Cranfield University

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PosiDAF for Algae Removal

School of Applied Sciences

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ABSTRACT

During algae blooms, coagulation is frequently unsuccessful resulting in poor flotation due to complex algal character. This thesis explores the link between algal character and conventional treatment and the potential for developing more appropriate algae treatment technologies. Specifically, dissolved air flotation (DAF) that has been adapted by dosing cationic chemicals to the saturator to modify bubble surfaces, such that it does not rely on coagulation, is investigated. This process is termed PosiDAF.

Analysis of dissolved algogenic organic matter (AOM) extracted from problematic species enabled investigation of the impact of morphology and AOM on coagulation-flocculation-flotation. Both increasing surface area and charge density of algae systems were related to increasing coagulant demand. Application of the appropriate coagulant demand ensured removal of all three components – cells, AOM and coagulant. Maintaining the zeta potential between -10 mV and +2 mV ensured optimum removal was obtained.

PosiDAF trials were conducted by dosing chemicals that had previously been shown to alter bubble charge, including coagulant, surfactant and polymer, to the saturator. Coagulants were unsuitable for use in PosiDAF as they did not remain at the bubble surface. Highly hydrophobic, cationic surfactants were observed to remove cells according to a theoretical model, such that removal improved with increasing bubble:particle ratio and with cell size. The polymer, polyDADMAC, achieved greater removal efficiencies than those predicted theoretically, attributed to an increase in the swept volume of the bubble. However, polyDADMAC was sensitive to changes in AOM composition. A chemical that combines attributes of both surfactant and polyDADMAC may overcome the barriers to PosiDAF implementation.

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ABBREVIATIONS AND NOTATION

Abbreviations

AOM – Algogenic Organic Matter

AF – *Asterionella formosa*

AS – Aluminium sulphate

BKC – Benzylkonium chloride

CB – Cyanobacteria

CCAP – Culture Collection for Algae and Protozoa

CMC – Critical Micelle Concentration

COCODAFF – Counter Current Dissolved Air Flotation and Filtration

CTAB – cetyltrimethlyammonium bromide

CV – *Chlorella vulgaris*

D - Diatoms

DAF – Dissolved Air Flotation

DI - Deionised

DiAF – Dispersed Air Flotation

DOC – Dissolved Organic Carbon

DOM – Dissolved Organic Matter

DBP – Disinfection Byproduct

DSS – Dodecyl sodium sulphate

DTAB – Dodecyltrimethylammonium bromide

DWI – Drinking Water Inspectorate

EM – Electrophoretic Mobility

EEM – Excitation-Emission Matrix

EPS – Extracellular Polymeric Substances

FAF – Fulvic Acid Fraction

FC – Ferric chloride

FS – Ferric sulphate

G – Green algae

GAC – Granular Activated Carbon

HAF – Humic Acid Fraction

HPI – Hydrophilic

HPO – Hydrophobic

IC – inorganic carbon

i.e.p. – isoelectric point

MA – *Microcystis aeruginosa*

MCLR – Microcystin-LR

MIB – Methylisoborneol

Msp. – *Melosira* sp.

MTAB – myristyltrimethylammonium bromide

MW – Molecular Weight

NOM – Natural Organic Matter

NTU – Nephelometric Turbidity Units

OSS – Octadecylsodium sulphate

OTAB – Octadecyltrimethyl ammonium bromide

PAC – Polyaluminium chloride

PAHCS – polyaluminium hydrogen chloride silicate

PEI - Polyethyleneimine

PFS – Polyferric sulphate

polyDADMAC – polydiallyldimethylammonium chloride

PVSA – Poly (vinylsulphonic acid) sodium salt

RGF – Rapid Gravity Filter

rpm – Revolutions per minute

SSA – Specific Surface Area

SSF – Slow Sand Filter

SMP – Soluble Microbial Products

SUVA – Specific Ultraviolet Absorbance

TC – Total Carbon

THM - Trihalomethane

THMP – Trihalomethane Precursor

TOC – Total Organic Carbon

TPI - Transphilic

UK – United Kingdom

WHO – World Health Organisation

WTW – Water Treatment Works

ZP – zeta potential

Notation

a – Activity

C - Concentration

d_b – Bubble diameter

G – Velocity Gradient

R_r – Recycle Ratio

ΔG – Standard Free Energy Change

γ – Surface Tension

Γ – Surface Excess

λ - Wavelength

κ – Debye-Hückel parameter

σ – Floc strength constant

α_s – Dissociation constant

$n_{p,e}$ – Number of particles in the effluent water

$n_{p,i}$ – Number of particles in the influent water

α_{pb} – Attachment efficiency

η_T – Dimensionless particle transport coefficient (Collision efficiency)

ϕb – Bubble volume concentration

v_b – Bubble rise velocity

t_{cz} – Time the bubble spends in the contact zone

CHAPTER 1: INTRODUCTION

1. INTRODUCTION

1.1 PROJECT BACKGROUND

Algae are photosynthetic organisms that survive in aquatic environments by utilising inorganic nutrients such as nitrogen and phosphorus (Manahan, 2000). They are ubiquitous in reservoirs and rivers supplying water treatment works (WTW) but do not interfere with unit processes whilst present at relatively low population densities. However, continuing eutrophic conditions, in addition to favourable environmental factors including photoperiod and wind activity, encourage seasonal algal blooms (Casterlin and Reynolds, 1977; Canovas *et al.*, 1996). During such events, algae populations can increase dramatically over a relatively short time span, significantly affecting the efficiency of operations. For example, increased coagulant demand and filter clogging are frequently reported (Bernhardt, 1984; Mouchet and Bonnélye, 1998). Additionally, drinking water produced during algae blooms can be of relatively poor quality as a result of: a) carry over of micro-cells and coagulant (Cheng and Chi, 2003); b) the generation of trihalomethane precursors (THMP) from chlorination of algal cells and associated algogenic organic matter (AOM) (Graham *et al.*, 1998); c) the release of offensive taste and odour compounds (Rosen *et al.*, 1992; Kim *et al.*, 1997); and d) toxin release (Haider *et al.*, 2003). Hence, it is important that algae are removed from the influent water during the initial treatment stages to ensure minimal impact on subsequent processes. Furthermore, the removal process should ideally remove the cells intact to contain undesirable toxins and taste and odour compounds.

Many WTWs in the UK utilise a combination of coagulation and flocculation followed by dissolved air flotation (DAF) for algae removal. DAF is frequently used to treat algae laden water as it takes advantage of both their natural tendency to float and the very low density flocs that form on coagulation (Haarhoff and Edzwald, 2004). The DAF process removes particles using microscopic bubbles that are produced by saturating recycled water with air at high pressure and subsequently releasing it at atmospheric pressure (Figure 1.1). The generated bubbles collect

particles, raising them to the surface. Particle collection is therefore related to bubble-particle collision and attachment efficiencies which are increased by enlarging particles and reducing the energy barrier to contact respectively (Haarhoff and Edzwald, 2004). The energy barrier is typically relatively high as typical influent pollutants including algae, natural organic matter (NOM) and clay, are negatively charged and thus electrostatically repelled by the negatively charged bubbles that are generated in the DAF process (Han and Dockko, 1999). Upstream coagulation and flocculation makes particles more floatable by improving collision and attachment efficiencies. This is achieved by the addition of a positively charged chemical, a coagulant, to the influent suspension which reduces the magnitude of the particle charge. Consequently, the system becomes destabilised and thus larger particles, called flocs, are formed (Gregory, 2006) and, most importantly it is claimed, the energy barrier to bubble-particle contact is lowered (Han and Dockko, 1999). However, inefficient flotation of algae is frequently reported by water treatment operators (Chipps, 2004; Holden, 2004) and is thus attributed to poor coagulation. Algae are active, biological entities that have widely differing morphological and physiological characteristics, as well as the ability to respond to changes in their immediate environment, making controlling cell surface charge and by inference coagulation particularly complicated (Pieterse and Cloot, 1997; Clasen *et al.*, 2000). Additionally, associated algogenic organic matter (AOM) that is excreted throughout the lifetime of an alga via metabolic processes and finally cell lysis, comprising proteins, polysaccharides (carbohydrates), lipids and nucleic acids (Fogg, 1983), has also been shown to interfere with coagulation (Bernhardt *et al.*, 1985). There have been only limited attempts to link the character of various algae species to treatment, the most comprehensive focussing on direct filtration (Bernhardt and Clasen, 1991). Furthermore, in contrast to NOM systems where the link between organic matter character and treatment is well known (Sharp *et al.*, 2006), there is a lack of understanding as to the most important characteristics of AOM with respect to water treatment and how this varies with species. Further research is therefore required to determine the impact of algae and associated AOM character on coagulation and flotation.

Difficulties experienced in coagulation have led to the conception of an alternative procedure, utilising collector surface modification rather than particle surface modification in physical removal processes including DAF and depth filtration. For example, a positively charged bubble and a negatively charged cell may similarly attract as a negatively charged bubble and positively charged algae cell do in conventional processes. Controlling collector surfaces might provide a generic solution which is less dependent on influent particle character, a particular advantage in the case of algae. To date, the majority of research in this regard has been undertaken with respect to depth filtration (Truesdail *et al.*, 1998). However, adaptation of filter media has proved difficult as contamination of the functionalised surfaces readily occurs, greatly reducing the lifetime of media if influent loadings are high (Chen *et al.*, 1998). This particular problem is averted in the case of the former as the collector surface is constantly renewed. Furthermore, the modification of a bubble surface by chemical addition enables existing technologies to be adapted on a seasonal basis.

Bubble surface modification may be achieved by dosing chemical direct to the saturator (Figure 1.1). Specifically, the addition of positively charged chemicals to functionalise the bubble surface is appropriate due to the negatively charged influent particles, and as such the process will be referred to as PosiDAF. Much research has focused on the generation of positively charged bubbles using metal coagulants (Li and Somasundaran, 1991; Han *et al.*, 2006), cationic surfactants (Kubota and Jameson, 1993; Cho *et al.*, 2005) and polymers (Malley, 1995). Only the latter study then applied the modified bubbles to particle removal by saturator dosing, where it was determined that for water comprising low concentrations of turbidity and humic acid, removal efficiency was comparable to that achieved by conventional techniques. Furthermore, when used in addition to conventional coagulation, removal efficiency of both humic acid and turbidity was always enhanced. DAF efficiency is a function of size in addition to charge, where a minimum influent particle diameter of 10-30 μm is frequently reported (Han and Dockko, 1999; Haarhoff and Edzwald, 2004). Hence, implementation of bubble surface modification may either completely remove the requirement for upstream coagulation for the majority of algae cells, which have a diameter of greater than 10 μm and are thus larger than colloidal natural organic matter (NOM) and turbidity, or be used as an additional treatment stage to

enhance removal and perhaps lower coagulant demand. Research on the application of PosiDAF for algae treatment is required to determine the potential for its commercial implementation.

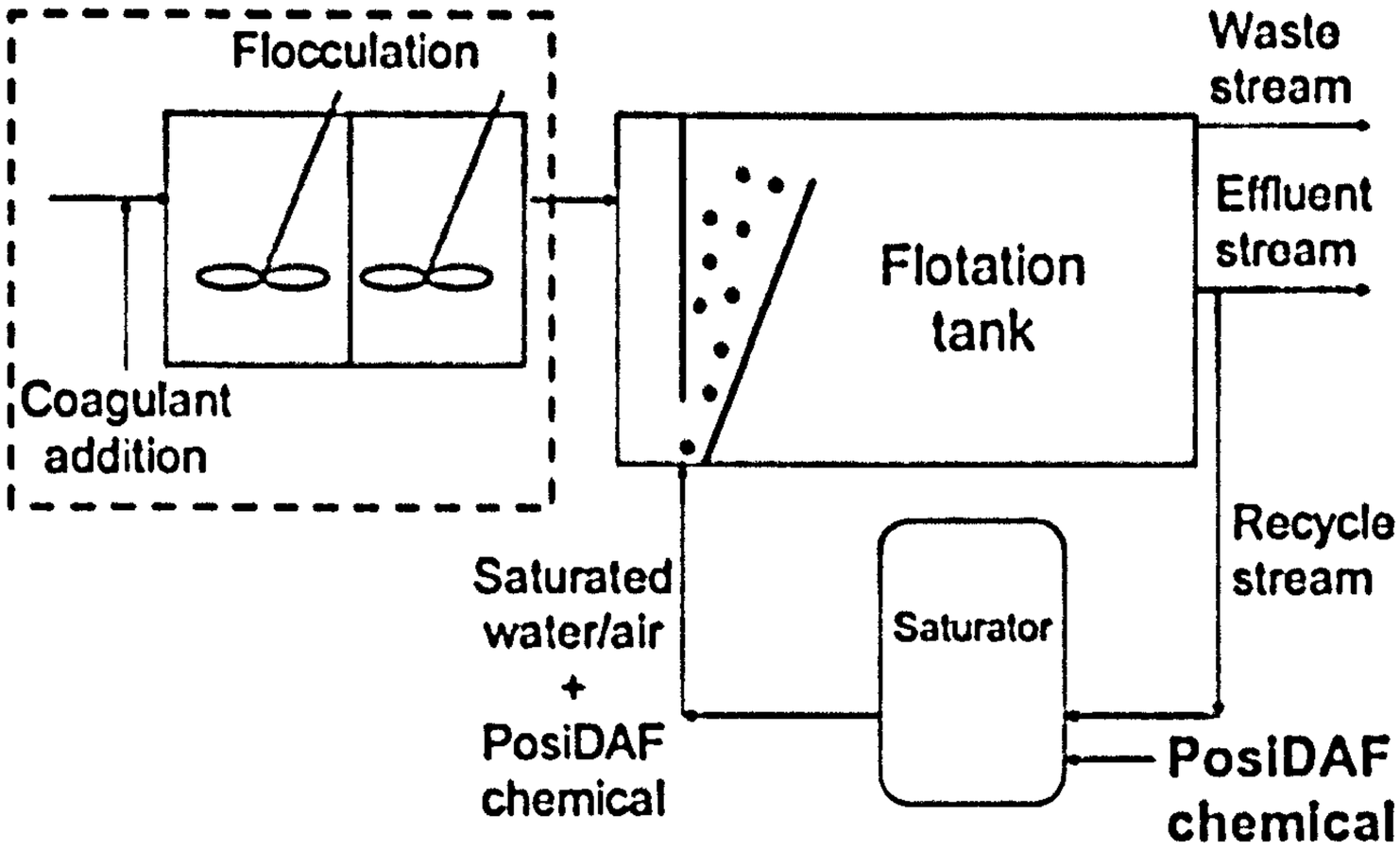

Figure 1.1 *A sketch demonstrating conventional unit operations of coagulation and flocculation with downstream DAF and the anticipated retrofit for operating as PosiDAF.*

1.2 PROJECT DEVELOPMENT

The work presented in this thesis brought together water companies including Anglian Water, Northumbrian Water, Thames Water Utilities Ltd. and Yorkshire Water as well as expertise from Cranfield University. The project initially investigated the treatment issues relating to algae blooms, identifying problematic species, and how algae character impacted on the removal efficiency by coagulation and flotation. Subsequently, the potential for applying PosiDAF for algae treatment was investigated through bench-scale investigations. Attention was paid to the influence of chemical functionality on removal efficiencies and in turn the impact of variable algae character on removal by PosiDAF.

