 2003 (DWI, 2000).

Contamination by bromate is commonly associated with disinfection by-product (DBP) formation during ozonation of potable water containing naturally occurring bromide. Treatment processes where bromate can be formed as a DBP include ozonation followed by hypochlorination and/or chloramination, or hypochlorination alone. Bromate may be formed by oxidation of bromide via the intermediate hypobromite (BrO^-), and also indirectly by ozone via free-radical attack. Following ozonation, bromate concentrations in potable waters can reach 60 $\mu g\,l^{-1}$ (Kirisits and Snoeyink, 1999). Several technologies have been evaluated for removal of bromate within this range, including filtration (Marhaba and Medlar, 1993), UV irradiation (Siddiqui and Amy, 1994), chemical reduction (Gordon *et al.*, 2002), and granular activated carbon (Kirisits *et al.*, 2000). However, many of these techniques have not been evaluated beyond controlled laboratory conditions and may prove unsuitable for cost-effective implementation at full-scale.

Bromate has not historically been reported as occurring naturally in surface waters (Hutchinson *et al.*, 1997) or aquifers, but recent analytical advances have led to detection of bromate both in the surface water environment (Kruithof and Meijers, 1995) and also within an aquifer in the UK. Despite being a powerful oxidant, reaction rates for natural bromate degradation do not appear significant (Lopez-Cueto *et al.*, 2001), which suggests bromate is conservative in both surface and groundwaters. However, biological bromate reduction has been shown to occur under anoxic conditions in suspended growth (Hijnen *et al.*, 1995), fixed film (Hijnen *et al.*, 1999) and biological activated carbon systems (Kirisits and Snoeyink, 1999). Bacterial denitrification is a more energetically favourable system, but in the absence of high oxygen and nitrate levels bromate reduction to bromide may be hypothesised to occur by use as a terminal electron acceptor during cellular respiration. Biological nitrate and perchlorate reduction systems have already demonstrated the potential of this approach for groundwater bioremediation (Hall, 1997; Polk *et al.*, 2001). It is therefore proposed a biological bromate reduction system using indigenous groundwater bacteria be investigated, for potential development into an *ex situ* groundwater remediation system.

Methods

A two-vessel anaerobic chemostat was used to investigate bromate reduction following glucose augmentation of groundwater under continuous culture conditions. Two trials were undertaken, with trial 1 focusing on carbon and bromate influent concentrations necessary to initiate bromate reduction. Trial 2 was subsequently completed using conditions experimentally determined during trial 1 as favourable for bromate reduction, with continuous flow maintained for 15 weeks (trial 2a) at steady states under bromate conditions between 1 and 128 $mg\,l^{-1}$, followed by 9 weeks (trial 2b) with constant conditions (32 $mg\,l^{-1}$ influent bromate). Anaerobic suspended growth was maintained by slow stirring and nitrogen sparging of reactors and influent supply. General operating conditions are given in Table 1.

Influent supply for both trials was provided by separate groundwater and glucose feeds, to minimise growth in supply lines. Groundwater represented 90% (trial 1) and 95% (trial 2) of influent flow, along with glucose solution concentrated by 10× and 20× for trials 1 and 2 respectively. The groundwater supply was sourced from a bromate contaminated aquifer, and was continuously augmented with nutrients ($NaH_2PO_4.2H_2O$ (300 $mg\,l^{-1}$); $MgSO_4.7H_2O$ (10 $mg\,l^{-1}$); H_3BO_4 (3 $mg\,l^{-1}$); $MnSO_4.H_2O$ (2 $mg\,l^{-1}$); $CuSO_4$ (0.4 $mg\,l^{-1}$); $ZnCl_2$ (0.2 $mg\,l^{-1}$); $CoCl_2.6H_2O$ (0.4 $mg\,l^{-1}$); $Na_2MoO_4.2H_2O$

Table 1 Operating conditions of the suspended growth chemostat system

Parameter	Value	
	Trial 1	Trial 2
Reactor volume	2.0 litres per reactor	2.0 litres per reactor
Reactor configuration	Two reactors operated as independent units	Two reactors in series, with effluent from reactor 1 providing influent for reactor 2
pH	Between 6.5–8.0. pH control not used	Between 6.5–8.5. pH control not used
Temperature	10 °C ± 4 °C	20 °C ± 2 °C
Dilution rate (Residence time)	0.05 hr^{-1} (20 hours)	0.025 hr^{-1} (40 hours – reactor 1); 0.013 hr^{-1} (80 hours – reactors 1 and 2 combined)
Flow rate	100 ml hr^{-1} to each reactor	50 ml hr^{-1} to combined system

(0.3 mg l^{-1}); $FeCl_3.6H_2O$ (1 mg l^{-1}); NH_4Cl (300 mg l^{-1})) to ensure growth limitation by either carbon (with $\leq$40 mg l^{-1} glucose addition) or nitrate only. Background influent groundwater nitrate was 30–40 mg l^{-1} as NO_3^- (trial 1) and 8 mg l^{-1} as NO_3^- (trial 2). No additional biomass was added.

One 10 ml sample from each reactor, removed via the basal outlets, was taken every 2–3 days for biomass (measured spectrophotometrically at 600 nm) and pH monitoring. For most bacteria absorbance at 600 nm approximates to microbial biomass concentration expressed as grams dry weight per litre, and this relationship was assumed in this case. Steady state conditions were deemed to have been reached after a minimum of five liquid volume changes (4 days (trial 1) or 8 days (trial 2)), and when biomass and pH readings had stabilised.

The first trial, with the two reactor units operated as independent systems, each with a 20 hour residence time, investigated bromate and nitrate degradation over a range of bromate and glucose concentrations. Three influent bromate concentrations (trialled in the order 78 mg l^{-1}, 1.4 mg l^{-1} and then 0.2 mg l^{-1}) were studied by spiking groundwater with potassium bromate where necessary. Seven glucose concentrations (10, 20, 30, 40, 52, 100 and 150 mg l^{-1}) were used at each bromate level, giving a total 21 sets of steady-state conditions. In addition, one trial was carried out 25 °C to investigate effects of increased temperature. Trial 2 maintained continuous culture conditions for a total (trial 2a plus trial 2b) of 24 weeks, with the two reactors operating as one unit in series (2 × 40 hour residence times giving a total of 80 hours). Glucose input to the two reactors during trial 2 was 100 mg l^{-1} for reactor 1 and a further 52 mg l^{-1} to reactor 2, thus ensuring carbon excess. Groundwater source was from the same aquifer as during trial 1, but from an area with anomalously low bromate concentrations, suggested to contain populations of bromate reducing bacteria.

Analysis of samples was undertaken upon attainment of each steady state for trial 1, and every 1–2 weeks during trial 2. A 20 ml (trial 1) or 10 ml (trial 2) glucose solution sample was taken and made up to 200 ml with groundwater to emulate influent supply. Effluent samples were collected during a 2 hour period from each reactor, taken over ice to minimise further bacterial activity. A supernatant sample of 200 ml (trial 1) or 100 ml (trial 2) was subsequently taken from each reactor via a basal outlet. Samples were analysed immediately for all determinands apart from bromate during trial 1, which was carried out at a later date, and subsequently frozen on the day of sampling for long-term storage. Analytical measurements and procedures are given in Table 2.

Results and discussion

Trial 1

At all three influent bromate concentrations biomass increased with increasing glucose concentration. Biomass data for the 1.4 mg l^{-1} bromate concentration are given in Figure 1. Dissolved oxygen (DO) concentrations measured in supernatant samples were below 2 mg l^{-1}, with influent levels ranging from 0.9 to 4.4 mg l^{-1}. Since some oxygen entrainment was likely during sampling, this suggests reactor DO concentrations were maintained below 1 mg l^{-1}.

Soluble total organic carbon (TOC) results showed much of the glucose was utilised at all three influent bromate concentrations. With influent glucose below 52 mg l^{-1} there was 5–10 mg l^{-1} glucose equivalent effluent residual, although it is not known whether this was glucose or fermentation products such as ethanol, acetate or lactose. With excess glucose ($\geq$52 mg l^{-1}), effluent TOC was within the range 13–82 mg l^{-1} as glucose equivalent, suggesting there was excess available electron donor for bromate reduction.

Table 2 Analytical procedures undertaken for bromate reduction chemostat trials 1 and 2

Parameter	Analytical procedure
pH	Portable pH meter (Hanna HI 8424)
Temperature	Influent – Temperature probe (Jenway 9071) Supernatant – Thermometer permanently mounted within reactor
DO	Influent and supernatant only – DO (dissolved oxygen) probe (Jenway 9071)
Biomass	Optical density at 600 nm (Jenway 6505 spectrophotometer)
Carbon	Total Organic Carbon (TOC) with filtered samples (0.45 μm) on Shimadzu TOC-V
Bromate and bromide	Trial 1 (78 mg l^{-1} bromate) – Shimadzu vp series HPLC system with UV detection at 220 nm, Hamilton PRP-X100 column, 50 μl injection and carbonate/bicarbonate eluent Trial 1 (1.4 mg l^{-1} & 0.2 mg l^{-1} bromate) - Shimadzu vp series HPLC system with post-column reaction and UV detection at 356 nm, Metrohm MetroSupp-5 column, 200 μl injection, sulphuric acid/ammonium molybdate tetrahydrate eluent and potassium iodide post-column reagent Trial 2 (Bromate and bromide) – Dionex ICS-2500 Ion Chromatography system with AS-9 HC column, 9 mM sodium carbonate eluent and conductivity detection
Nitrate and nitrite	Trial 1 – Merck cell tests (Tests 14563 (Nitrate) and 14547 (Nitrite)) Trial 2 – Dionex IC analysis simultaneously with bromate and bromide

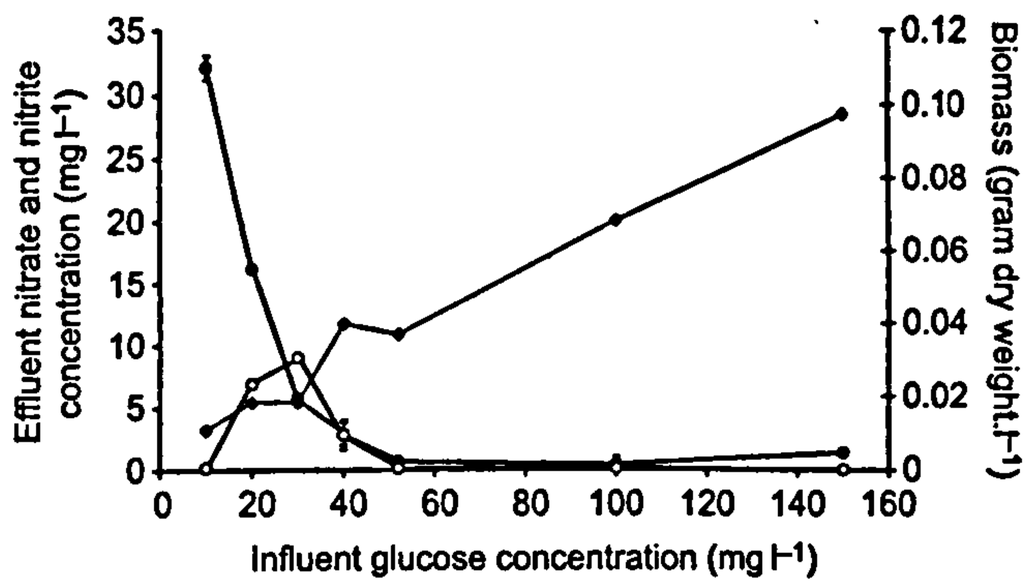

Figure 1 Nitrate (•) and nitrite (○) effluents and biomass readings (♦) at 1.4 mg l^{-1} bromate influent level during trial 1

Denitrification occurred at all glucose and bromate concentrations. Partial denitrification (12.3–91.0%) was observed with glucose addition under 40 mg l^{-1}. Nitrite production (0.02–8.9 mg l^{-1} as NO_2^-) was also noted under these conditions which is consistent with carbon limitation, whereby there is insufficient electron donor to completely reduce nitrate to nitrogen gas (Hall, 1997). Total nitrate removal to the limit of the test (experimentally determined as 0.6 mg l^{-1} nitrate as NO_3^-) was attained in nearly all cases when influent glucose was at least 40 mg l^{-1}, with no nitrite formation observed. Nitrate and nitrite data for the 1.4 mg l^{-1} bromate concentration are given in Figure 1.