1.3 AIMS AND OBJECTIVES

It is hypothesised that through a better understanding of the characteristics of algae systems the treatment capabilities of existing technologies can be enhanced and new technologies developed. The aim of this thesis is therefore to characterise algae systems and consequently examine the potential for linking character to treatment and developing novel treatment technologies. Accordingly, a series of objectives were identified:

1. To assess current knowledge on how varying algae characteristics impact on treatment processes used for algae removal.
2. To characterise key species for use in subsequent investigations.
3. To determine whether algae character can be linked to conventional treatment methods and treatability.
4. To investigate whether chemical dosing to the saturator can result in algae removal without pre-coagulation.
5. To investigate the link between chemical character and removal using PosiDAF.

1.4 THESIS PLAN

This thesis is presented as a series of chapters formatted as papers for publication. All papers were written by the primary author, Rita K. Henderson, and edited by Dr. Bruce Jefferson (supervisor). All experimental work was completed by Rita K. Henderson.

Initially, a literature review was conducted investigating how changing algae character influenced the treatability of algae by conventional chemical and physical processes and further evaluated whether algae could be categorised according to character that was applicable from a water treatment perspective as opposed to that of a biological viewpoint (Chapter 2, Paper 1 – submitted: Henderson, R. K., Parsons, S. A. and Jefferson, B. *The impact of variable algae functionality on treatment.* Water Research.). Further comprehension of algae treatment capabilities in the UK was

obtained by examining available historical data provided by the participating water companies. This allowed identification of the major problematic species which could then be further investigated in the ensuing experimental work (Chapter 2, Paper 2 – In press: Henderson, R. K., Chipps, M., Cornwell, N., Hitchins, P., Holden, B., Hurley, S., Parsons, S. A., Wetherill, A. and Jefferson, B. *Experiences of algae in UK waters: a treatment perspective.* Water and Environment Journal).

The character of algae was investigated by firstly making comparisons to colloids also found in raw water – NOM and clay. This gave further insight into how to proceed with respect to characterisation methods by knowledge transfer from these well-understood systems (Chapter 3, Paper 3 – published: R. Henderson, E. Sharp, P. Jarvis, S. Parsons and B. Jefferson (2006). *Identifying the linkage between particle characteristics and understanding coagulation performance.* Water Science and Technology: Water Supply, 6 (1), 31–38). Secondly, the AOM from all algae species was extracted and analysed for key components in order to allow comparisons between different species and also with NOM and soluble microbial products (SMP) from activated sludge biomass (Chapter 3, Paper 4 – submitted: Henderson, R. K., Baker, A., Parsons, S. A. and Jefferson, B. *Characterisation of algogenic organic matter (AOM) extracted from cyanobacteria, green algae and diatoms.* Water Research).

Chapter 4 then explored the coagulation and conventional flotation of the identified problematic species and how treatability related to algae characteristics. Examination of the conventional coagulation and flotation of these same algae species thus allowed evaluation as to the influence of both components – cell structure and AOM – on coagulation, and further enabled relationships between algae character and operational parameters, specifically coagulant dose, to be determined (Chapter 4, Paper 5 – submitted: Henderson, R. K., Parsons, S. A. and Jefferson, B. *Coagulation and flotation of algae: Impact of differing cell and algogenic organic matter (AOM) character.* Environmental Science and Technology). Based on the trends observed between coagulant demand and charge, the final paper of this chapter investigated the potential for utilising zeta potential measurements as a monitoring variable for

coagulation and flotation (Chapter 4, Paper 6 – submitted: Henderson, R. K., Parsons, S. A. and Jefferson, B. *Successful removal of algae using zeta potential.* Separation Science and Technology).

The final set of papers in Chapter 5 report an investigation into the potential for using PosiDAF for algae treatment. Firstly, a chemical trial was conducted by dosing various chemicals with different attributes into the saturator at different concentrations and analysing the resultant removal efficiencies. In this way, conclusions were drawn as to the most appropriate chemical characteristics for use in PosiDAF (Chapter 5, Paper 7 – ready to submit, on hold: Henderson, R. K., Parsons, S. A. and Jefferson, B. *The potential for using bubble modification chemicals in dissolved air flotation – PosiDAF.* Water Research). Having identified two types of chemicals as promising bubble surface modifiers, the final two papers examine each in turn with respect to mechanisms of removal, operational parameters and impact of varying algal character on removal (Chapter 5, Paper 8 – ready to submit, on hold: Henderson, R. K., Parsons, S. A. and Jefferson, B. *Surfactants as Bubble Surface Modifiers in the Flotation of Algae – PosiDAF.* Environmental Science and Technology; Chapter 5, Paper 9 – ready to submit, on hold: *Polymers as bubble surface modifiers in the flotation of algae– PosiDAF.* Water Research). The Intellectual Property Rights (IPR) of PosiDAF now resides with the centre's technology transfer company (Water Innovate Ltd.) and therefore the papers are on hold until the commercial opportunities have been fully explored.

The overall impact of the research with respect to algae treatment is then discussed generally, Chapter 6. A summary of the thesis plan is detailed in Table 1.1.

Table 1.1 *Summary of thesis plan.*

Chap -ter	Paper	Objective Addressed	Summary of Title	Journal	Status
2	1	1	The impact of variable algae functionality on treatment	Water Research	Submitted
	2		Experiences of algae in the UK	Water and Environment Journal	In press
3	3	2	The linkage between characteristics and coagulation	Water Science and Technology: Water Supply	Published
	4		Characterisation of AOM	Water Research	Submitted
4	5	3	Coagulation and flotation of algae: Impact of differing algae character	Environmental Science and Technology	Submitted
	6		Successful removal of algae using zeta potential	Separation Science and Technology	Submitted
5	7	4	The potential for using bubble modification chemicals in DAF	Water Research	Ready to submit – on hold
	8	5	Surfactants as Bubble Surface Modifiers	Environmental Science and Technology	Ready to submit – on hold
	9		Polymers as bubble surface modifiers	Water Research	Ready to submit – on hold
6	-	1, 2, 3, 4, 5	Implications for water treatment	-	-

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CHAPTER 2: BACKGROUND

Paper 1 Review – The Impact of Algae Functionality on Treatment

Submitted: *Water Research*

Paper 2 Experiences of Algae in the UK: A treatment perspective

In press: *CIWEM Journal*

2. BACKGROUND

2.1 REVIEW – THE IMPACT OF ALGAE FUNCTIONALITY ON TREATMENT

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ABSTRACT

This review examines the character of freshwater algal populations from a water treatment perspective and evaluates the impact of their varying character on their treatability. However, algae are traditionally classified according to biological descriptors which do not give information on surface characteristics that are important with respect to removal by water treatment processes. The characteristics shown to impact on treatment were morphology, motility, surface charge, cell density and the algogenic organic matter (AOM) composition and concentration. With the exception of density, these are not phyla specific. It was also shown that DAF was the most robust clarification method, where up to 99.8 % removal was achieved compared to 94 % for sedimentation when using cationic metal coagulants. However, successful clarification relied heavily on the optimisation of preceding coagulation and flocculation and coagulant demand was important in this respect. Comparison of all available data reveals a relationship between cell surface area and coagulant demand. It is thus suggested that cell surface area would provide a basis for regrouping algae such that the classification is informative with respect to water treatment. However, the absolute coagulant demand is a result of both surface area and AOM influences. The latter are relatively poorly understood in comparison to NOM systems and this remains a limit in current knowledge.

2.1.1 INTRODUCTION

Algae are photosynthetic, aquatic plants that utilise inorganic nutrients such as nitrogen and phosphorus (Manahan, 2000). Cyanobacteria are typically referred to as blue-green algae because they perform photosynthesis and are similar in size and colour, even though they are bacteria (World Health Organisation (WHO), 2004). Algae are ubiquitous in surface water but do not pose a problem to water treatment processes provided populations are relatively low. However, seasonal algal blooms can dramatically increase populations on relatively rapid timescales and as a result water treatment process efficiency can be impaired. On occasion this has led to the presence of algae in supply or even to the closure of a particular site. For example, in the Anglian Region of the UK, a cyanobacteria bloom of 400,000 cells mL^{-1} of *Microcystis* could not be treated, resulting in the treatment plant being out of service for an 8 week period (Greene and Hayes, 1981). Furthermore, algal cells and associated algogenic material are trihalomethane (THM) precursors (Bernhardt, 1984; Graham *et al.*, 1998), which has resulted in the restriction of chlorine usage. Similarly, the potential for toxin release by cyanobacteria, in particularly from *Microcystis*, has resulted in the World Health Organisation (WHO) setting a guideline value of 1 $\mu g\ L^{-1}$ for the associated toxin, microcystin-LR (MCLR) (WHO, 1998). Finally, the presence of offensive taste and odour compounds including 2-methylisoborneol (2-MIB) and geosmin in the resultant drinking water supply has also been attributed to high algae populations (Burlingame *et al.*, 1992; Rosen *et al.*, 1992; Kim *et al.*, 1997).

Algae are traditionally characterised according to differences in pigmentation and cell complexity arising as a result of evolution (Bellinger, 1992). Cyanobacteria existed prior to all algae phylum and are prokaryotic cells. Algae evolved as a result of primary endosymbiosis, whereby a prokaryotic cell engulfed a bacterium on two occasions to produce eukaryotic green and red algae phyla, differentiated by the quantity of chlorophyll *a* in the cell pigmentation. Current opinion is that secondary endosymbiosis, whereby eukaryotic cells engulfed other eukaryotic cells, produced phyla including diatoms, chrysophytes, cryptomonads, brown algae and

dinoflagellates (Palmer, 2003). While this classification method is satisfactory from a biologist's perspective, its usefulness with respect to water treatment is less, as algae are not grouped according to the characteristics that effect treatment processes. Within a particular phylum, the species can vary significantly in terms of their morphology and other important functionalities including the composition and quantity of excreted algogenic organic matter (AOM). This suggests that a water treatment process may well be able to successfully remove a number of species from particular phyla, while struggle with others.

It is required that algae are removed from drinking water, preferably during the initial stages to ensure minimal impact on subsequent processes. This review examines the character of freshwater algal populations from a water treatment perspective and evaluates the impact of their varying functionality, specifically their morphological, physiological as well as AOM character, on their treatability with respect to processes including sedimentation, flotation and filtration. The review will focus on the following phyla: green algae, blue-green algae and diatoms, which are regularly responsible for algae blooms in the UK. This review seeks to establish a basis for regrouping these algae such that the classification is informative with respect to water treatment.

2.1.2 ALGAE CHARACTER FROM A WATER TREATMENT PERSPECTIVE

All algae phyla have key characteristics that allow identification. For example, green algae are typified by the grass-green pigmentation that dominates as a result of the high chlorophyll *a* content, while cyanobacteria have a blue-green shade, accounting for the common reference to "blue-green algae", and diatoms are brown in colour (Bellinger, 1992). In addition to pigmentation, cell structure is important with respect to identification. Cyanobacteria are prokaryotic and as such have no internal cell organelles, such as chloroplasts. In contrast, green algae and diatoms contain chloroplasts that are arranged to give characteristic patterns that allow determination

to species level when combined with morphological and reproductive features. For example, *Spirogina* and *Zygnema* are both filamentous green algae differentiated by spiral shaped and star shaped chloroplasts respectively (John *et al.*, 2002). Additionally, diatoms have a hard outer cell wall due to high silica concentrations as well as distinct morphologies: centric and pennate. The former is cylindrical and radially symmetric, for example, *Stephanodiscus* and *Cyclotella*, while the latter is pen-shaped with bilateral symmetry, examples being *Synedra* and *Asterionella* (Cox, 1996).

Cell morphology, including shape, size, and appendages, is used for classification purposes but only once identified to phylum level as key cell shapes exist in all phyla. Single, spherical cells of less that 5 µm are common to both green algae and cyanobacteria e.g. *Chlorella* sp. and *Synechocystis minuscula* respectively (Table 2.1.1). Furthermore, single cells can colonise to produce more complex structures such as filaments. For example, the cyanobacteria *Anabaena*, the green algae *Spirogina* and the diatom *Melosira* can all form chains of cells (John *et al.*, 2002). Appendages including flagella, bristles or spines are also common among the different phyla. Spines can be found both on the green *Scenedesmus* and the diatom *Stephanodiscus* (Table 2.1.1). Of the three phyla examined in the current paper, only green algae are found with flagella facilitating motility. However, motion of a gliding nature is observed for both cyanobacteria, for example *Oscillatoria* and diatoms, known as "raphid pennates" e.g. *Navicula* (Cox, 1996).

Cell density is observed to vary from 1.07 g cm^{-3} to 1.14 g cm^{-3} for green algae and diatoms respectively (Table 2.1.1) (Edzwald and Wingler, 1990), although densities as low as 1.02 g cm^{-3} have been quoted for a "typical algal entity" (Pieterse and Cloot, 1997). In general, diatoms are heavier than the other species as a result of their silica-rich hard outer wall. However, cyanobacteria cells have the ability to adjust the content of water within the cell using gas vacuoles (John *et al.*, 2002), thus most cyanobacteria species have no absolute density and can even maintain a lower density than that of water.

Algae suspension surface charge is species but not phyla dependent, although all algae cells have a negative zeta potential at natural water pH (Table 2.1.1). This arises as a result of the dissociation of functional groups at the cell surface, particularly carboxylic acid groups in either the cell wall (Northcote *et al.*, 1958) or in AOM attached at the cell surface (Bernhardt *et al.*, 1985). Hence, algae suspensions behave according to an acidic dissociation model in that the surface charge only changes over acidic pHs (Hunter, 1994). The zeta potential of an algal cell is typically electronegative for pH 4-10, ranging from -10 mV for *Chlorella* to -35 mV for *Scenedesmus* and *Selenastrum* (Table 2.1.1). In general, an isoelectric point of around pH 3-4 is determined for all algae species (Liu *et al.*, 1999; Clasen *et al.*, 2000; Phoochinda and White, 2003). The stage of life cycle can also influence zeta potential. To illustrate, the diatom *Nitzschia* had a zeta potential of -30 mV at the initial growth phase, -35 mV in the log growth phase and -28 mV in the stationary phase (Konno, 1993) and the surface charge of *Chlorella* also became less negative on transition from log growth phase to stationary phase, measured as -1.6 to -1.4 $\mu mV\ s^{-1}\ cm^{-1}$ (-19.8 to -17.4 mV) (Edzwald and Wingler, 1990). It has been postulated that this phenomenon is due to variations in quantity and composition of AOM attached to the cell surface (Bernhardt *et al.*, 1985). This implies that it is the organic matter present that controls the surface charge as opposed to the cell surface itself.

AOM can influence the surface chemistry of mineral particles similar to natural organic matter (NOM) (Bernhardt *et al.*, 1985; Beckett and Le, 1990; Paralkar and Edzwald, 1996; Leppard, 1997), as well as promoting or inhibiting floc formation (Passow *et al.*, 1984; Bernhardt *et al.*, 1985; Kioerboe and Hansen, 1993) and chelating metal cations (Kaplan *et al.*, 1988; Gregor *et al.*, 1996; Leppard, 1997). Reported AOM concentrations range from 1.8 mg L^{-1} for the cyanobacteria *Synechocystis* to 81 mg L^{-1} from the green genus *Chlorella* (Hoyer *et al.*, 1985). Whilst actual levels vary, similar trends have been observed for all species examined to date. For example, the AOM concentration increases with the age of the algae while the uronic acid content measured within the AOM decreases with increasing population age (Figure 2.1.1). The latter observation is highly relevant with respect to algae character as it has been shown that increasing uronic acid content can be

correlated with greater metal complexing capacity whereby a marked difference in uronic acid content and hence complexing capacity was found between species of the same genera. Specifically, *Chlorella stigmatophora* and *Chlorella salina* were found to contain 30 % and 6 % uronic acid respectively (Kaplan *et al.*, 1988).

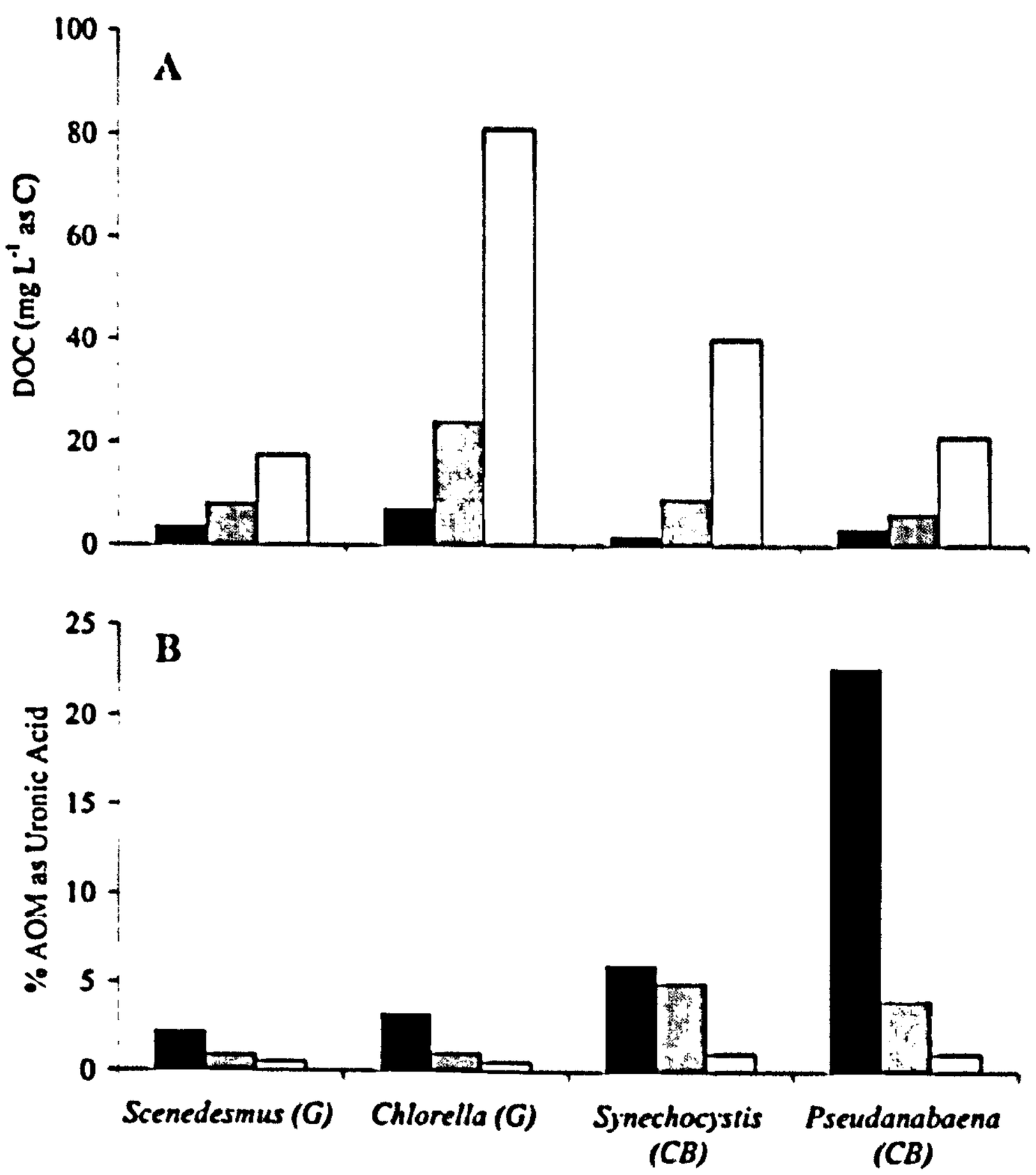

Figure 2.1.1 ***AOM character of green (G) algae, Scenedesmus and Chlorella, and cyanobacteria (CB), Synechocystis and Pseudanabaena, where A. demonstrate the increase in AOM concentration with algal age and B. demonstrates the decrease in uronic acid concentration with age (adapted from Hoyer et al., 1985).***

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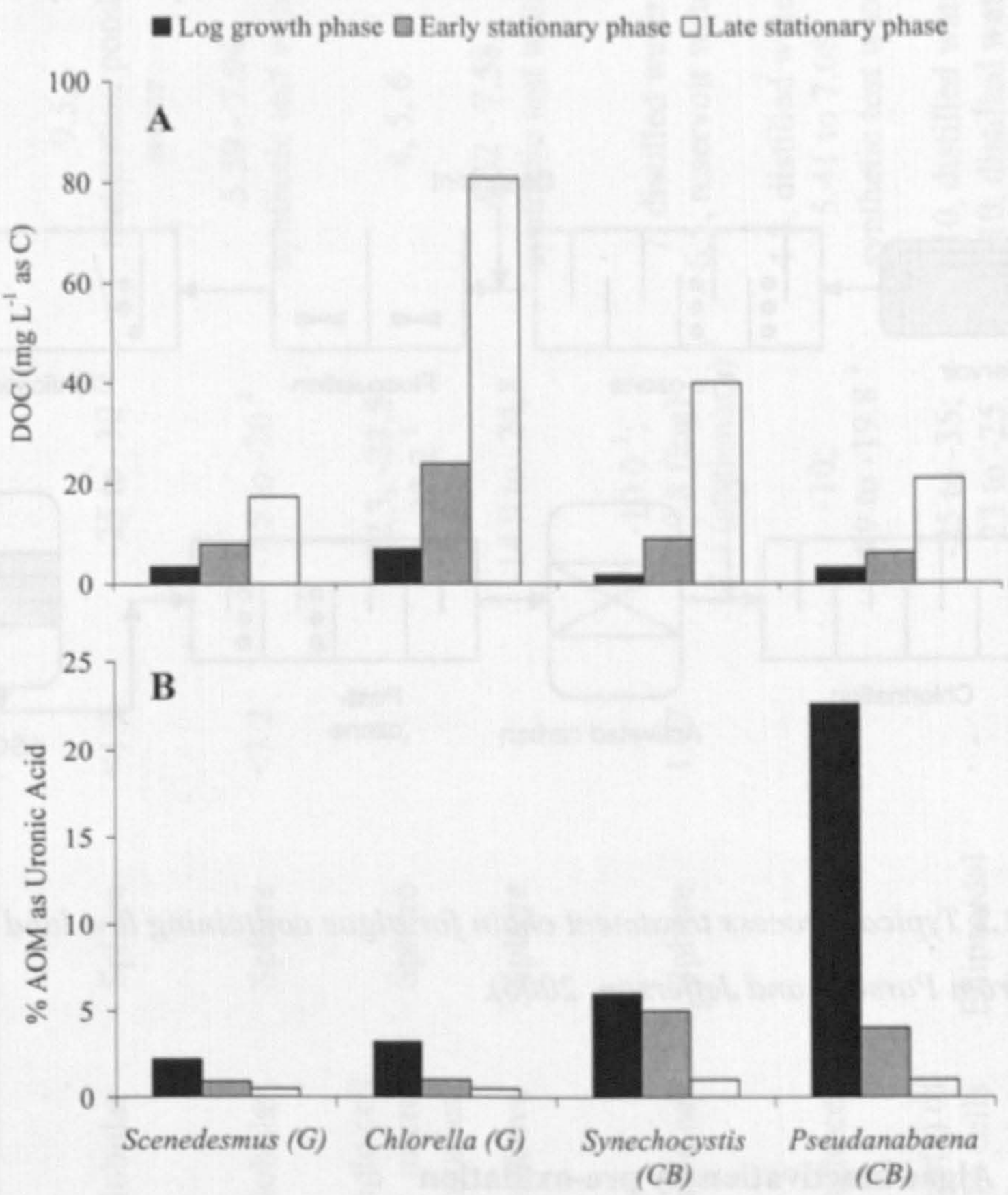


Figure 2.1.1 *AOM character of green (G) algae, Scenedesmus and Chlorella, and cyanobacteria (CB), Synechocystis and Pseudanabaena, where A. demonstrate the increase in AOM concentration with algal age and B. demonstrates the decrease in uronic acid concentration with age (adapted from Hoyer et al., 1985).*

2.1.3 ALGAE REMOVAL TREATMENT PROCESSES

Algae are typically removed using the following treatment chain: pre-oxidation, coagulation and flocculation, and clarification either by flotation, filtration or sedimentation (Figure 2.1.2). More recently, ozone and granular activated carbon (GAC) filters have been installed downstream of clarification, primarily for pesticide removal.