Bromate removal in excess of 5% was detected at the 150 mg l^{-1} glucose, 1.4 mg l^{-1} bromate steady-state condition, and also at all glucose concentrations with the 0.2 mg l^{-1} bromate feed. With 150 mg l^{-1} glucose addition, bromate concentrations were reduced to 700 μg l^{-1} and 15 μg l^{-1} from 1.4 mg l^{-1} and 0.2 mg l^{-1} respectively, thus removing a maximum 700 μg l^{-1} bromate. Specific rates of bromate and nitrate reduction in the presence of carbon excess are given in Table 3. Under carbon limiting conditions, reductions in bromate concentration of between 0–55 μg l^{-1} were noted, but this only represented a

Table 3 Comparison of selected specific bromate and nitrate removal rates by cultures under excess carbon conditions during chemostat trials 1 and 2b

Trial	Retention time (hours)	Bromate in feed (mg l^{-1})	Reactor temp. (°C)	Glucose addition (mg l^{-1})	qN (μmol N g dry wt^{-1} hr^{-1})	qBr (μmol Br g dry wt^{-1} hr^{-1})	qN/qBr
1	20	0.2	10	150	229	0.77	297
1	20	1.4	10	150	305	2.83	108
1	20	1.4	25	150	286	5.54	52
2b (9 Jan 04)	40	32	20	100	66.55	14.73	4.52
2b (9 Jan 04)	80	32	20	100 + 52	25.13	16.49	1.52
2b (18 Mar 04)	40	32	20	100	65.87	96.86	0.68
2b (18 Mar 04)	80	32	20	100 + 52	25.11	35.30	0.71

qN – Specific nitrate removal rate
qBr – Specific bromate removal rate
qN/qBr – Relative molar ratio of specific bromate removal to specific nitrate removal

maximum 27% of the influent bromate available. No decrease was observed with 78 mg l^{-1} influent bromate contamination, although small reductions may not have been evident above background noise.

Maximum specific bromate reduction rates observed in trial 1 were comparable with those obtained by Hijnen *et al.* (1995), who studied a suspended growth denitrifying enrichment culture grown in a medium containing 1 mg l^{-1} bromate, 5 mg l^{-1} nitrate and 100 mg l^{-1} ethanol at 20–25 °C. The relative molar ratio (qN/qBr) in this case was calculated as 174–202, compared with the ratios 108–297 obtained during trial 1 at 10 °C. Therefore, whilst bromate reduction concomitant with denitrification did occur, bromate reduction rates were comparatively very low. It is likely that, as in the case of the Hijnen *et al.* study, observed bromate reduction during trial 1 was due to 'co-metabolic' activity of nitrate reductase within denitrifiers and not the action of specific bromate degrading strains.

Physical conditions of the additional steady state run during trial 1 at 25 °C with 1.4 mg l^{-1} bromate, 150 mg l^{-1} glucose conditions were directly analogous to those at 10 °C. Specific rates are given in Table 3. The specific bromate removal rate at 25 °C was almost double that obtained at 10 °C, which showed increased bromate removal rate could be achieved by simply increasing temperature. Suppression by dominant denitrifying strains of any bromate reducing bacteria present at the lower temperature may have been occurring, leading to the residence time being insufficient to maintain viable populations. Alternatively, increased temperature may have made any co-metabolic bromate reducing activity within denitrifiers more favourable.

Trial 2

Following completion of trial 1, the chemostat system was run for an extended period under conditions previously described. It was anticipated these should provide favourable selection pressures for high rate bromate reducing (HRBD) strains within the groundwater, should they be present. Trial 2a, using bromate concentrations ranging from 1 mg l^{-1} to 128 mg l^{-1}, showed an increase in bromate reduction over that obtained during trial 1. With an 80 hour residence time, a maximum of 20 mg l^{-1} bromate reduction was observed from an initial concentration of 64 mg l^{-1}, with reduction to the detection limit attained under 1 mg l^{-1} and 2 mg l^{-1} influent bromate conditions. As effluent from reactor 1 was providing influent for reactor 2 it was hypothesised nitrate was removed within reactor 1 (40 hour residence time) containing a largely denitrifying culture, leaving a high bromate, low nitrate water supply for reactor 2.

During trial 2b, with bromate influent set at 32 mg l^{-1}, bromate removal within reactor 1 gradually increased until reduction to below 1 mg l^{-1} was occurring with the 40 hour residence time. Reduction within reactor 2 showed a corresponding decrease simply due to lower influent concentrations provided by the effluent from reactor 1. Effluent bromate concentrations from reactor 2 (total 80 hour residence time) were below the limit of detection (5 μg l^{-1}). Percentage bromate reduction within the system during this time is shown in Figure 2. Bromide concentrations showed a corresponding increase, up to a maximum of 22.01 mg l^{-1}. The overall average ratio of bromate removal to bromide increase was 0.631 (standard deviation 0.064, $n = 3$), which is very close to the stoichiometric bromate:bromide ratio of 0.63 mg Br^- per mg BrO_3^-. This suggests total bromate reduction to bromide with no intermediate formation, and was confirmed by an average ratio within reactor 2 of 0.601 (standard deviation 0.133, $n = 6$), although the corresponding ratio within reactor 1 was 0.838 (standard deviation 0.588, $n = 6$). During this period nitrate reduction continued with low (0.01–2.4 mg l^{-1}) but detectable effluent concentrations, suggesting nitrate reduction rate was no longer a factor in bromate reduction and that specific bromate reducing strains

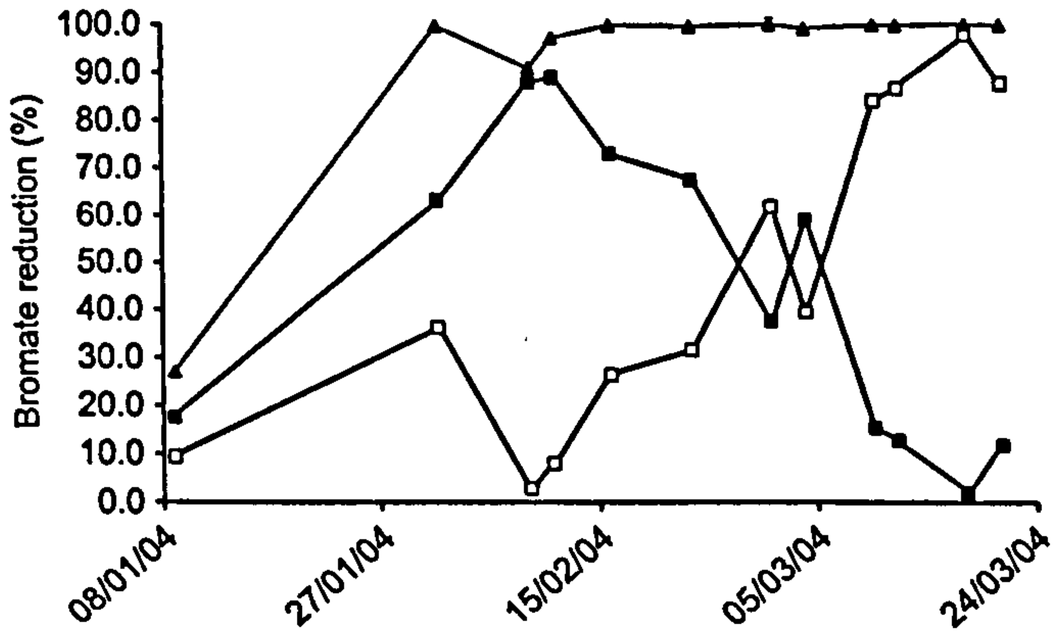

Figure 2 Percentage bromate reduction during trial 2b with 32 mg l^{-1} influent bromate and 40 hour residence time within chemostat reactor 1 (□), reactor 2 (■) and with combined 80 hour residence time (▲)

had started to predominate. Specific nitrate reduction rates reduced from those observed in trial 1 to 37.34–127.12 μmol N g dry wt^{-1} hr^{-1} and 11.09–38.22 μmol N g dry wt^{-1} hr^{-1} for 40 and 80 hour residence times respectively, although part of this reduction was due to a lower influent nitrate concentration. Nitrite production was consistently below the UK drinking water limit of 0.5 mg l^{-1}. An increase in specific bromate reduction rate to 5.30–160.48 μmol Br g dry wt^{-1} hr^{-1} and 16.49–50.94 μmol Br g dry wt^{-1} hr^{-1} for 40 and 80 hour residence times respectively led to the relative molar ratio (qN/qBr) within an 80 hour residence time reducing to 0.38–1.52, which is considerably more favourable than both ratios obtained during trial 1 (108–297) and those of 174–202 reported by Hijnen *et al.* (1995). Selected specific bromate and nitrate reduction rates during the 9 week run of trial 2b are given in Table 3.

The chemostat system was running as a continuous culture for approximately 15 months during the complete experimental period, which would have provided strong selection pressures for bacterial strains able to utilise bromate during cellular respiration. The culture produced during trial 1 was an efficient denitrification system, but low bromate reduction rates showed nitrate was preferentially utilised under anoxic conditions. It is likely these conditions favoured faster-growing strains, allowing denitrifying bacteria to predominate, with bromate reduction only occurring by 'co-metabolism' during denitrification. Trial 2 was an attempt at enrichment to favour selection of high bromate reduction by putative HRBD strains, with a higher temperature, longer retention times and a groundwater source specifically chosen for its bromate reduction potential. This approach proved successful with the specific rate of bromate reduction increasing, from a maximum during trial 1 of 5.54 μmol Br g dry wt^{-1} hr^{-1}, to 96.86 μmol Br g dry wt^{-1} hr^{-1} in reactor 2 at the end of trial 2b (peaking at 160.48 μmol Br g dry wt^{-1} hr^{-1}) with 50–99% denitrification and residual effluent carbon (24.22–32.53 mg l^{-1} as glucose equivalent).

The existence of microorganisms able to reduce bromate and conserve metabolic energy from the reaction has not currently been proven to our knowledge. However, following studies into the analogous oxyanions chlorate and perchlorate, several strains have been reported which may have this potential with these electron acceptors in a form of anaerobic respiration analogous to denitrification (Logan *et al.*, 2001). These strains can compete effectively for carbon even in the presence of nitrate, and suggest chlorate respiration may be carried out at comparable rates to denitrification. The current study does not prove, but may suggest a similar mechanism for bromate.

Ex situ processes for remediation of nitrate and perchlorate contaminated groundwater have been developed and trialled successfully (Hall, 1997; Polk *et al.*, 2001), and any potential groundwater bromate remediation strategy would follow similar design guidelines. *Ex situ* processes have the advantage of precise control of parameters including temperature, retention time and carbon addition, all shown as controlling variables in the current study. Development of a viable *ex situ* bromate bioreactor system will require both lower retention times and a capacity for dealing with lower bromate concentrations (up to $1-2\,mg\,l^{-1}$). A study by Hijnen *et al.* (1999) investigated reduction of $15-35\,\mu g\,l^{-1}$ bromate by an attached growth denitrification bioreactor system, and concluded removal rates were around 10–20 times greater than those achieved under suspended growth. Specific bromate removal rates ($2.8-8.3\,\mu g\,g^{-1}\,min^{-1}$) were not far below those obtained by Hijnen *et al.* (1995) using denitrifiers under suspended growth conditions with $1-5\,mg\,l^{-1}$ bromate ($11.1-23.6\,\mu g\,g^{-1}\,min^{-1}$), despite the lower bromate concentration and a lower temperature (12 °C compared with 20 °C). Therefore, fixed-films may prove more efficient in a full-scale remediation system for treatment of bromate contaminated groundwater.

Conclusions

The first experimental stage (trial 1) established bromate reduction within a suspended growth chemostat system did occur, although specific rates of 0.77–5.54 μmol Br g dry $wt^{-1}\,hr^{-1}$ were substantially lower than those for denitrification (229–305 μmol N g dry $wt^{-1}\,hr^{-1}$). Bromate reduction under carbon limiting conditions did not exceed 27% of influent bromate concentrations at $0.2\,mg\,l^{-1}$ and $1.4\,mg\,l^{-1}$, although 50–93% reduction occurred with carbon excess under otherwise identical conditions.