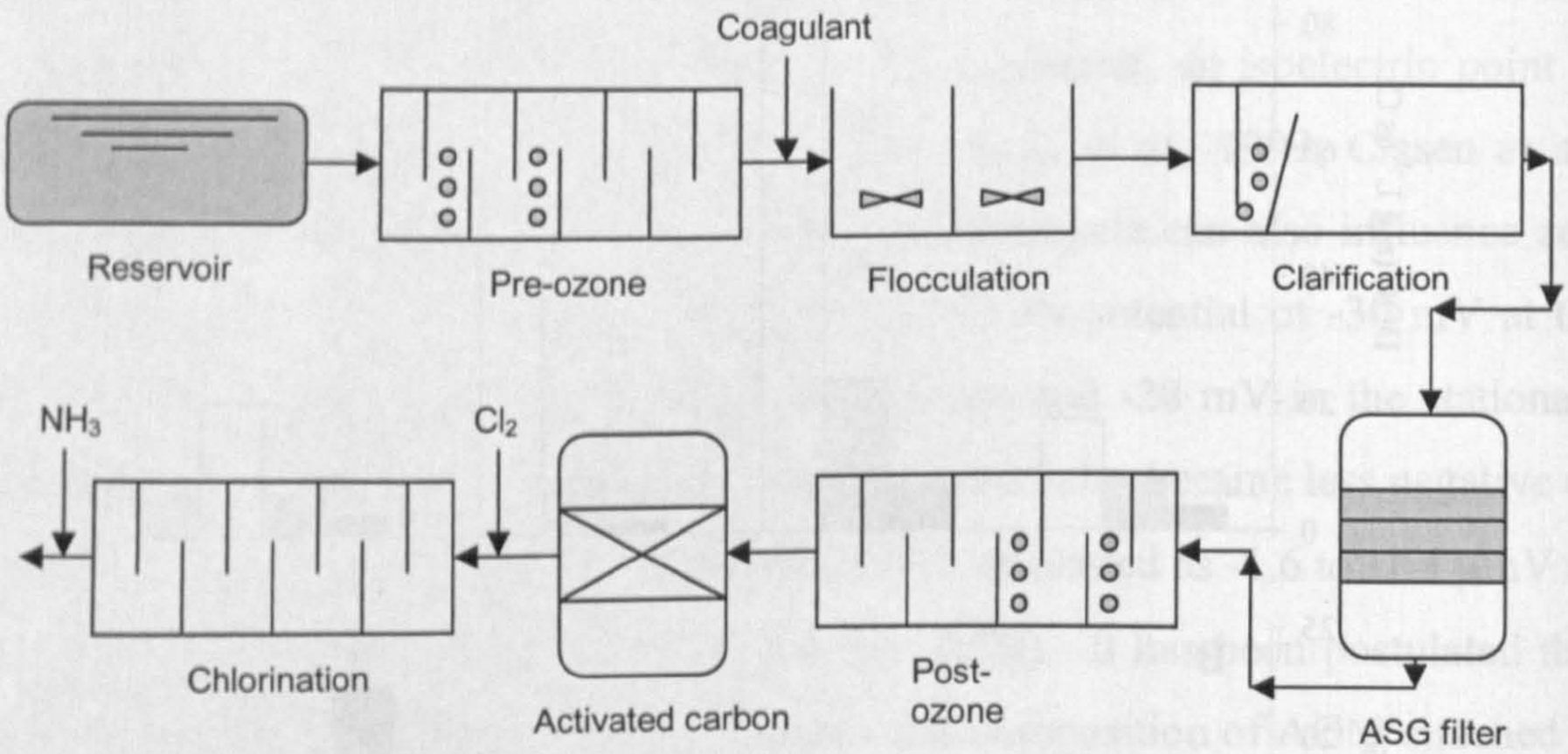


Figure 2.1.2 *Typical process treatment chain for algae containing low land water (adapted from Parsons and Jefferson, 2006).*

2.1.3.1 Algae inactivation by pre-oxidation

Pre-treatment, using oxidants such as ozone, chlorine, potassium permanganate and potassium ferrate, has been shown in many instances to improve algae removal as a result of "algal inactivation" (Table 2.1.2). For example, pre-ozonation improved *Scenedesmus quadricauda* removal by 99 % on sedimentation (Plummer and Edzwald, 2002). Improved removal was also demonstrated in a flotation/filtration pilot scale study, where inclusion of pre-ozonation increased removal of algae from 75 % to 93 % (Montiel and Welté, 1998). The improvement in algae removal has been attributed to four mechanisms as follows:

Table 2.1.1 *Characteristics of algae and other colloidal particles including size, shape, surface charge and isoelectric point (i.e.p.).*

Particle	Size (µm)	Colonial?	Shape	Density (g cm^{-3})	Zeta Potential, (mV)	Test Conditions (pH; test water)	Reference
Cyanobacteria							
Microcystis aeruginosa	3-7	Globular	Sphere	<1.2	-25 to -30	9.5, maturation pond water	Jameson (1999); Vlaški *et al.* (1996)
Microcystis	N/A	Globular	Sphere	<1.2	-7.5 to -26 [1]	5.59 - 7.94, synthetic test water	Clasen *et al.* (2000)
Synechocystis minuscula	6	Single cell or micro colonies	Sphere	-	-22.3, -28.5, -32.2 [1]	4, 5, 6	Bernhardt and Clasen (1994)
Synechocystis minuscula	5	As above	Sphere	-	-14.9 to -24.8	4.52 - 7.58, synthetic test water	Clasen *et al.* (2000)
Green algae							
Chlorella vulgaris	5.3	Single cell	Sphere	1.07	-10.0 [1]; -19.8 (log) -17.4 (stationary) [1]	7, distilled water; 6.5, reservoir water	Ives (1959); Edzwald and Wingler (1990)
Chlorella sp	3.5	Single cell	Sphere	1.07	-10; -14.9 to -19.8 [1]	4-8, distilled water; 5.41 to 7.08, synthetic test water	Liu *et al.* (1999); Clasen *et al.* (2000)
Scenedesmus quadricauda	13.1; 18 (d) 25 (l)	Row(s) of 4-16 cells with spines	Ellipsoidal	-	-25 to -35; -23 to -25	7-10, distilled water; 7-10, distilled water	Phoochinda and White (2003); Chen *et al.* (1998)

l = length; d = diameter

[1] Converted from electrophoretic mobility to zeta potential using Henry's Equation and a conversion factor of 12.4

Table 2.1.1 *Characteristics of algae and other colloidal particles including size, shape, surface charge and isoelectric point (i.e.p.) (cont.).*

Particles	Size (μm)	Colonial?	Shape	Density (g cm^{-3})	Zeta Potential, (mV)	Test Conditions (pH; test water)	Reference
Green algae							
Selenastrum capriconutum	2-3 (d), 6-8 (l)	Single cell	Crescent shaped	1.09	-35	6-10, 10^{-3}M $NaClO_4$ solution	Huang *et al.* (1999)
Diatoms							
Cyclotella sp	6.1	Chain	Sphere	1.14	-19.8 to -22.3 [1]	4-10, reservoir water	Edzwald and Wingler (1990)
Stephanodiscus hantzscii	8-20 (d), 40 (spines)	Chain	Disc shaped with spines	-	-12.4 [1]	7	Bernhardt and Clasen (1991)
Fragillaria crotonensis	2-3 (d), 40-150 (l)	Cells joined along length	Elongated	-	-18.6 [1]	7	Bernhardt and Clasen (1991)
Nitzschia linearis	35 (l)	No	Elongated	-	-30 (initial) -35 (log growth) -28 (stationary)	-	Konno (1993)
Syendra acus	4.5-6 (d), 100-300 (l)	No	Needle	1.1	-30 to -40	7.5-7.7	Jun *et al.* (2001)
Inorganic							
Kaolin	4.3; 15	N/A	Crystalline	2.67	-46; -13; -31 to 43.4 [1]	7, 10^{-3}M $NaClO_4$ solution; 7.1, tap water; 3-8, deionised water	Huang *et al.* (1999); Han *et al.* (2001); Black and Chen (1967)
Bentonite	-	N/A	Crystalline	-	-24.8 [1]	6, distilled water	Levy *et al.* (1992)
Silica	3.6	N/A	Sphere	-	-45	7, distilled water	Jameson (1999)

l = length; d = diameter

[1] Converted from electrophoretic mobility to zeta potential using Henry's Equation and a conversion factor of 12.4

a) A significant change in external cell architecture after oxidation. To illustrate, spinal appendages of green algae *Scenedesmus* and *Chlorococcum* cells become detached (Ma and Liu, 2002; Plummer and Edzwald, 2002); there may be noticeable damage to the outer cell, although cell wall perforation may not occur until after relatively high ozone doses (Plummer and Edzwald, 2002); and, row organisation of *Scenedesmus* colonies may be disturbed (Ma and Liu, 2002).

b) The motion of flagellated species including *Rhodomonas minuta, Cryptomonas* sp., *Euglena* sp. and the gliding action of species such as *Navicula* sp. and *Nitzschia* sp. is completely impeded (Petruševski *et al.*, 1996). In fact, in all cases examining the removal of flagellated algae treated with pre-oxidation removal was improved by 85 % to 95 % depending on the oxidant employed (Steynberg *et al.*, 1996).

c) The excretion of chitin containing fibrils by diatoms including *Cyclotella* and *Stephanodiscus hantzschii* has been shown to occur to a much greater degree relative to normal rate of release on pre-oxidation. This particular polymeric material has a glue-like consistency and as such behaves as a coagulant aid in a similar manner to anionic and non-anionic polyelectrolytes and thus improves agglomeration (Petruševski *et al.*, 1996).

d) AOM may be degraded such that it no longer impairs flocculation as was observed for the diatom *Fragillaria* and the blue-green species *Pseudanabaena* at a dose of 0.8 mg mg^{-1} C (Hoyer *et al.*, 1987).

However, there are a number of drawbacks to pre-oxidation. Disinfection by-products (DBPs) can form when using chlorine or chlorine dioxide, specifically trihalomethanes (THMs). For example, blooms of the diatom *Asterionella formosa* (10^5 cells mL^{-1}) and the blue-green algae *Anabaena flos-aquae* (10^6 cells mL^{-1}) produced 0.27 and 0.45 mg chloroform mg^{-1} TOC respectively from the cell material while 0.15 and 0.35 mg chloroform mg^{-1} TOC respectively were produced from associated AOM (Graham *et al.*, 1998). Ozone was investigated as an alternative oxidant; however, the dissolved organic carbon (DOC) content of the green algae *Scenedesmus quadricauda* increased by to 400 %, increasing the THM formation potential by 34 % (Plummer and Edzwald, 2002). Other alternatives are potassium permanganate and potassium ferrate oxidants however these have been demonstrated

to cause increases in residual manganese and turbidity. Petruševski *et al.* (1996) noted that permanganate dose and residual manganese were positively correlated, although it was observed that the subsequent application of cationic polymer ensured that residual manganese levels were acceptable.

Irrespective of oxidant utilised, overdosing can not only induce cell lysis, releasing undesirable toxins or taste and odour compounds, but also degrade AOM to the extent that compounds with interfering properties including mono and dicarboxylic acids and glycaric acids are formed (Hoyer *et al.*, 1987). The optimum dose is that which achieves cell modification without cell lysis and this has been shown to be species dependent. To illustrate, application of a dose of 3 mg L^{-1} of ozone to 100,000 cells mL^{-1} of the diatom *Cyclotella* did not cause significant damage to the relatively strong, silica containing, cell wall, however the same dose applied to 100,000 cells mL^{-1} of the green algae *Scenedesmus* caused significant alterations to cell morphology and in some instances induced cell lysis (Plummer and Edzwald, 2002). It was observed for the AOM of green algae *Dictyosphaerium* that a dose of as little as 0.3 mg ozone mg^{-1} AOM as C impaired flocculation (Hoyer *et al.*, 1987).

The sensitivity of pre-oxidation success in relation to algae species indicates that successful application of pre-oxidation treatment is highly dependent on an evaluation of the influent algae system. However, the recent inclusion in many water treatment works of processes such as post-ozone and granular activated carbon (GAC) allows treatment of increased DOC content that may contribute to DBP precursor formation. For example, GAC has been demonstrated to remove exo-MCLR to below the given the current guideline value of 1 μg L^{-1} as MCLR (Lambert *et al.*, 1996). It has also been demonstrated that excreted odorous compounds such as geosmin and 2-MIB can be removed by adsorption onto granular activated carbon with post-ozonation, using an empty bed contact time of 15 minutes and ozone dose of 3-5 mg O_3 L^{-1} with a hydraulic retention time of 7.5 minutes (Ando *et al.*, 1992; Kim *et al.*, 1997).

Table 2.1.2 *Observations on pre-oxidisation of algae suspensions.*

Species	Cell quantity	Oxidant	Dose (mg L^{-1})	Observations	Reference
Green Algae					
Chlamydomonas sp.	50 µg L^{-1} as chlorophyll *a*	ClO_2	0.8	90 % improvement (ascribed to immobilisation)	Steynberg *et al.* (1996)
		Cl_2	2	85 % improvement	
Scenedesmus	2×10^6 cells mL^{-1}	O_3	4.6	Coagulant demand halved	Sukenik *et al.* (1987)
		ClO_2	5	Coagulant demand quartered	
		Cl_2	2-20	Coagulant demand increased	
Scenedesmus quadricauda	20,000 cells mL^{-1}	O_3	1.2	99 % improvement	Plummer and Edzwald (2002)
		Cl_2	1.0	10 % improvement	
Scenedesmus sp.	3.9×10^8 cells mL^{-1}	K_2FeO_4	5	Get enhanced algae removal at all coagulant dosages, ~10 %	Ma and Liu (2002)
Green algae mixture	Up to 70,000 cells mL^{-1}	O_3	1	Increase in removal from 75 % to 93 %	Montiel and Welté (1998)
Diatom					
Cyclotella sp.	20,000 cells mL^{-1}	O_3	0-3	No improvement	Plummer and Edzwald (2002)
		Cl_2	0-3	No improvement	
Euglenophyta					
Euglena gracilis	50 µg L^{-1} as chlorophyll *a*	ClO_2	0.8	90 % improvement (ascribed to immobilisation)	Steynberg *et al.* (1996)
		Cl_2	2	95 % improvement	

2.1.3.2 Coagulation/Flocculation

Ives (1959) conducted some of the original research into the significance of surface charge with respect to algal coagulation. It was suggested that the coagulation mechanism was "one of mutual attraction and charge neutralisation of the algae and the incipient hydroxide flocculi" and as such the hydroxide precipitant should be positively charged. In a more recent study, the flocculation mechanism of *Synechocystis miniuscula* (diameter of 6 μm) using alum coagulant was investigated. It was determined that at pH 5 charge reversal was achieved with 7 mg L^{-1} as Al and cells started to form distinctive aggregates. It was suggested that aggregation occurred as a result of the cationic aluminium hydroxo complexes interacting with the algal surface, in accordance with the principle of adsorption coagulation with charge neutralisation (Bernhardt and Clasen, 1994).

Certain coagulation experiments have taken surface charge into account by measuring the zeta potential when examining conditions for optimal removal. On comparing three such studies (Edzwald and Wingler, 1990; Bernhardt and Clasen, 1991; Mouchet and Bonnélye, 1998), it was observed that on adjusting the zeta potential of an algal suspension with a specific operational range, removal was significantly improved (Figure 2.1.3). The range was noted to alter depending on the clarification procedure. To illustrate, for both sedimentation examples the bands are fairly narrow, with optimum removal occurring at between -5 to 0 mV and -8 to 0 mV for Seine and Nile river water respectively (Mouchet and Bonnélye, 1998). Use of flotation as opposed to sedimentation widened the zeta potential range for optimum removal; such that successful removal was obtained at more negative zeta potential ranges of -15 to 0 mV. Additionally, the residual turbidities were much lower for DAF processes than for sedimentation (Figure 2.1.3). This occurred irrespective of phyla, where green algae were represented by *Chlorella vulgaris* and *Pediastrum*, diatoms by *Cyclotella* and *Melosira* and cyanobacteria by *Synechocystis miniscula*. Furthermore, the varying morphology did not appear to impact on the requirement to operate within a specific zeta potential range for whilst *Chlorella* and *Synechocystis* are micro-algae

with spherical cells, *Cyclotella*, *Melosira* and *Pediastrum* are barrel shaped with spines, filamentous and disc shaped respectively.

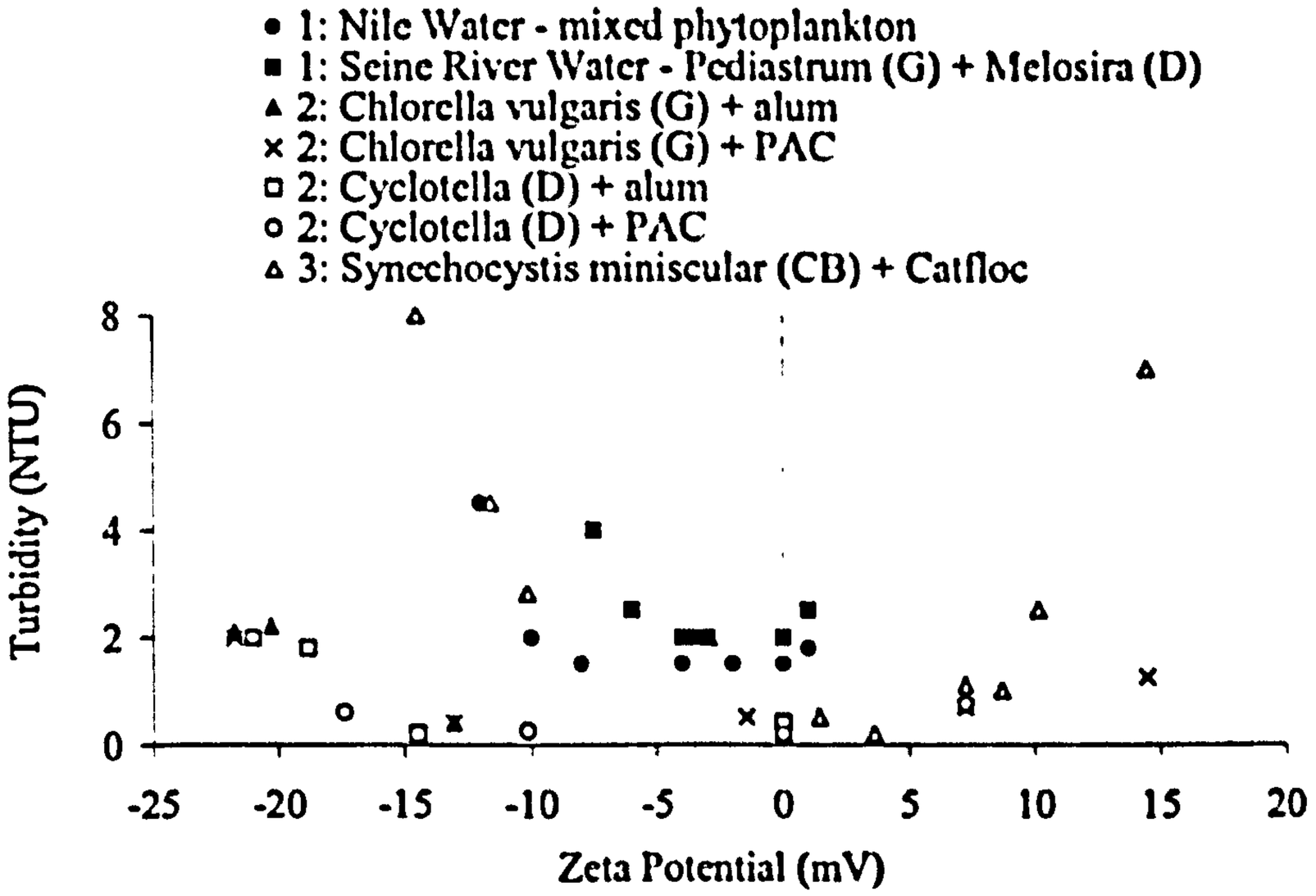


Figure 2.1.3 *Turbidity vs zeta potential for various species (CB = cyanobacteria; G = green; D = diatom), coagulants and removal processes.*

The relationship between algae cell destabilisation by reduction of the magnitude of the zeta potential and coagulant dose has been shown to be time dependent, particularly for relatively low aluminium doses (Clasen *et al.*, 2000). For example, on dosing 1 mg L^{-1} of Al to 1 × 10^6 cells mL^{-1} of the spherical cyanobacteria cells, *Synechocystis*, the zeta potential took over 6 minutes to decrease to the final value. The time lag was reduced to 2 minutes when the dose was increased to 10 mg L^{-1}. This phenomenon was attributed to algae actively influencing the surface charge by ion transfer across the cell membrane to restore the negative charge, as has been discussed in previous studies (Pieterse and Cloot, 1997; Ulberg and Marochko, 1999). It was supposed that higher Al dosages disrupted the cell repair mechanism, leading to more immediate destabilisation. This observation held for four additional species of

algae – the spherical green algae *Chlorella* and other cyanobacteria species including spherical *Microcystis* and filamentous *Pseudanabaena* and *Planktothrix*. It was further concluded that algae may therefore need more time to flocculate than other particles. This was demonstrated when comparing kaolin, natural organic matter (NOM) and the spherical green algae *Chlorella vulgaris*, where steady state floc size was achieved after four, five and 25 minutes, respectively (Henderson *et al.*, 2006). Furthermore, no observable growth was observed for the *C. vulgaris* until after seven minutes.

It has been demonstrated that the impact of some algal characteristics means removal by charge neutralisation mechanisms is unfeasible. Removal by charge neutralisation can be obtained if the algal cell is spherical, free from protruding appendages or polymeric substances and microscopic in size (Bernhardt and Clasen, 1991; Pieterse and Cloot, 1997). Deviation from this optimum conformation is common among algae cells and hence the optimum removal conditions cannot always be predicted by charge measurement data (Table 2.1.3). In many instances, even increasing the coagulant dose to enable removal by sweep flocculation mechanisms does not improve removal. For example, the diatoms *Asterionella formosa* and *Fragillaria crotonensis* are so large that filter clogging occurs rapidly and increasing coagulant addition only decreases run times (Bernhardt and Clasen, 1991). Pieterse and Cloot (1997) noted that large algal cells such as the aforementioned can no longer be treated as colloidal entities, and therefore discussion of system "destabilisation" may not be appropriate. They identified the likelihood of the presence of an additional short range force of mutual attraction (universal gravitation) favouring the coagulation process.

Table 2.1.3 *Alternative algae flocculation mechanisms (adapted from Bernhardt and Clasen, 1991).*

Algal Species	Cell Description	Removal Difficulty	Flocculation Mechanism
Diatoms			
Stephanodiscus hantzschii	Small, spherical cell with bristles of up to 40 µm in length	Steric interaction prevents direct cell contact	Sweep coagulation, hydroxide floc fills gaps between bristles – charge neutralisation occurs after 120 mg alum L^{-1} whereas 80-100 mg alum L^{-1} gives longest filter run time.
Asterionella formosa and *Fragillaria crotonensis*	Individual cells congregate to form large colonies	Easily removed, however, filter clogging and surface filtration dominate due to large size	Low dosage of alum (10 mg alum L^{-1}) required for elimination – charge neutralisation occurs when 20 mg alum L^{-1} has been dosed
Cyanobacteria			
Oscillatoria rubenscens	Small cells but large filaments reaching up to several mm in length	Large filaments exceed the size of metal hydroxide flocs	Addition of anionic or non-ionic flocculant aid reduced alum dose from 100 mg L^{-1} to 10 mg L^{-1}
Green Algae			
Dictyosphaerium pulchellum	Cell is coated in macromolecular compounds (AOM) which give surface a gelatinous consistency	Compounds have similar properties to a weak anionic polymer. On metal addition, hydroxide flocs can breakthrough filter due to chelation	As AOM acts as coagulant aid, metal dose required is significantly less, unless AOM concentration exceeds 1-2 mg L^{-1} as C
Rhodophyta			
Rhodomonas	Algae have flagella	Flagellates are able to liberate themselves from floc aggregates	Large metal salt dosages only achieve 50 % removal at best by sweep coagulation – require inactivation by oxidation

AOM acting as a polymer aid can also decrease the amount of coagulant required, for example, the green algae *Dictyosphaerium pulchellum* had an AOM composition that enhanced flocculation, when present in small concentrations (0.1-2.0 mg L^{-1} as C). However, at increased AOM concentrations (>1-2 mg L^{-1} as C) flocculation was inhibited for a dose of 3 mg L^{-1} as Fe, and only when 10 mg L^{-1} as Fe had been added was coagulation satisfactory (Bernhardt *et al.*, 1985). This was attributed to either steric hindrance or to metal complexation. Another study determined that the coagulant demand did not wholly correlate with cell surface area as 0.25 mg L^{-1} as Al was required for destabilisation of *Pseudanabaena* compared to 1 mg L^{-1} for a similar surface area of *Synechocystis* – both of which are cyanobacteria, the former filamentous and the latter spherical (Clasen *et al.*, 2000).

A variety of chemicals have been used to coagulate algal particles including trivalent metal cations (such as aluminium sulphate (AS), ferric sulphate (FS) and ferric chloride (FC)) and inorganic polymers, for example polyaluminium chloride (PAC) and polyferric sulphate (PFS). On examining the removal efficiency for various algae of differing character by sedimentation for a range of metal-based coagulants, there does not appear to be a relationship between either algae phyla or algae character and coagulant type (see Table 2.1.4 for specific test conditions) (Figure 2.1.4). For example, FS removes both *M. aeruginosa* and *A. formosa* by 62 and 63 % despite the fact that they are a cyanobacterium and a diatom, with simple spherical and complex colonial morphologies, respectively. Overall, coagulation using AS consistently achieved >75 % removal irrespective of the species (Jiang *et al.*, 1993; Jiang and Graham, 1998; Liu *et al.*, 2001; Drikas *et al.*, 2001; Jun *et al.*, 2001), contrasting to FS and FC which achieved only between 62-74 % removal respectively (Jiang *et al.*, 1993; Jiang and Graham, 1998; Jun *et al.*, 2001). This suggests that alum is less sensitive to changing algae character. However, when ferric was applied in its polymerised form (PFS), removal of each species tested was improved relative to the addition of FS by 21-27 % (Jiang *et al*, 1993; Jiang and Graham, 1998). In contrast, when alum was applied as a polymer (PAC rather than AS), a general decrease in removal efficiency of 13-14 % was observed (Jiang *et al.*, 1993; Jun *et al.*, 2001) with the exception of *Chlorella* sp. (Liu *et al.*, 2001).