An extended enrichment period (24 weeks) with bromate concentrations initially between $1-128\,mg\,l^{-1}$ for 15 weeks (trial 2a) followed by a steady state at $32\,mg\,l^{-1}$ for 9 weeks (trial 2b) showed increased specific bromate reduction rates over those obtained during trial 1, with a peak of 160.48 μmol Br g dry $wt^{-1}\,hr^{-1}$ from a $32\,mg\,l^{-1}$ bromate influent. Effluent mass balances confirmed reduction of bromate to bromide with no intermediate production.

Whilst long residence times (40–80 hours) were used with glucose in excess, the high bromate concentrations reduced ($1-32\,mg\,l^{-1}$) suggest for the first time that bacterial strains capable of specifically reducing bromate in the presence of nitrate were present.

The application of favourable conditions for an extended time period in a suspended growth bioreactor has shown the occurrence and development of biological bromate reduction within groundwater. Use of a fixed-film bioreactor with acclimated inoculum and further optimisation of influent conditions may allow development of an efficient *ex situ* bioremediation system for a bromate contaminated aquifer.

References

DWI (2000). *The Water Supply (Water Quality) Regulations 2000* (WWW document). http://www.dwi.gov.uk/regs/si3184/3184.htm (accessed 5th August 2003).

Gordon, G., Gauw, R., Emmert, G., Walters, B. and Bubnis, B. (2002). Chemical reduction methods for bromate ion removal. *J. Am. Water Works Assoc.*, **94**, 91–98.

Hall, T. (1997). *Water Treatment Processes and Practices.* WRc, Swindon, UK.

Hijnen, W., Voogt, R., Veenendaal, H., van der Jagt, H. and van der Kooij, D. (1995). Bromate reduction by denitrifying bacteria. *App. Environ. Micro.*, **61**, 239–244.

Hijnen, W., Jong, R. and van der Kooij, D. (1999). Bromate removal in a denitrifying bioreactor used in water treatment. *Water Res.*, **33**, 1049–1053.

Hutchinson, T., Hutchings, M. and Moore, K. (1997). A review of the effects of bromate on aquatic organisms and toxicity of bromate to oyster (*Crassostrea gigas*) embryos. *Ecotox. Environ. Safety*, **38**, 238–243.

IARC (1999). *Potassium Bromate (Summary of Data Reported and Evaluation)* (WWW document). http://www-cie.iarc.fr/htdocs/monographs/vol73/73-17.html (accessed 26th July 2003).

Kirisits, M. and Snoeyink, V. (1999). Reduction of bromate in a BAC filter. *J. Am. Water Works Assoc.*, **91**, 74–84.

Kirisits, M., Snoeyink, V. and Kruithof, J. (2000). The reduction of bromate by granular activated carbon. *Water Res.*, **34**, 4250–4260.

Kruithof, J. and Meijers, R. (1995). Presence and formation of bromate in Dutch drinking water treatment. *Water Supply*, **13**, 117–125.

Kurokawa, Y., Takayama, S., Konishi, Y., Hiasa, Y., Asahini, S., Takahashi, M., Maekawa, A. and Hayashi, Y. (1986). Long-term *in vivo* carcinogenicity tests of potassium bromate, sodium hypochlorite, and sodium chlorite conducted in Japan. *Environ. Health Persp.*, **69**, 221–235.

Logan, B.E., Zhang, H., Mulvaney, P., Milner, M.G., Head, I.M. and Unz, R.F. (2001). Kinetics of perchlorate- and chlorate respiring bacteria. *App. Environ. Micro.*, **67**(6), 2499–2506.

Lopez-Cueto, G., Ostra, M. and Ubide, C. (2001). New way of application of the bromate-bromide mixture in kinetic analysis. *Anal. Chim. Acta*, **445**, 117–126.

Marhaba, T.F. and Medlar, S.J. (1993). Treatment/removal of bromate and bromide ions from drinking water. In: *Proc. AWWA Water Qual. Technol. Conf.*, Miami, 1993.

McCann, B. (1999). By-product blues. *Water 21*, July, 15–18.

Polk, J., Murray, C., Onewokae, C., Tolbert, D.E., Togna, A.P., Guarini, W.J., Frisch, S. and Del Vecchio, M. (2001). Case study of *ex-situ* biological treatment of perchlorate-contaminated groundwater. In: *Proc. 4th Tri-services Environ. Tech. Symposium*, 18–20 July 2001, San Diego.

Siddiqui, M. and Amy, G. (1994). Removal of bromate during drinking water treatment by UV irradiation. In: *IOA Regional Conf. Proc.*, Zurich, 31 Aug – 2 Sept 1994.

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Remediation of bromate-contaminated groundwater in an ex situ fixed-film bioreactor

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Abstract

Use of a pilot-scale fixed-film bioreactor was investigated for remediation of bromate contamination within groundwater. Bromate reduction with stoichiometric production of bromide was observed, providing supporting evidence for complete reduction of bromate with no production of stable intermediates. Reduction of 87–90% bromate from an influent concentration of 1.1 mg L^{-1} was observed with retention times of 40–80 h. Lower retention times led to decreases in bromate reduction capability, with 11.5% removal at a 10 h retention time. Nitrate reduction of 76–99% from a 30.7 mg L^{-1} as NO_3^- influent was observed at retention times of 10–80 h, although an increase in nitrite production to 2.7 mg L^{-1} occurred with a 10 h retention time. Backwashing was not required, with the large plastic packing media able to accommodate biomass accumulation without decreases in operational efficiency. This study has provided proof of concept and demonstrated the potential of biological bromate reduction by fixed-film processes for remediation of a bromate contaminated groundwater source.

Keywords: Bromate; Bromide; Bioreactor; Fixed-film; Groundwater; Pilot-scale

1. Introduction

Contamination by bromate (BrO_3^-), an oxyanion of bromine, is commonly associated with ozonation processes during water treatment, where it is formed as a disinfection by-product (DBP) by oxidation of naturally occurring bromide (Br^-). It can also be produced during ozonation following addition of chemicals such as calcium chloride containing bromide as an impurity. Despite being a thermodynamically powerful oxidant, once in solution, bromate is highly stable at room temperature (DSP, 1999). Due to evidence of carcinogenicity (Kurokawa et al., 1986), bromate has been classed as a Group 2B carcinogen by the World Health Organisation (IARC, 1999). Maximum concentrations in potable water are limited to 10 μg L^{-1} in countries including the UK, the United States and Canada.

Bromate has not historically been perceived as an environmental contaminant, and is not reported as occurring naturally in surface waters (Hutchinson et al., 1997) or aquifers. However, advances in the application of ion chromatography (IC) analytical techniques and the impetus of tighter legislation have together led to enhanced monitoring programmes. As a consequence, bromate has been detected within both surface water (Kruithof and Meijers, 1995) and more recently within a UK chalk aquifer. Significant bromate contamination

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of industrial origin has led to formation of a plume within this aquifer and is currently affecting potable water abstraction in the area.

Techniques including filtration (Marhaba and Medlar, 1993), ultraviolet (UV) irradiation (Siddiqui and Amy, 1994), chemical reduction (Gordon et al., 2002), and granular activated carbon (Kirisits et al., 2000) have previously been investigated for removal of bromate from water. However, many of these methods have not been evaluated beyond laboratory scale, and were developed specifically for removal of bromate as a DBP from potable water, where concentrations within the range 10–60 μg L^{-1} may be detected (Kirisits and Snoeyink, 1999).

Nitrate (NO_3^-) and perchlorate (ClO_4^-) are also oxyanions known as groundwater pollutants when discharged to the environment either accidentally or intentionally (Korom, 1992; Urbansky, 2002). The processes of nitrate (Hiscock et al., 1991) and perchlorate (Logan, 1998) reduction by bacteria during anaerobic respiration can be successfully harnessed within ex situ water treatment systems. For example, biological perchlorate treatment has been developed using both fixed and fluidised bed bioreactors (Xu et al., 2003). A perchlorate reduction system utilising a fluidised bed reactor with ethanol addition as carbon source has been approved for drinking water treatment in California (Sakaji, 2002), and other pilot and full-scale systems have been tested for groundwater remediation (Hatzinger et al., 2000; Brown et al., 2002; Nerenberg et al., 2002; Min et al., 2004).

Biological bromate reduction to bromide has been shown to occur, but is much more sparsely understood. Hijnen et al. (1995, 1999) first demonstrated bromate reduction in a denitrifying bioreactor supplemented with ethanol using both mixed and pure cultures. Although batch studies initially indicated a bromate reduction rate over 100 times lower than that of nitrate reduction (Hijnen et al., 1995), continuous-flow trials suggested the two rates could be comparable at similar influent concentrations (Hijnen et al., 1999). Biological bromate reduction has also been observed on biological activated carbon (BAC) filters (Kirisits and Snoeyink, 1999; Kirisits et al., 2001). In neither case was reduction by specific bromate reducing strains demonstrated, and the hypothesis that nitrate and bromate competed for use as electron acceptors was put forward (Kirisits et al., 2001).

In this paper, studies are described to harness biomass acclimatised to bromate contamination, for reduction of bromate to bromide in contaminated groundwater within a fixed-film pilot-scale bioreactor system. Two reactors were operated between June and October 2004, treating groundwater from a contaminated aquifer containing approximately 1 mg L^{-1} bromate influent. Following inoculation of the reactors with biomass and a 23 day period of acclimation in batch culture, the ability of the system to reduce bromate to bromide was evaluated. The effects of retention time (RT) on efficacy of the system, supplied with carbon in excess, were investigated, and bromate removal rates examined. This study is to our knowledge the first study to investigate bromate removal from groundwater in a pilot-scale bioreactor system.

2. Materials and methods

2.1. Bioreactors

Trials were conducted using two identical bioreactors (Reactors 1 and 2) operated as separate, parallel systems to treat bromate-contaminated groundwater. Fig. 1 shows a schematic of one reactor. The experimental rig was housed in an unheated indoor test facility at Cranfield University in the UK. The two cylindrical reactors used in this trial had a height of 1.4 m and internal diameter of 0.2 m. For continuous flow operation they were packed with Etapak 210 (Koch-Glitsch UK, Stoke-on-Trent, UK), a random plastic media with a diameter of 63 mm, surface area of 200 m^2 m^{-3} and voidage of 96%. The media was packed within each reactor to give a bed height of 1.2 m and volume of 36 L.

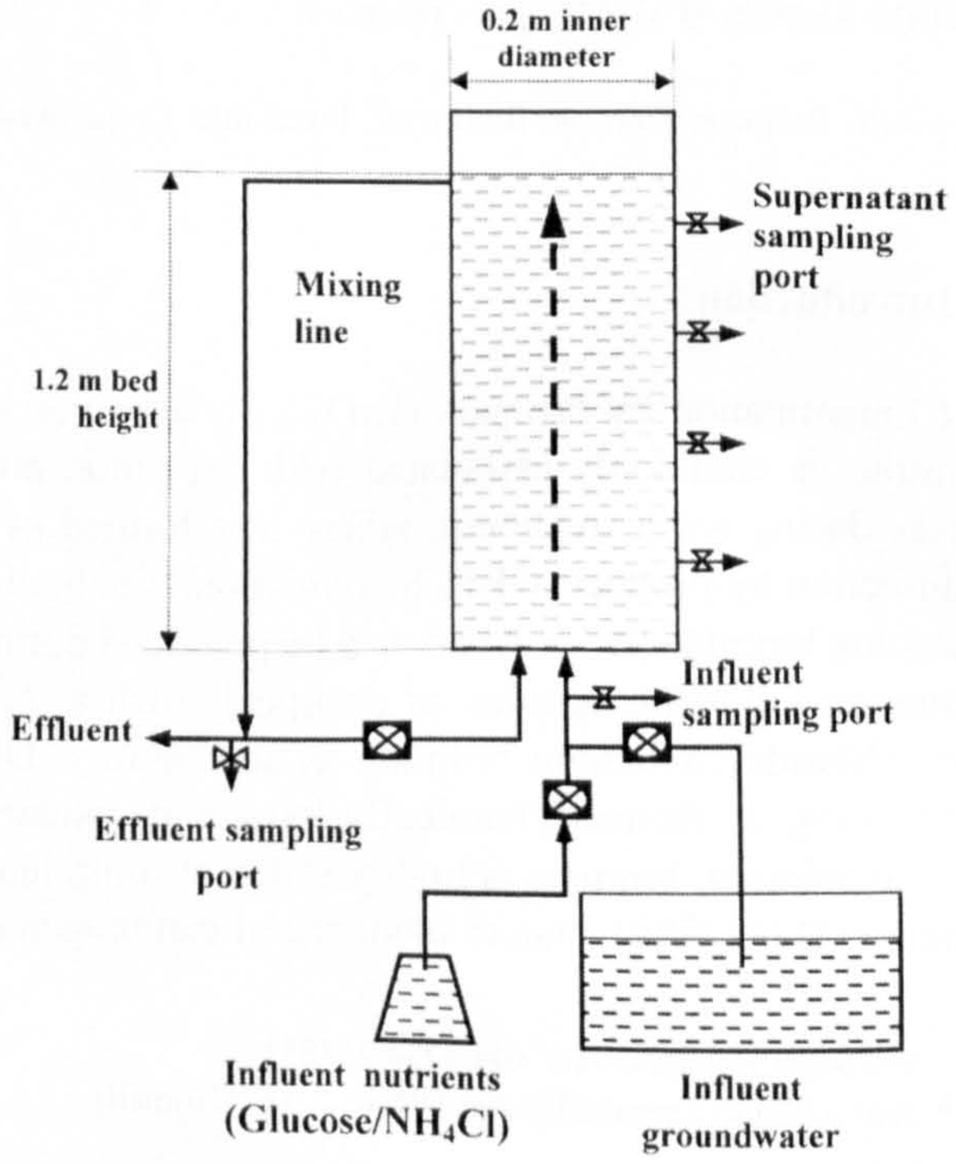

Fig. 1. Schematic of bioreactor.

Groundwater was pumped into each reactor at flow rates of 7.1–57.0 mL min^{-1}, with nutrient stock solution added at 0.38–3.0 mL min^{-1} via a separate influent supply pump. The nutrient stock solution contained 2 g L^{-1} glucose as a carbon source and 2 g L^{-1} ammonium chloride (NH_4Cl) as a nitrogen source (both laboratory grade; Fisher Scientific, Loughborough, UK), giving a molar C/N ratio of 1.78:1. This was added at a rate to give a groundwater/amendments dilution ratio of 20:1, and thus a 100 mg L^{-1} final influent concentration of both glucose and NH_4Cl. It was assumed trace metals were present in the groundwater at sufficient concentrations for bacterial requirements. No attempt was made to optimise addition of carbon (which was added in excess), with the aim of the study focussing on ability and performance of the reactors in removing bromate contamination.

Influent flow rates gave reactor retention times of 10–80 h, with mixing provided by a peristaltic pump which continuously recirculated the reactor contents at a flow rate of 0.7 L min^{-1}. Effluent flow was through an overflow with a one-way valve on the mixing line. No backwashing facility was present within the reactor setup. Four sample ports were spaced evenly up the side of each reactor but, due to the recirculation leading to mixing within the vessels, only the top port was used for supernatant samples.

2.2. Influent groundwater supplies

Two groundwater supplies (GW-1 and GW-2) with elevated concentrations of bromate and bromide were obtained from a contaminated aquifer in the UK. Selected properties of the two groundwater sources, including influent anion concentrations of interest, are given in Table 1. Groundwater GW-1, utilised for initial batch operation (Phase A) only, was collected in 25 L jerry cans and stored at 7 °C prior to use for up to 60 days. Groundwater GW-2 was pumped into a covered holding tank (1.05 m^3) for transportation to the test facility and was stored within the facility at ambient temperature for a maximum of 50 days prior to use.

2.3. Inoculum

Inoculum was obtained from a laboratory-scale bromate reducing suspended-growth chemostat system which had been in operation for approximately 15 months. The chemostat was operated using groundwater GW-1, with bromate reduction of up to 48 mg L^{-1} at a 40 h RT attained within this enrichment culture prior to transfer of inoculum. Experimental setup and chemostat operating parameters have previously been described in Butler et al. (2005). Optical density (absorbance at 600 nm) of the inoculum at time of addition was 0.06±0.02. Optical density readings for the purposes of this trial were assumed to directly approximate to microbial biomass concentration expressed as g dry wt L^{-1}.

Table 1
Selected properties of influent groundwater supplies (GW-1 and GW-2), plus supernatant samples measured at an 80 h RT

	GW-1[a]	GW-2[b]	Reactor 1[c]	Reactor 2[d]
pH	6.9	7.3±0.3	7.2±0.3	7.3
Total organic carbon (mg L^{-1})	19.9	47.2±2.7	14.3±11.8	12.4
Bromate as BrO_3 (mg L^{-1})	1.4	1.1±0.3	0.2±0.1	0.1
Bromide as Br (mg L^{-1})	16.6	2.3±0.4	2.8±0.4	3.8
Total oxidised nitrogen as NO_3 (mg L^{-1})	8.6	30.7±4.7	<0.3	<0.3
Nitrite as NO_2 (mg L^{-1})	0.08	<0.06	<0.06	<0.06
Sulphate as SO_4 (mg L^{-1})	74.8	24.5±2.8	21.5±3.9	21.1

Errors given as ±1 S.D.
[a] Single sample collected on May 7, 2004.
[b] $n=8$ (TOC: $n=5$).
[c] $n=3$.
[d] Single sample collected on September 15, 2004.

2.4. Reactor operation

The period of reactor operation was split into two phases, with an initial 23 day start-up period of batch configuration identified as Phase A (Days A0–A22), and the subsequent 98 day fixed-film continuous flow operation given as Phase B (Days 0–97). Phase A was run as a suspended growth system using reactor 2 only, with plastic media added to both reactors for fixed-film operation during Phase B. Within Phase B a 63 day period of acclimation to fixed-film operation (Days 0–62) was followed by an investigation into effects of RT on reactor operation (Days 63–97). These periods were designated phases Bi and Bii, respectively.

2.4.1. Phase A – Inoculation and batch suspended growth period

On Day A0, Reactor 2 was completely filled with groundwater GW-1 plus 25 mg L^{-1} bromate as BrO_3^- (laboratory grade potassium bromate; Fisher Scientific, Loughborough, UK) and 100 mg L^{-1} glucose (laboratory grade; Fisher Scientific, Loughborough, UK). 200 ml bacterial inoculum was added on Days A1,

A2 and A5. The reactor was then left under batch suspended growth conditions to allow acclimation and growth for a total of 23 days. Additional glucose solution was added on Days A15, A19, A20 and A21, to give a final reactor concentration of 100 mg L^{-1} in each case. The reactor was continuously sparged with oxygen-free nitrogen (BOC Ltd, Guildford, UK). On the final day of Phase A (Day A22), 50% of reactor 2 contents were transferred to reactor 1, with both reactors then filled with plastic media on Day 0 (start of Phase B), sealed and set into continuous flow using groundwater source GW-2 and an RT of 80 h. Sparging with nitrogen was discontinued with completion of Phase A.

2.4.2. Phase B – Continuous flow fixed-film reactor operation

Upon commencement of Phase B, reactor 1 was designated the control reactor and operated at an 80 h RT for the duration of the trial. Reactor 2 was operated at an 80 h RT during Phase Bi (Days 0 – 62), followed by a series of steady states at retention times of 80, 60, 40, 20 and 10 h (Phase Bii; Days 63 – 97). A steady state was deemed to have been achieved after a minimum of 3 liquid volume changes under steady flow conditions. Both reactors were operated as batch systems with manual glucose addition on Days 45 – 56, due to a nutrient pump failure on Day 42 which led to partial washing out of the biomass. Reactor 1 was operated only intermittently in continuous flow mode until Day 74, due to an observed susceptibility of the biomass to wash out under continuous flow conditions. Steady state conditions were achieved in reactor 1 between Days 81 and 97.

2.5. Analytical procedures

Supernatant samples were collected every 1–2 days via the top sampling port on each reactor for pH (Hanna HI 8424 pH meter), optical density (Jenway 2505 spectrophotometer) and temperature monitoring. Groundwater and nutrient solution influent flow rates were also monitored every 1–2 days. Influent, supernatant and effluent samples were taken every 5–7 days and/or upon attainment of a steady state condition. Analysis of these samples included parameters described above, plus dissolved oxygen (DO; Jenway 9071 DO meter), soluble Total Organic Carbon (TOC; Shimadzu TOC-5000A) and the anions bromate, bromide, nitrate, nitrite and sulphate. All procedures apart from TOC and anion analysis were carried out immediately following sample collection. Where analysis was not undertaken on the day of collection, samples were frozen and stored at −20 °C. Simultaneous anion analysis was completed using a Dionex ICS-2500 IC system incorporating an ED50 electrochemical detector and AS9-HC analytical column, with a 9 mM sodium carbonate eluent, 1 mL min^{-1} flow rate and 250 μL sample injection. Errors were calculated as ±1 S.D.

3. Results

3.1. General operating conditions

Steady-state samples were taken from both within the reactor (supernatant) and from the effluent line. Results from the two sets of readings were similar, with the example of both bromate and bromide data all being within ±6% during Phase B. Supernatant readings provided the more complete data set so have been used throughout. Operation of the reactors was under ambient temperature conditions, with an average reactor temperature of 20.1±2.2 °C (n=19). Reactor temperature decreased during the experimental period due to the time of year, with a maximum of 24.2 °C observed on Day 33 and minimum of 15.2 °C on Day 97. An average pH of 7.3±0.4 and 7.4±0.4 was observed for reactors 1 and 2, respectively, with no pH control required to maintain consistent pH readings. Influent DO concentration during Phase B was 5.6±0.6 mg L^{-1} with no attempt made to reduce DO within groundwater prior to introduction into the reactors. Supernatant DO in the control reactor between Days 81 and 97, measured immediately following sample collection, was within the range 0.4–1.1 mg L^{-1}. This suggests largely anoxic conditions were present within a reactor under steady-state conditions.

3.2. Phase A – Inoculation and batch suspended growth period

Phase A was carried out in Reactor 2 only, under batch suspended growth conditions. Biomass readings during this period increased from 0.04 g dry wt. L^{-1} (Day A0) to 0.08 g dry wt. L^{-1} (Day A22). An increase in bromate and nitrate concentrations over Days A0–A2 from initial values of 26.6 mg L^{-1} and 9.3 mg L^{-1} to 28.5 mg L^{-1} and 10.9 mg L^{-1}, respectively, can be explained by addition of inoculum containing bromate and nitrate on Days A1 and A2. Following this short lag phase, both bromate and nitrate reduction were occurring by Day A5. Nitrate concentrations declined over the first 5 days, with a maximum of 3 mg L^{-1} remaining. Nitrite concentrations did not rise above 0.2 mg L^{-1}, suggesting the occurrence of total denitrification.

Bromate concentrations exhibited a continuing decrease over the remaining period of batch operation. Simultaneously a stoichiometric increase in bromide concentration from an initial level of 10.4 mg L^{-1} was observed. Anion concentrations during Phase A are given in Fig. 2.

3.3. Phase B – Continuous flow fixed-film reactor operation

3.3.1. Reactor 1

The experimental aim for reactor 1 was operation as a continuous flow fixed-film reactor with a RT of 80 h, for use as a control. However, following commissioning a susceptibility to washing out of the biomass was noted, measured as a decrease in optical density and increase in supernatant bromate concentration. Consequently, the reactor was operated as a batch system between Days 29–38 and Days 65–74. Failure of the nutrient pump led to a further period of batch operation between Days 45 and 56 to regenerate biomass. Sustainable bromate and nitrate reduction was not achieved until following the third period of batch operation, on Day 75. Reactor 1 was considered in steady-state operation for Days 81–97, during which period average percentage bromate and nitrate reduction of 85.0% and >99.2%, respectively, was obtained. Nitrite concentrations during this period were always below 0.06 mg L^{-1}, suggesting total denitrification to nitrogen gas was occurring at all times. Bromide supernatant concentrations were in excess of influent (2.3±0.4 mg L^{-1}), with an average of 2.8± 0.4 mg L^{-1} during the steady-state period.

3.3.2. Reactor 2 – Phase Bi (80 h retention time)

Subsequent to addition of plastic media and commencement of continuous flow conditions on Day 0 (80 h RT), bromate reduction from a 1.1±0.3 mg L^{-1} influent to the 0.01 mg L^{-1} detection limit was observed on Days 4 – 18. Between Days 18 and 26 an average supernatant concentration of 0.5±0.02 mg L^{-1} was achieved, with subsequent steady-state supernatant concentrations of 0.2±0.1 mg L^{-1} between Days 26 and 43. The nutrient pump failure on Day 42 led to the requirement for a period of batch operation between Days 45 and 56. However, biomass recovery was rapid, with only 3 reactor volumes (240 h) required to regain previous reduction rates. Fig. 3 shows bromate and nitrate reduction between Days 45 and 62, immediately following the period of nutrient pump failure. Bromide concentrations were in excess of 3 mg L^{-1} at all times during Phase Bi, with supernatant nitrate concentrations not exceeding 4 mg L^{-1} at any time apart from during the period of nutrient pump failure.