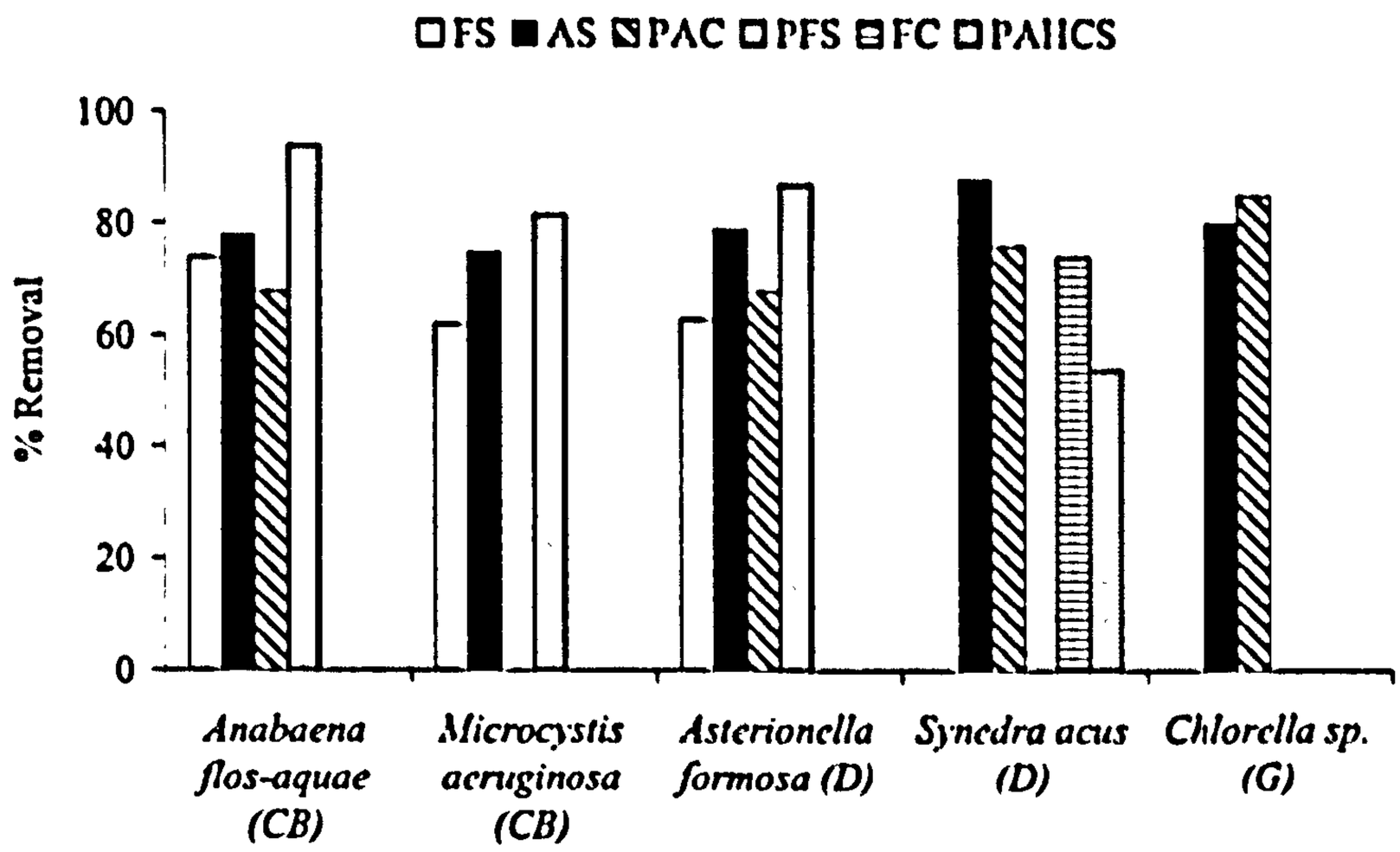


Figure 2.1.4 *Comparison of the removal efficiency by sedimentation of cyanobacteria (CB) (Jiang and Graham, 1998; Drikas et al., 2001), diatoms (D) (Jiang et al., 1993; Jun et al., 2001) and green algae (Liu et al., 1999) using ferric sulphate (FS), aluminium sulphate (AS), polyaluminium chloride (PAC), polyferric sulphate (PFS), ferric chloride (FC), and polyaluminium hydrogen chloride silicate.*

There is evidence to suggest that the use of cationic organic polymers will aid coagulation of algae – again, this appears to be irrespective of the differing algae characteristics. For example, the use of Superfloc C-573 with ferric generated a removal efficiency of 98.9 % for the spherical cyanobacteria *M. aeruginosa* (Vlaški *et al.*, 1996) whilst the use of cationic polymer C-599A alongside alum improved the removal of the needle-shaped diatom *S. acus* from 88 % (for alum alone) to 99 % (Jun *et al.*, 2001) (Table 2.1.4). However, it is noted that in the former example, filtration was also applied which is likely to have helped improve removal efficiency. Additionally, employing chitosan alone obtained removal efficiencies of 90 % for a mixture of species of differing character – the green species of spherical celled *Chlorella* and filamentous *Spirulina*, and the filamentous cyanobacteria *Oscillatoria* (Divakaran and Pillai, 2002). Conversely, the use of anionic and non-ionic polymers was not reported to improve removal of algae. Hence, it was concluded that the use of cationic polymer improved charge neutralisation and interparticle bridging thus

incorporating the cells into flocs more efficiently, producing settleable flocs of greater density, size and strength (Jun *et al.*, 2001).

2.1.3.3 Clarification processes

There are three clarification processes typically used for removing algae flocs – sedimentation, flotation and direct filtration. Overall, dissolved air flotation tends to have the most efficient removal rates, consistently removing greater than 90 % of cells. Sedimentation reliably removed between 70-80 % of cells, while it is suggested that direct filtration is the most susceptible to changing algae character (Figure 2.1.5). Filtration successfully removes the larger *Stephanodiscus hantszchii*, while micro-algae penetrate the filter to a significant extent, and motile *Rhodomonas* almost completely pervades the filter.

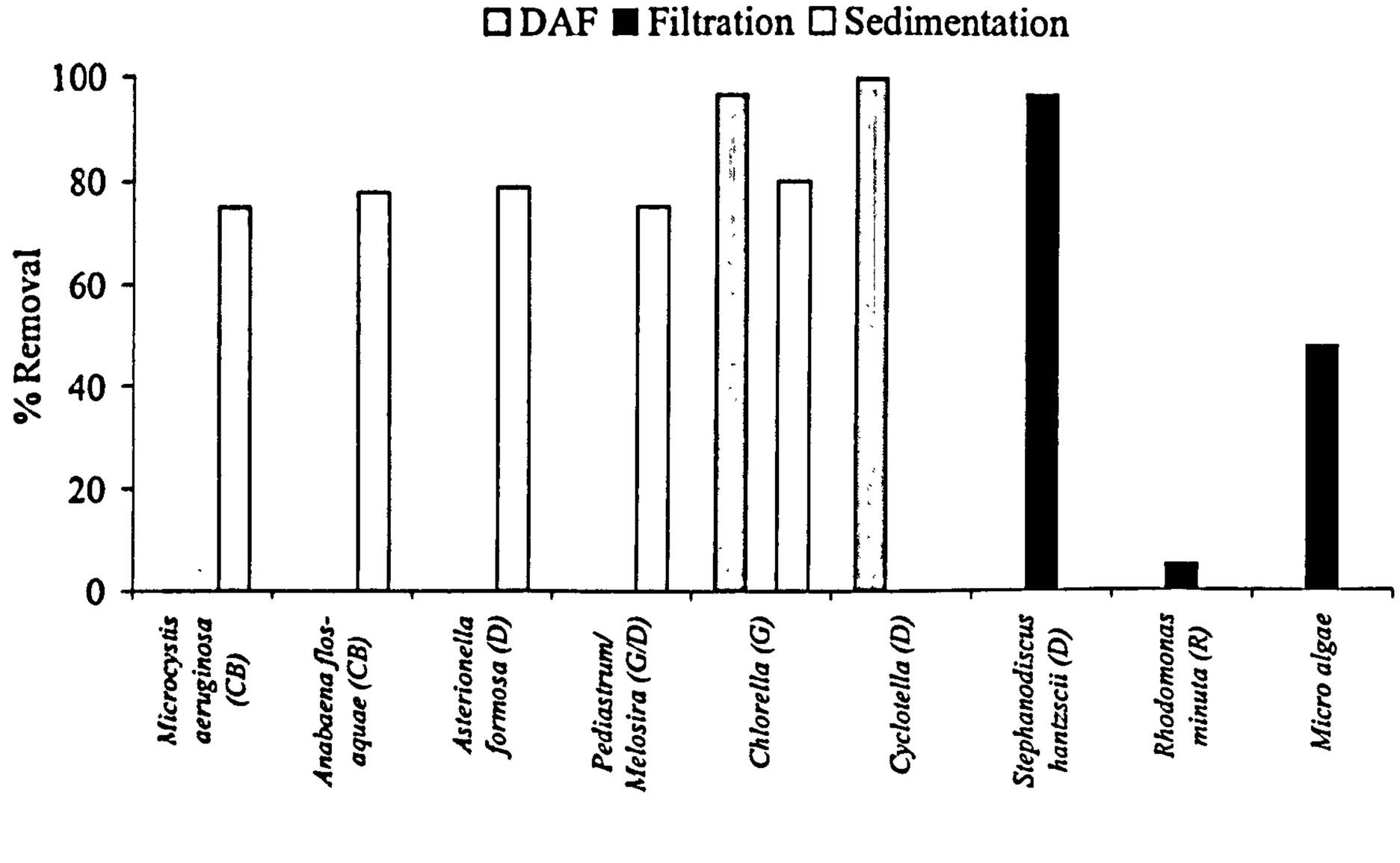


Figure 2.1.5 *Comparison of removal efficiency using DAF (Edzwald and Wingler, 1990), filtration (Petruševski et al., 1993) and sedimentation (Jiang et al., 1993; Mouchet and Bonnélye, 1998; Drikas et al., 2001) for a variety of species (CB = cyanobacteria; D = diatom; G = green; CP = cryptophyta).*

2.1.3.3.1 Sedimentation

Sedimentation is the most traditional separation technique and relies on efficient coagulation and flocculation to produce dense flocs with good settling properties (Vlaški *et al.*, 1997). It is difficult to achieve dense flocs from algae cells given the cell density can be as low as 1.02 g cm^{-3} (Pieterse and Cloot, 1997). For example, in a flotation study, Edzwald (1993) estimated that a *Cyclotella*-aluminium floc had a density of 1.1 g cm^{-3}; half that of a typical turbidity floc of approximately 2.2 g cm^{-3} (assuming a solid density of 2.67 g cm^{-3} (Huang *et al.*, 1999) and volume fraction of the solid within the aggregate of 70 %). This low density is likely to be responsible for the relatively inefficient removal rates reported for sedimentation studies of 63 % to 94 %. Improved removal (99 and 98.9 %) was only obtained when a cationic polymer was utilised and in the latter an additional filtration step (Vlaški *et al.*, 1996; Jun *et al.*, 2001) (Table 2.1.4).

In general, settlement achieved between 70 and 80 % removal efficiency for settlement times varying from 10 minutes to 2 hours when using aluminium sulphate for a variety of species (Jiang *et al.*, 1993; Mouchet and Bonnélye, 1998; Liu *et al.*, 1999; Drikas *et al.*, 2001) (Figure 2.1.5 and Table 2.1.4). Interestingly, there were no significant variations observed on the basis of morphology. For example, the spherical microscopic species, *Microcystis* and *Chlorella* (Table 2.1.1), were not removed to any greater extent than cells with the more complex structures of *Asterionella* (star shaped diatom cell colony) or *Pediastrum* (disc shaped green algal colony). This is despite the differences in algal density that would be affecting the floc properties. For example, *M. aeruginosa* contains gas vacuoles to aid buoyancy thus lowering the density whereas the diatoms *A. formosa* will have a relatively higher density as a result of its heavy cell wall (Table 2.1.1). Autoflotation has also been observed to impact on the floc settleability whereby oxygen produced by algae during photosynthetic processes can exceed the saturation level leading to bubble formation within flocs (Jodłowski, 2002), thus reducing settling rates.

Table 2.1.4 *Removal efficiency of algae by coagulation/flocculation and sedimentation.*

Algae	Source Water	Algae Quantity (cells mL^{-1})	pH	Coagulant Dose (mg L^{-1})	Rapid Mix (rpm; s)	Flocculation (rpm; min)	Settling Time (mins)[1]	% Removal	Reference
Cyanobacteria									
Microcystis aeruginosa	reservoir water	5.0×10^5 to 1.5×10^6	7.2	10.3 as Al ($Al_2(SO_4)_3$)	230; 60	25;14	15	75	Drikas *et al.* (2001)
Microcystis aeruginosa	reservoir water	1×10^4; 3-3.5 NTU	8	10 as Fe; 1.0 as Superfloc C-573	G:10^3 s^{-1}; 30	G: 30 s^{-1}; ≥30 mins	60; filtration 10 m h^{-1}; 200mm sand bed	98.9	Vlaški *et al.* (1996)
Microcystis aeruginosa	growth media	5.8×10^4; 6.1 NTU	7.5	5 as Fe ($Fe_2(SO_4)_3$)	300; 60	35; 20	60	62	Jiang and Graham (1998)
Microcystis aeruginosa	growth media	5.8×10^4; 6.1 NTU	7.5	5 as Fe (PFS)	300; 60	35; 20	60	81.6	Jiang and Graham (1998)
Anabaena flos-aquae	growth media	2×10^5	7.5	11.2 as Fe ($Fe_2(SO_4)_3$)	300; 120	35; 25	120	74	Jiang *et al.* (1993)
Anabaena flos-aquae	growth media	2×10^5	7.5	5.4 as Al ($Al_2(SO_4)_3$)	300; 120	35; 25	120	78	Jiang *et al.* (1993)
Anabaena flos-aquae	growth media	2×10^5	7.5	11.2 as Fe (PFS)	300; 120	35; 25	120	94	Jiang *et al.* (1993)
Anabaena flos-aquae	growth media	2×10^5	7.5	5.4 as Al (PACl)	300; 120	35; 25	120	68	Jiang *et al.* (1993)

[1] N.B. No loading rate data was available for Table 3.3 as all report results obtained from jar test experiments.