3.3.3. Reactor 2 – Phase Bii (effect of retention time)

Reactor 2 was operated at an identical (80 h) RT to reactor 1 between Days 63 and 68, to investigate reproducibility of the system. Results obtained, given in Table 1, confirmed the two reactors were exhibiting similar performance characteristics. Bromate and nitrate reduction within reactor 2 at the 80 h RT was 87.2% and 98.1%, respectively. Lower retention times of 60 and 40 h led to little alteration, with percentage bromate and nitrate removals of 86.9–90.3% and 98.4%, respectively. Under a 20 h RT minimal alteration in nitrate reduction capacity was noted (97.9%), although a slight reduction in efficiency to 76.5% was observed at the 10 h RT. In tandem, an increase in nitrite production was observed, leading to supernatant concentrations of 2.7 mg L^{-1} and suggesting total denitrification was not occurring. A loss in bromate reduction capacity at a 20 h RT was observed, although 50.2% reduction of

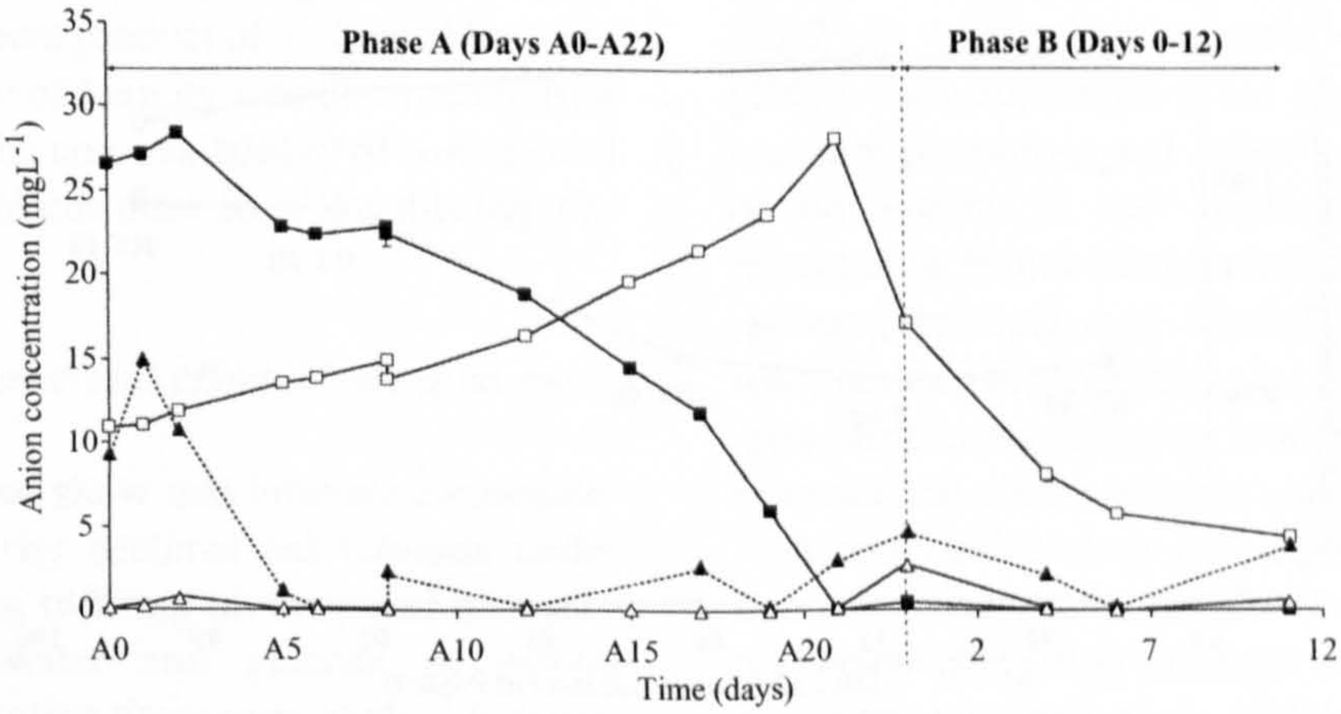

Fig. 2. Reactor 2 supernatant bromate (■), bromide (□), nitrate (▲) and nitrite (△) concentrations during Phase A (batch operation) and Phase B Days 0–12 (continuous flow operation).

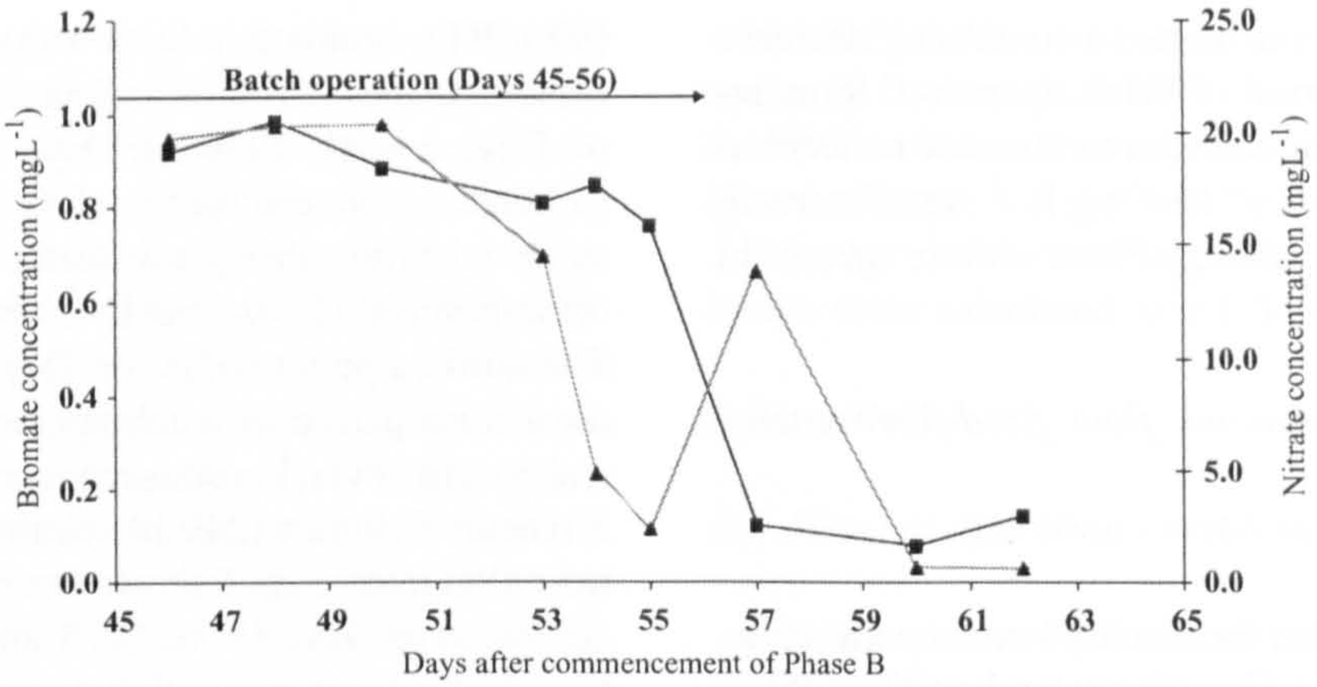

Fig. 3. Effect of nutrient pump failure (Day 42 for 65 h) and subsequent temporary batch operation on reactor 2 supernatant bromate (■) and nitrate (▲) concentrations. Bromate and nitrate supernatant concentrations of 0.1 and 0.3 mg L^{-1} respectively prior to pump failure.

influent concentrations was still noted. Under a 10 h RT, this loss of capacity became much more marked, leading to only 11.5% reduction. Sulphate reduction was noted at all retention times apart from 10 h, with a maximum reduction of 15.3 mg L^{-1} at a 20 h RT from an average influent of 23.9±2.5 mg L^{-1}. Fig. 4 shows bromate and bromide concentrations during phase Bii. TOC concentration in the supernatant was always >10 mg L^{-1}, indicating that overall carbon levels were always in excess.

4. Discussion

4.1. Reactor startup

Bromate reduction during Phase A (Reactor 2) was observed 2–5 days after commencement of the trial, with nitrate reduction after only 2 days. The inoculum was previously known to have bromate reducing capabilities, and this result confirmed that the enrichment culture produced during previous trials (Butler et al., 2005) retained this capability when transferred to a new environment. The composition of the enrichment culture is currently unknown, but studies are underway to identify strains involved.

During Phase A, a biomass layer would have formed on the inner wall of reactor 2 even prior to addition of the plastic media. This surface biofilm appears to have facilitated biomass maintenance under the continuous flow conditions of phase B, and also provided a buffer against influent quality perturbations such as loss of nutrient influent on Day 42. There was insufficient time for a biofilm to form on any reactor 1 surface prior to commencement of continuous flow during Phase B. This appears to have made the biomass much more prone to washing out until Day 75, by which time it is likely a biofilm had formed on both reactor walls and the packing media. Following the

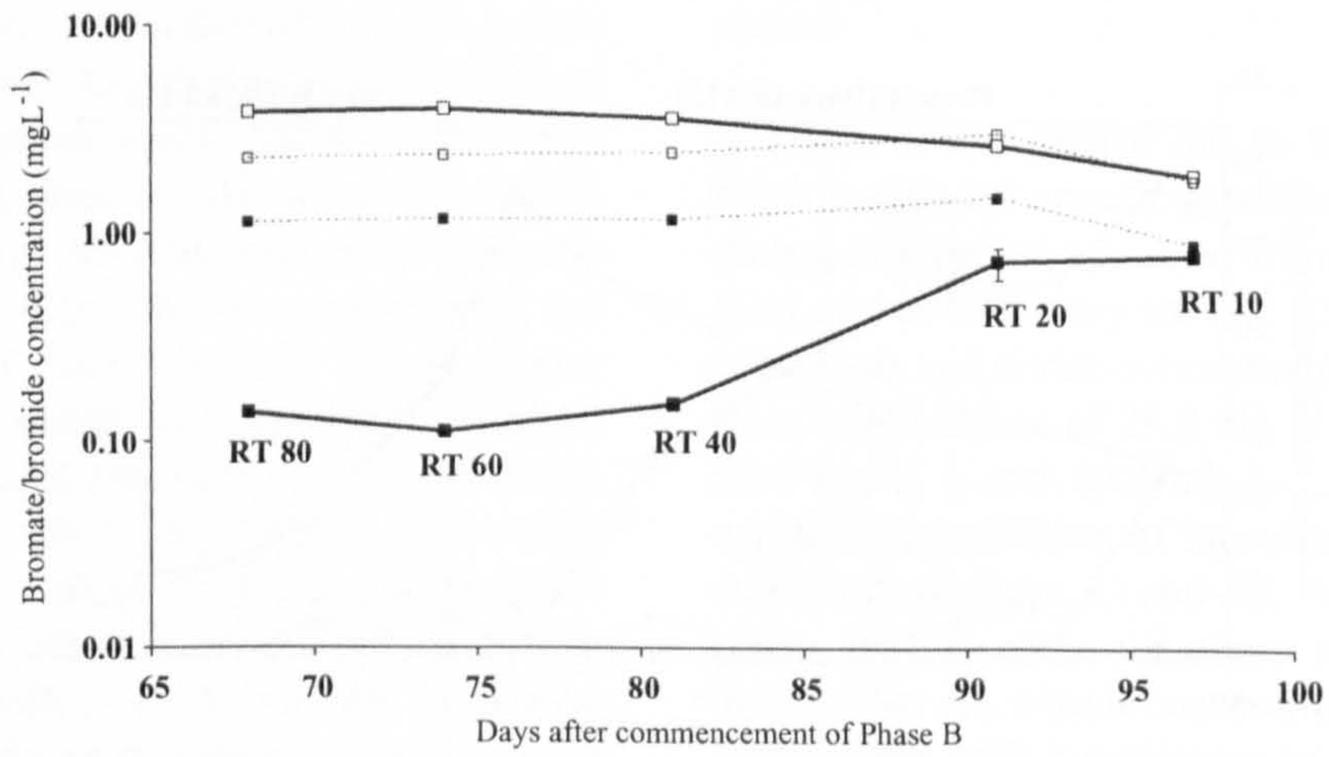

Fig. 4. Reactor 2 bromate (■) and bromide (□) concentrations during Phase Bii at retention times from 80 h (RT80) to 10 h (RT10). Supernatant concentrations indicated by solid lines and (—) influent concentrations by dashed lines (- - -).

nutrient supply interruption, reactor 2 recovered faster than reactor 1, which also required a further period of batch operation (Days 65–74) for bromate reducing ability to be regained. It is known that fixed-film reactors are generally less sensitive to variations in influent supplies than suspended growth systems, and as such are useful for the treatment of slow-growing and specialised microbial populations (Hyman and Dupont, 2001). Current trials have confirmed that, for sustainable operation of a continuous flow fixed-film bromate bioreactor, an initial batch growth phase is critical to allow biofilm establishment.

4.2. Stoichiometry

A logarithmic decrease of bromate simultaneously with a stoichiometric increase in bromide (101% recovery) following a lag phase of around 30 h was noted by van Ginkel et al. (2005) using a mixed bacterial population in 100 ml activated sludge batch cultures spiked with approximately 40 mg L^{-1} bromate. A longer lag phase of 2–5 days was observed in this study, likely due to the continued addition of inoculum over days 1–5 and the much larger volumes involved. However, similar stoichiometry was observed. A theoretical stoichiometric bromate/bromide ratio can be calculated as 0.63 mg Br^- per mg BrO_3^- for total bromate reduction to bromide. During the batch culture phase an overall ratio of bromate removal to bromide increase of 0.64 was obtained, which confirmed the previous figure of 0.63 obtained by Butler et al. (2005). Stoichiometric reduction of bromate and bromide production supports the hypothesis advanced by Hijnen et al. (1995) that bromate is fully reduced to bromide by bacteria possessing the ability, without the formation of stable intermediates such as bromite (BrO_2^-) and hypobromite (BrO^-). van Ginkel et al. (2005) suggested that toxic bromite may be a transient product of biological bromate reduction. No evidence of toxicity was observed during the current study, but the non-availability of commercial bromite analytical standards does not allow this hypothesis to be tested.

4.3. Reactor performance and effect of retention time

The results presented show that bromate contamination can be removed by acclimatised biomass under steady-state conditions, utilising an unspiked bromate-contaminated groundwater and glucose as carbon source. A range of retention times were studied to ascertain optimum operating efficiency of the system, with the choice of 10–80 h based on previous studies (Butler et al., 2005) and intended to maximise potential for attaining bromate reduction in this previously untested pilot-scale system. Groundwater used for the trial contained considerably more nitrate than bromate (an average of 30.7 mg L^{-1} nitrate as NO_3^- as opposed to 1.1 mg L^{-1} bromate) so conditions could be thought to favour predomination of denitrifiers. However, despite the reduction of >97% nitrate at all but the 10 h RT, in excess of 50% bromate contamination was also removed.

Optimum retention time for bromate reduction was shown to be within the range 20–40 h, with the higher values giving superior bromate removal (90.3% against 50.2%) but lower throughput and vice versa. For the enrichment culture utilised in these trials, a 10 h RT was unsuitable due to poor bromate reduction and high nitrite production, in excess of the current UK nitrite drinking water limit of 0.5 mg L^{-1}. Other trials have concluded lower retention times are sufficient for bromate reduction, with Kirisits and Snoeyink (1999) suggesting a 25 min empty bed contact time, and Hijnen et al. (1999) a 25- to 50 min contact time. However, the bromate concentrations used in these trials were low (20–35 μg L^{-1}). The only trial to date utilising high bromate concentrations (294 mg L^{-1} bromate) used a 48 h RT (van Ginkel et al., 2005), which is comparable to the current study.

Bromate removal rates can be expressed as bromate reduced per minute (μg L^{-1} min^{-1}), which allows comparisons with previous studies (Table 2). The highest bromate removal rate obtained in the current study under continuous flow conditions, at the 40 h RT, was 0.4 μg L^{-1} min^{-1}. This value is directly comparable to rates obtained in previous continuous flow studies (Hijnen et al., 1999; Kirisits and Snoeyink, 1999). A higher removal rate of 13.7 μg L^{-1} min^{-1} was achieved by Butler et al. (2005), with a significantly higher rate of 102.2 μg L^{-1} min^{-1} reported by van Ginkel et al. (2005). However, both these studies used higher bromate concentrations and either low (8 mg L^{-1} as NO_3^-) or no nitrate in the influent, conditions possibly expected to favour predomination of strains capable of reducing bromate over denitrifiers. The current study was not designed to achieve maximal bromate reduction rates, instead investigating bromate reduction at realistic bromate and nitrate influent concentrations.

A comparable study investigating perchlorate reduction in a fixed film bioreactor concluded regular (weekly) backwashing was essential in both sand and plastic media reactors (Min et al., 2004). Backwashing was not carried out for the duration of current trials, and no detrimental effect in operational efficiency was noted.

Table 2
Comparison of bromate removal rates in selected batch, suspended growth and fixed film bioreactors

Influent bromate concentration (mg L^{-1})	Type of reactor[a]	Temperature (°C)	Retention/ experimental time	Removal rate (μg L^{-1} min^{-1})	Reference	Notes
1	B	20	>50 days	0.01	Hijnen et al., 1995	
5	B	20	>150 days	0.02		
0.02	F	12	18 min	0.3	Hijnen et al. 1999	
0.04	F	12	18 min	0.8		
0.04	F	12	26 min	0.7		
0.02	F	n/d	25 min	0.7	Kirisits and Snoeyink, 1999	BAC filter
0.02	F	n/d	49 min	0.4		
1.4	S	10	20 h	0.6	Butler et al., 2005	
32.9	S	20	40 h	13.7		
294.4	S	20	48 h	102.2	van Ginkel et al., 2005	
22.9	B	20.1	16 days	1.0	Current study	Phase A (Days A5–A21)
1.1	F	20.1	10 h	0.2	Current study	Phase Bii
1.1	F	20.1	40 h	0.4	Current study	Phase Bii

n/d=no data.
[a] Batch (B), suspended growth (S) or fixed film reactor (F).

The low surface area and open structure of the plastic media utilised, coupled with a continuous water flow caused by use of a mixing line would have allowed natural sloughing action to maintain a constant biofilm thickness. Negating the requirement for backwashing would reduce both capital and operation costs in a full-scale system, thereby reducing downtime.

Few comparative studies have been published on optimal packing media for anion reduction, although a variety of packing materials including glass beads (Logan and LaPoint, 2004), diatomaceous earth (Celite R-635; Giblin et al., 2000) and sand have been trialled in potable water nitrate and perchlorate bioreactors. Min et al. (2004) concluded a plastic media was easier to use than sand, which was prone to clogging due to biofilm growth, provided less consistent perchlorate removal and produced higher backpressures. Another study observed that use of a combined sand and Etapak 210 media increased denitrification efficiency by 27% over a pure sand bed (2–3 mm diameter) and reduced backwashing requirements in an autotrophic fixed-bed bioreactor (Rajapske and Scutt, 1999). Current trials showed Etapak 210 to be an effective packing media for a bromate bioremediation system, although comparative trials would be necessary to identify whether other media types allow superior reduction efficiency.

5. Conclusions

Bromate contamination of approximately 1 mg L^{-1} within real groundwater samples was successfully removed by biological reduction to bromide using acclimatised biomass in a fixed-film bioreactor with ambient temperature and addition of only a simple glucose/NH_4Cl mixture as nutrient source. Bromate was stoichiometrically reduced to bromide, with an optimum retention time of 20–40 h leading to reduction of 50–90% influent bromate. A lower RT of 10 h led to significant loss of reduction efficiency. Nitrate was also successfully reduced by >97% (20–80 h RT) from a 31 mg L^{-1} influent concentration. Batch operation during initial reactor startup was crucial to subsequent successful operation, as lack of a biofilm at commencement of continuous flow conditions apparently led to a propensity for loss of bromate reduction. Backwashing was not essential for efficient operation using an open plastic packing media.

Biological bromate reduction is currently a sparsely studied subject, and contamination of groundwater with bromate is not known as a widespread problem. However, the aquifer contamination incident described in this paper has provided an impetus for development of relevant technologies. The current process has the potential for reduction of bromate concentrations higher than those previously investigated for ozonation processes. It has shown that bromate can be remediated from groundwater under pilot-scale conditions using a fixed-film system and glucose as carbon source. Utilisation for remediation of potable water would require significant further optimisation to minimise carbon and biomass residues in treated water. However, this system is initially envisaged as a tool for source reduction in a bromate contaminated aquifer using a process of pump, treat and either reinject into the aquifer or pump to sewer. Use in this capacity could provide a simple and cost-effective methodology for gradual reduction of bromate concentration within a contaminated aquifer.

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References

Brown J, Snoeyink V, Kirisits M. Abiotic and biotic perchlorate removal in an activated carbon filter. J Am Water Works Assoc 2002;94(2):70–9.

Butler R, Godley A, Lake R, Lytton L, Cartmell E. Remediation of bromate contaminated groundwater. Proceedings of the 4th International Groundwater Quality Conference, Waterloo, Canada, July 2004, vol. 297. IAHS Publications; 2005.

DSP (Depository Services Program (Canada)). Citing internet resources (www document), http://dsp.psd.pwgsc.gc.ca/collection/H48-10-1-18-1999E.pdf (Accessed 17/3/04); 1999.

Giblin T, Herman D, Deshusses M, Frankenberger W. Removal of perchlorate in ground water with a flow-through bioreactor. J Environ Qual 2000;29(2):578–83.

Gordon G, Gauw R, Emmert G, Walters B, Bubnis B. Chemical reduction methods for bromate ion removal. J Am Water Works Assoc 2002;94(2):91–8.

Hatzinger P, Greene M, Frisch S, Togna M, Manning J, Guarini W. Biological treatment of perchlorate-contaminated groundwater using fluidized bed reactors. 2nd International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, California, USA, 22–25 May; 2000.

Hijnen W, Voogt R, Veenendaal H, van der Jagt H, van der Kooij D. Bromate reduction by denitrifying bacteria. Appl Environ Microbiolv 1995;61(1):239–44.

Hijnen W, Jong R, van der Kooij D. Bromate removal in a denitrifying bioreactor used in water treatment. Water Res 1999;33(4):1049–53.

Hiscock K, Lloyd J, Lerner D. Review of natural and artificial denitrification of groundwater. Water Res 1991;25(9):1099–111.

Hutchinson T, Hutchings M, Moore K. A review of the effects of bromate on aquatic organisms and toxicity of bromate to oyster (*Crassostrea gigas*) embryos. Ecotoxicol Environ Saf 1997;38(3):238–43.

Hyman M, Dupont RR. Groundwater and soil remediation: process design and cost estimating of proven technologies. Reston, USA: ASCE Press; 2001.

IARC. Potassium bromate (summary of data reported and evaluation), vol. 73. Lyon: International Agency for Research on Cancer; 1999.

Kirisits M, Snoeyink V. Reduction of bromate in a BAC filter. J Am Water Works Assoc 1999;91(8):74–84.

Kirisits M, Snoeyink V, Kruithof J. The reduction of bromate by granular activated carbon. Water Res 2000;34(17):4250–60.

Kirisits M, Snoeyink V, Inan H, Chee-Sanford J, Raskin L, Brown J. Water quality factors affecting bromate reduction in biologically active carbon filters. Water Res 2001;35(4):891–900.

Korom S. Natural denitrification in the saturated zone – a review. Water Resour Res 1992;28(6):1657–68.

Kruithof J, Meijers R. Bromate formation by ozonation and advanced oxidation and potential options in drinking water treatment. Water Supply 1995;13(1):93–103.

Kurokawa Y, Takayama S, Konishi Y, Hiasa Y, Asahini S, Takahashi M, et al. Long-term in vivo carcinogenicity tests of potassium bromate, sodium hypochlorite, and sodium chlorite conducted in Japan. Environ Health Perspect 1986;69:221–35.

Logan B. A review of chlorate- and perchlorate-respiring microorganisms. Bioremediat J 1998;2(2):69–79.

Logan B, LaPoint D. Treatment of perchlorate and nitrate-contaminated groundwater in an autotrophic, gas phase, packed-bed bioreactor. Water Res 2004;36(14):3647–53.