Table 2.1.4 *Removal efficiency of algae by coagulation/flocculation and sedimentation (cont.).*

Algae	Source Water	Algae Quantity (cells mL^{-1})	pH	Coagulant Dose (mg L^{-1})	Rapid Mix (rpm; s)	Flocculation (rpm; mins)	Settling Time (mins)[1]	% Removal	Reference
Diatoms									
Asterionella formosa	growth media	2×10^5	7.5	11.2 as Fe ($Fe_2(SO_4)_3$)	300; 120	35; 25	120	63	Jiang *et al.* (1993)
Asterionella formosa	growth media	2×10^5	7.5	5.4 as Al ($Al_2(SO_4)_3$)	300; 120	35; 25	120	79	Jiang *et al.* (1993)
Asterionella formosa	growth media	2×10^5	7.5	5.4 as Al (PAC)	300; 120	35; 25	120	68	Jiang *et al.* (1993)
Asterionella formosa	growth media	2×10^5	7.5	11.2 as Fe (PFS)	300; 120	35; 25	120	87	Jiang *et al.* (1993)
Synedra acus/ Melosira	reservoir water	1500	6.8	1.62 as Al ($Al_2(SO_4)_3$)	135; 60	45; 10	30	88	Jun *et al.* (2001)
Synedra acus	reservoir water	1040	7	2.16 as Al (PAC)	135; 60	45; 10	30	76	Jun *et al.* (2001)
Synedra acus	reservoir water	760	7	2.16 as Al (PAHCS)	135; 60	45; 10	30	54	Jun *et al.* (2001)
Synedra acus	reservoir water	1040	5.3	14 as Fe ($FeCl_3$)	135; 60	45; 10	30	74	Jun *et al.* (2001)
Synedra acus	reservoir water	1480	6.8	2.16 as Al ($Al_2(SO_4)_3$); 0.25 as +ve polymer	135; 60	45; 10	30	99	Jun *et al.* (2001)

[1] N.B. No loading rate data was available for Table 3.3 as all report results obtained from jar test experiments.

Table 2.1.4 *Removal efficiency of algae by coagulation/flocculation and sedimentation (cont.).*

Algae	Source Water	Algae Quantity (cells mL^{-1})	pH	Coagulant Dose (mg L^{-1})	Rapid Mix (rpm; s)	Flocculation (rpm; min)	Settling Time (mins)[1]	% Removal	Reference
Chlorophyta									
Chlorella sp	DI water; 0.05 M $NaNO_3$	6.8×10^5	8	8 as Al ($Al_2(SO_4)_3$)	100; 120	25; 20	30	80	Liu *et al.* (1999)
Chlorella sp	DI water; 0.05 M $NaNO_3$	6.8×10^5	7	8 as Al (PAC)	100; 120	25; 20	30	85	Liu *et al.* (1999)
Mixture									
Spirulina, Oscillatoria, Chlorella	nutrient media, tap water; 14 mg L^{-1} as $CaCO_3$	55 NTU	7	5.0 as Chitosan	5 s	60; 30	30	90	Divakaran and Pillai (2002)
Pediastrum clathratum and *Melosira*	Seine River water	$1\text{-}3 \times 10^4$	--	9.5 as Al	--	--	10	70-80	Mouchet and Bonnélye (1998)

[1] N.B. No loading rate data was available for Table 3.3 as all report results obtained from jar test experiments.

The settling rates of algae species, specifically those of two slender diatoms – *Synedra acus* and *Nitzschia linearis* – of 125 μm and 35 μm in length respectively, have been investigated (Konno *et al.*, 1993). Both cells were observed to settle vertically but at very different rates – 17 μm s^{-1} and 40 μm s^{-1} correspondingly. The sedimentation efficiency of *S. acus* was very poor, attributed to the very slow cell settling rate relative to other algae cells. Moreover, a further study investigating *S. acus* removal by coagulation/flocculation and sedimentation observed that 50 % of flocculated *S. acus* cells either remained solitary or within a non-settleable floc (Jun *et al.*, 2001).

2.1.3.3.2 Dissolved Air Flotation

Dissolved Air Flotation (DAF) has become much more popular in terms of algae removal over the last decade, taking advantage of the low density of algae (Edzwald, 1993). Another advantage with respect to flotation is that the aeration can assist in removing volatile organics providing an improved taste in the treated water (Schofield, 2001). This has relevance when considering the reduction of taste and odour compounds which are typically volatile organic substances.

Studies investigating algae of varying character, for example, the spherical cyanobacteria *M. aeruginosa* and green *C. vulgaris* and the bristled, barrel shaped diatom *Cyclotella*, showed that removal efficiencies were all in excess of 94 %, reaching maximum removal efficiencies of 99.8 % (Edzwald and Wingler, 1990; Vlaški *et al.*, 1996). Overall, flotation performs significantly better than sedimentation as removal efficiency sees a 15-20 % improvement in comparison (Figure 2.1.5). In contrast, one study found that sedimentation achieved better removal of *M. aeruginosa* (98.9 %) than flotation (94.5 %) (Vlaški *et al.*, 1996), although different operating conditions were applied. Specifically, a flocculation time of ≥30 minutes compared to 8 mins, ferric dose of 10 mg L^{-1} versus 5 mg L^{-1} and cationic polymer dose of 1 mg L^{-1} versus 0.5 mg L^{-1} for the sedimentation and flotation experiments respectively (Table 2.1.4 and 2.1.5). The robustness of the process may be attributed to the ability of DAF to float particles of 30 μm or more (Han *et al.*, 2001), such that if flocculation has not been successful, flotation of resulting small flocs and larger cells will take place.

A summary of DAF performance at several sites at Severn Trent Water determined that good removal (80-98 %) was obtained at most sites fed with eutrophic water. On one occasion only 54 % removal was achieved at a site dominated by *Volvox*, a flagellated colonial green which can exceed 1000 µm, and the large filamentous diatom, *Melosira* (Markham *et al.*, 1997). The poor performance was attributed to a lack of pre-oxidation which may have been required to immobilise *Volvox*. Furthermore, poor removal (37 %) of the filamentous cyanobacteria, *Aphanizomenon* and *Anabaena*, was recorded prior to increasing the DAF recycle ratio from 3 % to 7-10 %, upon which an improvement to over 86 % was observed (Markham *et al.*, 1997). One study investigating the simultaneous removal of various species in reservoir water observed that removal varied between 46-80 % and was very dependent on algal character (Figure 2.1.6) (Kempeneers *et al.*, 2001). The lowest removal efficiency (46 %) was observed for the motile *Chlamydomonas*, supporting the theory that flagellated algae will escape flocs and swim through the clarification process. Removal of the remaining species can be related to the cell morphology, specifically size. For example, *Synedra*, a needle-shaped diatom (Table 2.1.1), was removed by only 58 %. However, these cells have been previously reported to be very difficult to coagulate, requiring cationic polymer to obtain efficient incorporation into the floc (Jun *et al.*, 2001) and are therefore likely to be solitary. Individual *Syendra* cells are known to settle vertically (Konno, 1993), such that a rising bubble may collide only with the 5 µm wide tip of a cell, greatly decreasing the likelihood of collision and explaining the low removal rate. The low removal rate of *Asterionella* (66 %) could also be explained in this way, as in one dimension the cell width is only 2-3 µm (Table 2.1.1), despite a long cell length of 30-70 µm. The larger colonies (approximately 30-40 µm by 15-25 µm) of diatoms *Cyclotella/Stephanodiscus* and green algae *Scenedesmus* saw an increase in removal (76 % and 71 % respectively), while the largest species present, the filamentous diatom *Melosira*, was removed by 80 %.

Table 2.1.5 *Removal efficiency of algae by coagulation/flocculation and dissolved air flotation (DAF).*

Algae	Source Water	Algae Quantity (cells mL^{-1})	pH	Coagulant Dose (mg L^{-1})	Rapid Mix (rpm; s)	Slow Stir (rpm; min)	Flotation/ R_r/Bubble Concentration (min; %; ppm)	% Removal	Reference
Chlorophyta									
Chlorella vulgaris	reservoir water	$1.1\text{-}1.3\times10^5$	5.5	0.5as Al (PACl)	400; 120	30; 5	10; 8; 4600	97-99	Edzwald and Wingler (1990)
Chlorella vulgaris	reservoir water	$1.1\text{-}1.3\times10^5$	6.5	1.6 as Al ($Al_2(SO_4)_3$)	400; 120	30; 5	10; 8; 4600	96.8	Edzwald and Wingler (1990)
Diatoms									
Cyclotella sp.	reservoir water	$4.7\text{-}5.3\times10^4$	5.5	0.5-1 as Al (PACl)	400; 120	30; 5	10; 8; 4600	97-99	Edzwald and Wingler (1990)
Cyclotella sp.	reservoir water	$4.7\text{-}5.3\times10^4$	6.5	1.6 as Al ($Al_2(SO_4)_3$)	400; 120	30; 5	10; 8; 4600	99.8	Edzwald and Wingler (1990)
Cyanophyta									
Microcystis aeruginosa	reservoir water	1×10^4; 3-3.5 NTU	8	5 as Fe; 0.5 as Superfloc C-573	G:10^3 s^{-1} 30	G: 10 s^{-1}; 8 mins	5; 7; Pressure: 600 kP; Filtration: 10 m h^{-1}	94.5	Vlaški *et al.* (1996)
Mixture									
Melosira	reservoir water	1.5×10^5	7.7	1.30 as Al(PACl)	Static mix	3; > 7.2	9.5; 6; Pressure: 6 bar; Loading rate: 15 m h^{-1}	80	Kempeneers *et al.* (2001)
Cyclotella		1.2×10^6						76	
Synedra		4.0×10^4						58	
Asterionella		3.4×10^4						66	
Chlamy-domonas		4.2×10^4						46	
Scenedesmus		2.6×10^4						71	

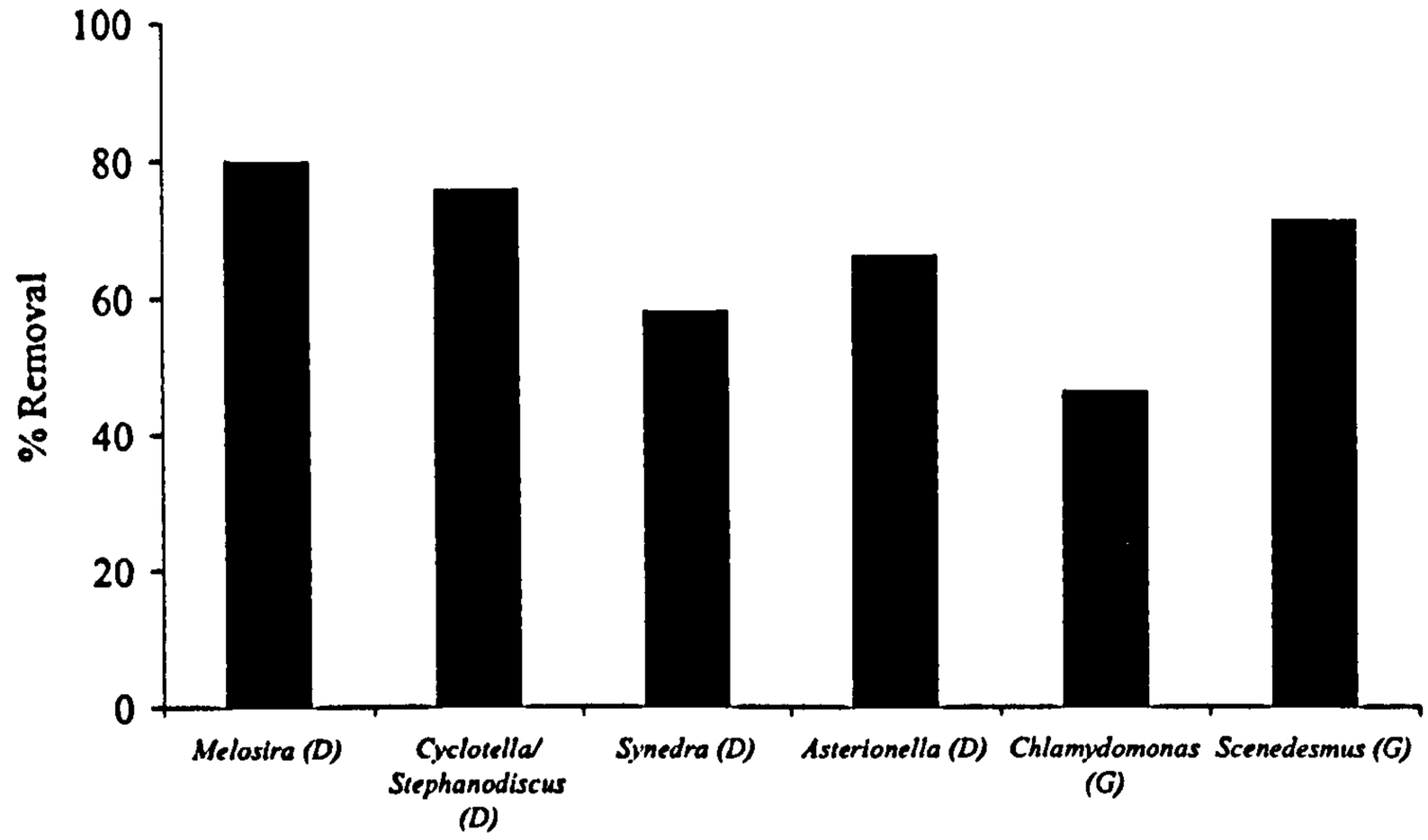


Figure 2.1.6 *% removal of algal species within a reservoir water using PAC and DAF (adapted from Kempeneers et al., 2001).*

2.1.3.3.3 Filtration

Filtration can be applied as a polishing step after either settlement or flotation or used directly as a clarification process. Direct filtration appears to be the clarification process most susceptible to variations in algal functionality (Figure 2.1.5) where one study demonstrated that small spherical green micro-algae were only removed by 48 % in a dual media filter after dosing 1 mg L^{-1} as Fe, while *Stephanodiscus hantzschii* were removed by 97 % given their larger size and *Rhodomonas minuta* were removed by only 5 % on account of their flagella (Table 2.1.6) (Petruševski *et al.*, 1993). Similarly, motile *Crypotomonas* and *Rhodomonas* could only be removed by 50 %, even after adding large doses of metal salts and metal-hydroxide formations (Bernhardt and Clasen, 1991). It was further noted that anionic flocculants did not aid removal. Filter penetration has also been observed whereby the proportion of a population of microscopic cyanobacteria increased from approximately 10-50 % of the total algal population to 85-100 % on settlement and filtration (Mouchet and Bonnélye, 1998), indicating that the filter preferentially removed other species, while these smaller cells were allowed through the filter.