Marhaba T, Medlar S. Treatment/removal of bromate and bromide ions from drinking water. Proceedings AWWA Water Quality Technology Conference, Miami, USA; 1993. p. 297–320.

Min B, Evans P, Chu A, Logan B. Perchlorate removal in sand and plastic media bioreactors. Water Res 2004;38:47–60.

Nerenberg R, Rittmann B, Najm I. Perchlorate reduction in a hydrogen-based membrane biofilm reactor. J Am Water Works Assoc 2002;94(11):103–14.

Rajapske J, Scutt J. Denitrification with natural gas and various new growth media. Water Res 1999;33(18):3723–34.

Sakaji, 2002. Citing internet resources (www document), http://www.safedrinkingwater.com /community/Aerojetletter040222.pdf (Accessed 12/5/05); 2002.

Siddiqui M, Amy G. Removal of bromate during drinking water treatment by UV irradiation. IOA Regional Conference Proceedings, Zurich, Switzerland; 1994. p. 49–62.

Urbansky E. Perchlorate as an environmental contaminant. Environ Sci Pollut Res 2002;9(3):187–92.

van Ginkel C, van Haperen A, van der Togt B. Reduction of bromate to bromide coupled to acetate oxidation by anaerobic mixed microbial cultures. Water Res 2005;39:59–64.

Xu J, Song Y, Min B, Steinberg L, Logan B. Microbial degradation of perchlorate: principles and applications. Environ Eng Sci 2003;20(5):405–22.

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Remediation of bromate contaminated groundwater

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Abstract Bromate is classed by the World Health Organization as a "possible human" carcinogen, with drinking water limits of 10 μg l^{-1} now implemented in areas including the UK, United States and Canada. Concentrations within a recently discovered UK groundwater bromate plume exceed these guidelines. However, knowledge on bromate remediation is restricted to low-level potable water contamination (10–60 μg l^{-1}) as a by-product of ozonation. The current project is examining a potential groundwater remediation methodology utilising biological bromate reduction to bromide by augmentation of indigenous microbial populations. Initial trials have focused on a glucose-enhanced anaerobic suspended-growth chemostat system. A maximum bromate reduction of 0.7 mg l^{-1} was obtained under carbon excess conditions at 10°C, with specific reduction rates (≤2.83 μmol Br/Ab600 h^{-1}) low compared to denitrification (≤305 μmol N/Ab600 h^{-1}). However, further studies investigating optimized conditions have now produced an enhanced culture capable of reducing over 30 mg l^{-1} bromate within a 40 h retention time. Work is now planned or underway to investigate potential process efficiencies for both *in situ* and *ex situ* applications, and to isolate and characterize bacterial strains possessing a putative bromate reduction pathway.

Keywords bromate; bioremediation; chemostat; groundwater

INTRODUCTION

Contamination by bromate (BrO_3^-) is commonly associated with disinfection by-product (DBP) formation during ozonation of potable water, where formation may occur by oxidation of bromide via the intermediate hypobromite (BrO^-), and also indirectly by ozone via free-radical attack. Following evidence of tumour induction in rats and mice (Kurokawa *et al.*, 1986), bromate was classified as a Group 2B or "possible human" carcinogen (IARC, 1999), and legislation limiting drinking water contamination has been implemented in a number of countries. EU directive 98/83/EC (Drinking Water Directive) specifies 25 μg l^{-1} by 2003 and 10 μg l^{-1} by 2008, and both the UK Water Supply (Water Quality) Regulations 2000 and Canadian Interim Maximum Admissible Concentrations (IMAC) set an upper limit of 10 μg l^{-1}.

Bromate has not historically been noted in surface waters (Hutchinson *et al.*, 1997) or aquifers, but recent analytical advances have led to detection of bromate in the surface water environment (Kruithof & Meijers, 1995). In addition, bromate contamination of industrial origin has been discovered in a UK chalk aquifer, forming a point source contamination plume and affecting water abstraction in the area.

Although several technologies have previously been evaluated for bromate removal, including filtration (Marhaba & Medlar, 1993), UV irradiation (Siddiqui & Amy, 1994) and chemical reduction (Gordon *et al.*, 2002), most have not been evaluated beyond controlled laboratory conditions for low-level (<60 μg l^{-1}) contamination and may prove unsuitable for cost-effective implementation at aquifer scale.

Biological bromate reduction has previously been shown to occur under anoxic conditions in suspended growth (Hijnen *et al.*, 1995), fixed film (Hijnen *et al.*, 1999) and biological activated carbon systems (Kirisits & Snoeyink, 1999). Although bacterial denitrification is more energetically favourable, in the absence of high oxygen and nitrate levels bromate reduction to bromide may be hypothesized to occur by use as a terminal electron acceptor during cellular respiration. Biological nitrate and perchlorate reduction systems have already demonstrated the potential of this approach for groundwater bioremediation (Hall, 1997; Polk *et al.*, 2001). It is therefore proposed a biological bromate reduction system using indigenous groundwater bacteria be investigated, for potential development into an *in situ* or *ex situ* groundwater remediation system.

METHODS

A two-vessel anaerobic suspended-growth chemostat was used to investigate bromate reduction following glucose augmentation of groundwater under continuous culture conditions. Two trials were undertaken, with phase I focusing on carbon and bromate influent concentrations necessary to initiate bromate reduction. Phase II subsequently used conditions experimentally determined during phase I as favourable for bromate reduction, with continuous flow maintained for 15 weeks (phase IIa) at steady states with bromate contamination of 1–128 mg l^{-1}, followed by 30 weeks (phase IIb) under more constant conditions (32 or 48 mg l^{-1} influent bromate). General operating conditions are given in Table 1.

Table 1 Operating conditions of the suspended growth chemostat system.

Parameter	Trial		
	Phase I	Phase IIa	Phase IIb
Reactor volume	2.0 l volume per reactor with slow overhead stirring		
Sparging	Nitrogen sparging to give reactor dissolved oxygen content ≤1 mg l^{-1}		
Reactor configuration	Two reactors operated as independent units	Two reactors in series	
Water source	A (see text)	B (see text)	
pH	Between 6.5–8.0	Between 6.5–8.5	
Temperature	10°C ± 3°C	20°C ± 2°C	
Residence time	20 h	40 h – reactor 1 80 h – reactors 1 and 2	
Flow rate	100 ml h^{-1} to each reactor	50 ml h^{-1} to combined system	
Bromate	0.2 / 1.4 / 78 mg l^{-1}	1–128 mg l^{-1}	32 / 48 mg l^{-1}
Glucose	10–150 mg l^{-1}	52 mg l^{-1} (both reactors)	100 mg l^{-1} (reactor 1) 52 mg l^{-1} (reactor 2)
Nitrate	30–40 mg l^{-1}	40 mg l^{-1} (spiked)	8 mg l^{-1}
Total run time	8 weeks	15 weeks	30 weeks

Influent supply for both trials was provided by separate groundwater and glucose feeds (to minimise growth in supply lines), with groundwater volumes representing 90% (phase I) or 95% (phase II) of the input. The groundwater supply was from a bromate contaminated aquifer, and was continuously augmented with nutrients ($NaH_2PO_4.2H_2O$ (300 mg l^{-1}); $MgSO_4.7H_2O$ (10 mg l^{-1}); H_3BO_4 (3 mg l^{-1}); $MnSO_4.H_2O$ (2 mg l^{-1}); $CuSO_4$ (0.4 mg l^{-1}); $ZnCl_2$ (0.2 mg l^{-1}); $CoCl_2.6H_2O$ (0.4 mg l^{-1}); $Na_2MoO_4.2H_2O$ (0.3 mg l^{-1}); $FeCl_3.6H_2O$ (1 mg l^{-1}); NH_4Cl (300 mg l^{-1})) to ensure growth limitation by either carbon (with ≤40 mg l^{-1} glucose addition) or nitrate only. No additional biomass was added.

One 10 ml sample from each reactor was taken every 2–3 days for biomass (measured spectrophotometrically at 600 nm) and pH monitoring, with steady state conditions deemed to have been reached when readings had stabilized (minimum five liquid volume changes). For most bacteria absorbance at 600 nm approximates to microbial biomass concentration expressed as grams dry weight per litre, and this relationship was assumed in this study.

Phase I was operated with the two reactors as independent systems, each with a 20-h residence time. Three influent bromate (0.2–78 mg l^{-1}) and seven glucose (10–150 mg l^{-1}) concentrations were trialled, giving 21 steady-state conditions. Phase II maintained continuous conditions for a total 45 weeks (phase IIa plus phase IIb), with the two reactors operating as one unit in series (2 × 40 h—total 80 h residence time). Influent bromate, nitrate and glucose conditions are given in Table 1. Groundwater source B was from the same contaminated aquifer as source A, but from an area with anomalously low bromate concentrations, suggested to contain bromate reducing bacteria.

Analysis of samples was undertaken upon attainment of each steady state for phase I, and every 1–2 weeks during phase II. Influent, supernatant and effluent (taken over ice to minimize further bacterial activity) samples were collected in each case. Analytical measurements and procedures are given in Table 2.

Table 2 Analytical procedures for bromate reduction chemostat trials.

Parameter	Analytical procedure
pH	Portable pH meter (Hanna HI 8424)
Temperature	Influent – Temperature probe (Jenway 9071) Supernatant – Thermometer permanently mounted within reactor
DO	Influent and supernatant only – DO (dissolved oxygen) probe (Jenway 9071)
Biomass	Optical density at 600 nm (Jenway 6505 spectrophotometer)
Carbon	Total Organic Carbon with filtered samples (0.45 μm) on Shimadzu TOC-V
Bromate and bromide	Phase I - Shimadzu vp series HPLC system with post-column reaction and UV detection at 356 nm, Metrohm MetroSupp-5 column, 200 μl injection, H_2SO_4/ammonium molybdate tetrahydrate eluent and KI post-column reagent Phase II – Dionex ICS-2500 Ion Chromatography system with AS-9 HC column, 9mM Na_2CO_3 eluent and conductivity detection
Nitrate and nitrite	Phase I – Merck cell tests (Tests 14563 (Nitrate) and 14547 (Nitrite)) Phase II – Dionex IC analysis simultaneously with bromate and bromide

RESULTS

Phase I

At all three influent bromate concentrations biomass increased with increasing glucose concentration. Soluble total organic carbon (TOC) results showed much of the glucose was utilised, leaving an effluent residual of 5–10 mg l^{-1} as glucose equivalent (influent glucose ≤52 mg l^{-1}) and 13–82 mg l^{-1} as glucose equivalent (influent glucose >52 mg l^{-1}). Denitrification occurred at all glucose and bromate concentrations, with reduction to below 0.6 mg l^{-1} nitrate (as NO_3^-) under carbon excess conditions (≥40 mg l^{-1} glucose). Partial denitrification (12.3–91.0%) was observed with glucose under 40 mg l^{-1} (carbon limitation), with nitrite production (0.02–8.9 mg l^{-1} as NO_2^-) also noted under these conditions.

Bromate removal in excess of 5% was detected at the 150 mg l^{-1} glucose, 1.4 mg l^{-1} bromate steady-state condition, and also at all glucose concentrations with the 0.2 mg l^{-1} bromate feed. With 150 mg l^{-1} glucose addition, bromate concentrations were reduced to 700 μg l^{-1} and 15 μg l^{-1} from 1.4 mg l^{-1} and 0.2 mg l^{-1}, respectively. Specific rates of bromate and nitrate reduction with 150 mg l^{-1} glucose addition are given in Table 3. These are comparable to those obtained by Hijnen *et al.* (1995), using a suspended-growth denitrifying enrichment culture containing 1 mg l^{-1} bromate, 5 mg l^{-1} nitrate and 100 mg l^{-1} ethanol at 20–25°C. The relative molar ratio (qN/qBr) obtained by Hijnen *et al.* was 174–202, compared with 108–297 obtained during the current study at 10°C. Therefore, whilst bromate reduction concomitant with denitrification did occur, bromate specific rates were comparatively very low. It is likely that, as in the Hijnen *et al.* study, reduction was due to "co-metabolic" activity of nitrate reductase within denitrifiers and not the action of specific bromate degraders.

Phase II

Following completion of phase I, the chemostat system was run under constant conditions for an extended period. It was anticipated this should provide favourable

Table 3 Comparison of selected specific bromate and nitrate removal rates by cultures under excess carbon conditions.

Phase	Retention time (hours)	Bromate in feed (mg l^{-1})	Temp (°C)	Glucose addition (mg l^{-1})	qN (μmol N g dry wt^{-1} h^{-1})	qBr (μmol Br g dry wt^{-1} h^{-1})	qN/qBr
I	20	0.2	10	150	229	0.77	297
I	20	1.4	10	150	305	2.83	108
IIa	80	1	20	52 + 52	150.06	1.76	85.49
IIa	80	64	20	52 + 52	118.46	30.33	3.91
IIb (09/01/04)	40	32	20	100	79.21	14.74	5.37
IIb (09/01/04)	80	32	20	100 + 52	23.61	13.26	1.78
IIb (15/06/04)	40	48	20	100	52.49	113.27	0.46
IIb (15/06/04)	80	48	20	100 + 52	16.04	41.80	0.38

qN, Specific nitrate removal rate; qBr, Specific bromate removal rate; qN/qBr, Relative molar ratio of specific bromate removal to specific nitrate removal.

selection pressures for high rate bromate degrading (HRBD) strains within the groundwater, should they be present. Phase IIa, using bromate concentrations between 1 and 128 mg l^{-1}, showed an increase in bromate reduction over that obtained during phase I. With an 80 h residence time, a maximum 20 mg l^{-1} bromate reduction was observed from an initial concentration of 64 mg l^{-1}, with reduction to the detection limit (5 μg l^{-1}) attained under 1 mg l^{-1} and 2 mg l^{-1} influent bromate conditions. As effluent from reactor 1 was providing influent for reactor 2 it was hypothesized nitrate was removed within reactor 1 (40-h residence time) by a largely denitrifying culture, leaving a high bromate, low nitrate water supply for reactor 2.

During phase IIb, with bromate influent set at 32 mg l^{-1} and subsequently 48 mg l^{-1}, bromate removal within reactor 1 gradually increased until reduction to below 1 mg l^{-1} was occurring with the 40-h residence time (Fig. 1). Effluent from reactor 2 (total 80 h residence time) was consistently below the bromate detection limit for the final 18 weeks. Bromide concentrations increased in concert with bromate reduction, up to a maximum of 30.66 mg l^{-1}. The overall average ratio of bromate removal to bromide increase was 0.636 (standard deviation 0.387), which is very close to the stoichiometric bromate:bromide ratio of 0.63 mg Br^- per mg BrO_3^- and confirmed total bromate reduction to bromide with no intermediate formation. Nitrate reduction continued with low (0.01–5.31 mg l^{-1}) but detectable effluent concentrations. Specific nitrate reduction rates declined from those observed in phase I of 229–305 μmol N g dry weight^{-1} h^{-1}, to 1.01–142.12 μmol N g dry weight^{-1} h^{-1} and 16.04–34.15 μmol N g dry weight^{-1} h^{-1} for 40- and 80-h residence times, respectively, although part of this reduction was due to lower influent nitrate concentrations. Nitrite production was consistently below the UK drinking water limit of 0.5 mg l^{-1}. An increase in specific bromate reduction rate from 0.77–5.54 μmol Br g dry weight^{-1} h^{-1} (phase I) to 5.31–160.51 μmol Br g dry weight^{-1} h^{-1} and 13.26–72.09 μmol Br g dry weight^{-1} h^{-1} for 40- and 80-h residence times, respectively, led to relative molar ratios (qN/qBr) within an 80-h residence time reducing to 0.38–1.78. Selected specific bromate and nitrate reduction rates are given in Table 3.

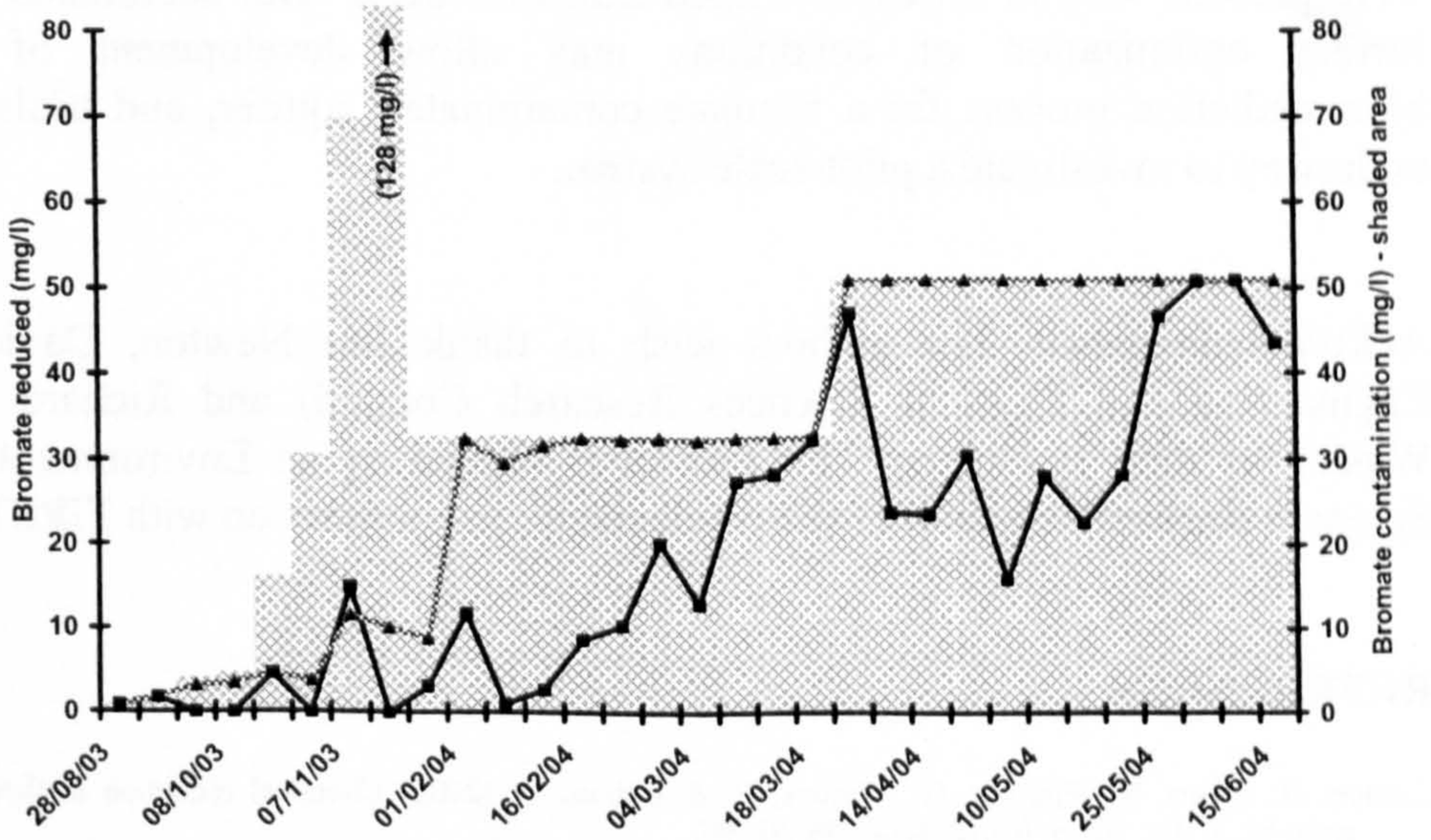

Fig. 1 Bromate reduction with 40 h (■) and total 80 h (▲) residence times under varying and stable bromate contamination (shaded area).

DISCUSSION

The suspended-growth chemostat system was running for approximately 15 months during the experimental period, which would have provided strong selection pressures for bacteria able to utilise bromate during cellular respiration. The culture produced during phase I was an efficient denitrification system, but nitrate was preferentially utilised under anoxic conditions, suggesting bromate reduction only occurred by "co-metabolism" during denitrification. Phase II was an attempt at enrichment to favour selection of high bromate reduction by putative HRBD strains and, during this time, the specific rate of bromate reduction increased by in excess of an order of magnitude.

The existence of microorganisms able to reduce bromate in a form of anaerobic respiration analogous to denitrification and conserve metabolic energy from the reaction has not currently been proven to our knowledge. However, studies into the analogous oxyanions chlorate and perchlorate have shown several strains which may have this potential (Logan *et al.*, 2001). These strains can compete for carbon, even in the presence of nitrate, and suggest chlorate respiration may be carried out at comparable rates to denitrification. The current study does not prove, but may suggest a similar mechanism for bromate.

Both *in situ* (Tompkins *et al.*, 2001) and *ex situ* (Hall, 1997; Polk *et al.*, 2001) processes for remediation of nitrate and perchlorate contaminated groundwater have been developed and trialled successfully, and any potential groundwater bromate remediation strategy would follow similar design guidelines. *Ex situ* processes have the advantage of precise control of parameters including temperature, retention time and carbon addition, all shown as controlling variables in the current study. In addition, fixed-films may prove more efficient than suspended-growth in a remediation system for bromate contaminated groundwater, as a study by Hijnen *et al.* (1999) concluded removal rates were around 10–20 times greater with a fixed-film system.

Whilst long residence times (40–80 h) were used with glucose in excess in this study, the high bromate concentrations reduced (1–48 mg l^{-1}) suggest for the first time that bacterial strains capable of specifically reducing bromate in the presence of nitrate were present. Use of an *ex situ* fixed-film bioreactor with acclimated inoculum and further optimization of conditions may allow development of an efficient bioremediation process for a bromate contaminated aquifer, and trials are currently underway to investigate a pilot-scale system.

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REFERENCES

Gordon, G., Gauw, R., Emmert, G., Walters, B. & Bubnis, B. (2002) Chemical reduction methods for bromate ion removal. *J. Am. Water Works Assoc.* **94**, 91–98.

Hall, T. (1997) *Water Treatment Processes and Practices*. WRc, Swindon, UK.

Hijnen, W., Voogt, R., Veenendaal, H., van der Jagt, H. & van der Kooij, D. (1995) Bromate reduction by denitrifying bacteria. *Appl. Environ. Micro.* **61**, 239–244.

Hijnen, W., Jong, R. & van der Kooij, D. (1999) Bromate removal in a denitrifying bioreactor used in water treatment. *Water Res.* **33**, 1049–1053.

Hutchinson, T., Hutchings, M. & Moore, K. (1997) A review of the effects of bromate on aquatic organisms and toxicity of bromate to oyster (*Crassostrea gigas*) embryos. *Ecotox. Environ. Safety* **38**, 238–243.

IARC (1999) Potassium Bromate (Summary of Data Reported and Evaluation) (www document). http://www-cie.iarc.fr/htdocs/monographs/vol73/73-17.html.

Kirisits, M. & Snoeyink, V. (1999) Reduction of bromate in a BAC filter. *J. Am. Water Works Assoc.* **91**, 74–84.

Kruithof, J. & Meijers, R. (1995) Presence and formation of bromate in Dutch drinking water treatment. *Water Supply* **13**, 117–125.

Kurokawa, Y., Takayama, S., Konishi, Y., Hiasa, Y., Asahini, S., Takahashi, M., Maekawa, A. & Hayashi, Y. (1986) Long-term *in vivo* carcinogenicity tests of potassium bromate, sodium hypochlorite, and sodium chlorite conducted in Japan. *Environ. Health Persp.* **69**, 221–235.

Logan, B. E., Zhang, H., Mulvaney, P., Milner, M. G., Head, I. M. & Unz, R. F. (2001) Kinetics of perchlorate- and chlorate respiring bacteria. *Appl. Environ. Micro.* **67**(6), 2499–2506.

Marhaba, T. F. & Medlar, S. J. (1993) Treatment/removal of bromate and bromide ions from drinking water. In: *Proc. AWWA Water Qual. Technol. Conf.* (Miami, Florida, USA).

Polk, J., Murray, C., Onewokae, C., Tolbert, D. E., Togna, A. P., Guarini, W. J., Frisch, S. & Del Vecchio, M. (2001) Case study of *ex situ* biological treatment of perchlorate-contaminated groundwater. In: *Proc. fourth Tri-services Environ. Tech. Symposium* (18-20 July 2001, San Diego, USA).

Siddiqui, M. & Amy, G. (1994) Removal of bromate during drinking water treatment by UV irradiation. In: *IOA Regional Conf. Proc.* (Zurich, Switzerland, 31 August–2 September 1994).

Tompkins, J., Smith, S., Cartmell, E. & Wheater, H. (2001) *In-situ* bioremediation is a viable option for denitrification of chalk groundwaters. *Quart. J. Eng. Geol. Hydrogeol.* **34**, 111–125.