A further difficulty is filter blockage by large algae species. Blockages alter the removal mechanism from one of depth to surface filtration, thus dramatically reducing

run times. For example, a cell population of 2700 cells mL^{-1} of the filamentous diatom, *Melosira*, at Wahnbach Reservoir, Germany, resulted in reduction of the filter run time from 30 to 8 hours, which was further reduced to 4 hours as a result of a simultaneous influx of smaller cyanobacteria *Coelosphaerium naegelianum* that required the addition of a much greater flocculant dose (Bernhardt, 1984). Similarly, as little as 250-1000 cells mL^{-1} of the needle-like diatom, *Synedra acus*, blocked filters resulting in run times decreasing from 35 hours to 3.5 hours (Jun *et al.*, 2001). Diatoms alone are not responsible for the problem, as the presence of flagellated colonial green algae, *Volvox*, which can exceed 1000µm, reduced run times from 24 to 11 hours (Markham *et al.*, 1997). The filterability of two green algae – the spherical *Chlorella* and colonial *Dictyosphaerium* – was compared in one bench scale study. Interestingly, while termination of filtration of the former was always a result of penetration, it was a result of blockage for the latter (Kunicane *et al.*, 1986). Additionally, the study determined that *Chlorella* was most likely to breakthrough the filter whilst in the early growth phase as opposed to its stationary phase and that as the culture age of *Dictyosphaerium* increased, a decrease in filter run time was observed from 33 to 3.5 minutes. It was observed that both cells passed through the filter in the absence of coagulation/flocculation; hence, the filter clogging effects were attributed to the specific ferric-algae floc character.

However, successful algae removal using direct filtration has been achieved. Algae were removed by 95 % using an activated carbon, sand and gravel rapid sand filter with a filtration rate of 10 m h^{-1} (Table 2.1.6) (Klute and Neis, 1983). However, in this study, the species of algae present in the tested water were not identified and hence this high removal efficiency may have been due to the absence of problematic species such as motile algae. Good rates of removal (approximately 95 %) were achieved using filtration during a pilot study conducted at Loch Leven. The filter media comprised anthracite and sand with a filtration rate of 5 m h^{-1} (Table 2.1.6). It was concluded that in order to maintain this rate of removal a coagulant dose of up to 1.68 mg L^{-1} as Al was required in addition to pre-chlorination. However, this generally resulted in the failure to achieve an acceptable aluminium residual of less than 0.1 mg L^{-1} (Johnson *et al.*, 1977).

Table 2.1.6 *Removal efficiency of algae by coagulation/flocculation and filtration.*

Algae	Source Water	Algae Quantity (cells mL^{-1})	Coagulant Dose (mg L^{-1})	Rapid Mix (rpm; s)	Flocculation (rpm; min)	Media	Loading Rate (m h^{-1})	% Removal	Reference
Cyanobacteria									
Synechococcus	reservoir water	~1.2×10^6 clumps mL^{-1}	Pre-chlorination 1.25-1.68 as Al ($Al_2(SO_4)_3$)	Flash mixing	--	0.4 m No. 2 anthracite; 0.45 m 16/30 sand	5; Run time 42 hours	95 (not consistent)	Johnson *et al.* (1977)
Diatom									
Stephanodiscus hantzscii	natural water	2000	1 as Fe ($FeCl_3$)	G:1000 s^{-1}; 30 s	G: 10 s^{-1}; 7 mins	200 mm; anthracite/ sand	10	97	Petruševski *et al.* (1993)
Green									
μ-algae (< 3μm)	natural water	3000	1 as Fe ($FeCl_3$)	G:1000 s^{-1}; 30 s	G: 10 s^{-1}; 7 mins	200 mm; anthracite/ sand	10	48	Petruševski *et al.* (1993)
Rhodophyta									
Rhodomonas minuta	natural water	500	1 as Fe ($FeCl_3$)	G:1000 s^{-1}; 30 s	G: 10 s^{-1}; 7 mins	200 mm; anthracite/ sand	10	5	Petruševski *et al.* (1993)
Algae (species not specified)	--	Various algal quantities	100 as Al (PAC); 0.5 as 423 K (50% ionic groups)	Static mix	--	0.35 m activated carbon; 0.7 m sand; 0.1 m gravel	10	95	Klute and Neis (1983)

Many Thames Water sites treating algae employ two-stage filtration and to predict process performance a "treatability index" has been implemented. The index is a function of filter loading rate and clarification coefficient such that a higher index corresponds to a shorter run length and thus a lower throughput (Ta and Woodward, 1998). The clarification coefficient was obtained by measuring the first order decay of algae concentration through a filter. In general, the highest clarification coefficients were associated with closely packed colonial algae, such as *Melosira*, or large single celled algae, such as the green species, *Closterium*. The clarification coefficient was found to be closely associated with shape and size, such that for unknown algae estimations could be deduced based on the square of the algae size. It was emphasised that this index was only applicable for waters that had not been coagulated.

2.1.4 DISCUSSION

Key algae characteristics that have been reported to influence the performance of a water treatment works include: morphology, specifically size, shape and any additional appendages; motility, either by gliding or flagellated species; surface charge as measured by the zeta potential; cell density; and AOM composition and concentration. It has been demonstrated that these characteristics in general have no relation to the phyla from which the species originates and as such monitoring algae on the basis of parameters such as cell count and chlorophyll *a* will give limited data with which to optimise treatment processes. One exception to this is density which was shown to increase in the order cyanobacteria (on account of their gas vacuoles)<green algae<diatoms (on account of their hard, silica rich cell wall).

Taking each treatment process in turn, the specific algae character that affects it can be summarised as follows:

- Preoxidation: no conclusive relationship with character
- Coagulation/flocculation: morphology, motility, AOM, surface charge
- Sedimentation: morphology, motility, density

- Flotation: morphology, motility
- Filtration: morphology, motility

Pre-oxidation studies have been inconclusive to date with respect to why certain species respond well to oxidation while others do not. However, it has been shown to have a large impact on the character of the algae, in terms of morphology, motility and EOM concentration, which has in turn a significant effect on downstream processes, particularly coagulation, for which these characteristics are critical. There is a general consensus that the use of pre-oxidation specifically for motile species is advisable to impede locomotion and facilitate downstream coagulation (Petruševski *et al.*, 1996; Steynberg *et al.*, 1996). Coagulation is effected by changes in all the various algae characteristics and thus is most susceptible to changes in influent algae species. Furthermore, as downstream clarification processes are vulnerable to algae character, specifically that of morphology and motility, if coagulation fails the treatment chain may breakdown. The most robust process is flotation, not only because of the low density of algae cells (Edzwald and Wingler, 1990), but for the reason that micro bubbles are able to float particles as small as 30 μm (Han *et al.*, 2001), providing a buffer to small flocs and larger solitary cells if coagulation is unsuccessful.

Overall, the key process to consider with respect to algae removal is coagulation. References in the literature were frequently made with respect to the importance of coagulant demand and how this could alter as a result of the algae character described, resulting in many studies investigating the optimum dose for a variety of species. There was controversy particularly over the importance of cell surface area with respect to coagulant demand. This was highlighted in one experiment where coagulant demand was measured in relation to cell surface area as opposed to the more conventional cell number (Clasen *et al.*, 2000), as a result of a previous study emphasising the importance of cell morphology (Bernhardt and Clasen, 1991). However, Clasen *et al.* (2000) concluded that there was not a relationship between cell surface area and coagulant demand. In contrast, Ta and Woodward (1998) have

developed a "treatability index" that is based on assessing influent algae according to size.

Further elucidation of the relationship between cell surface area and optimum coagulant demand is shown in Figure 2.1.7. For all analogous studies, the average cell surface area was calculated based on cell dimensions reported in the studies (where possible) and on corresponding geometric shapes, a technique used previously (Clasen *et al.*, 2000) and compared with the reported optimum coagulant doses (Table 2.1.7). Overall a reasonable log-log relationship was observed between cell surface area and coagulant demand (Figure 2.1.7) whereby very low coagulant doses per cell were required for correspondingly low cell surface areas and vice versa. This demonstrates that cell surface area can be used to predict an approximate coagulant demand, although additional data points would allow a more accurate relationship between surface area and coagulant demand to be established. However, the actual demand varies depending on other characteristics, primarily that of AOM character. It is expected that differences in AOM composition and concentration would impact on the coagulant demand either by affecting the surface charge of the algae (Bernhardt *et al.*, 1985), complexing the metal coagulant (Kaplan *et al.*, 1988) or sterically interfering with coagulation (Bernardt *et al.*, 1985; Bernhardt and Clasen, 1991). The impact of AOM thus accounts for deviations from the log-log correlation observed in Figure 2.1.7. Hence, it should be emphasised that while surface area dominates the coagulant demand, AOM interference is observable.

Overall, cell surface area provides a relatively simple basis for reassessing algae such that the classification is informative with respect to water treatment. This proposition is corroborated by the success of the treatability index already established at Thames Water, where algae are classified according to the square of the algae size (roughly approximate to the area) in order to estimate their filterability (Ta and Woodward, 1998). Cell size and surface area can therefore be used to estimate firstly the coagulant demand required to ensure optimum removal, and secondly, the filter treatment capability in the absence of coagulation.

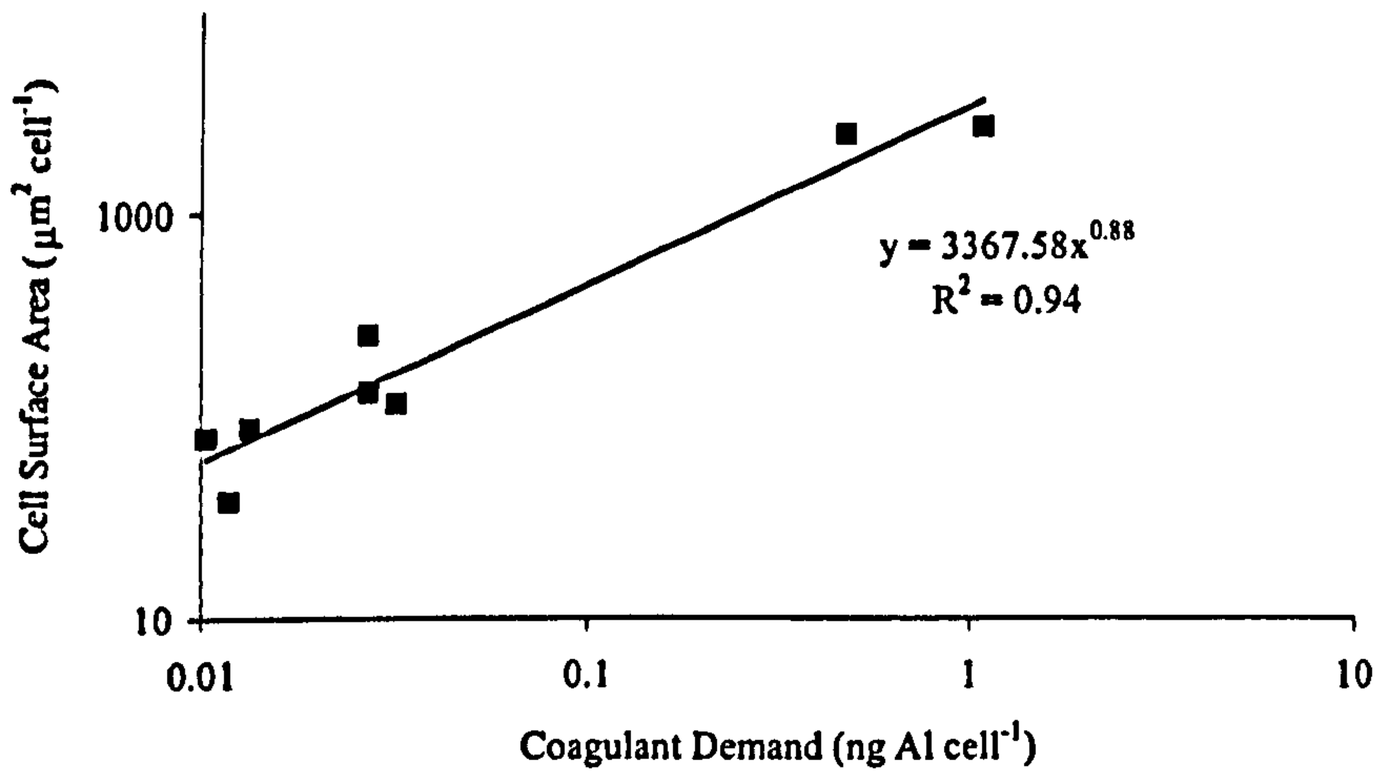


Figure 2.1.7 *The correlation between aluminium dose per cell and cell surface area as calculated in Table 2.1.7.*

It is of note that only the relationship between optimum dose of aluminium sulphate at approximately pH 7 with no pre-oxidation and that of surface area has been examined, as there was insufficient data to compare with alternative coagulants or with pre-oxidised algae systems. Further work is required to determine how the coagulant dose – surface area relationship would differ under these varying conditions. The role of surface area in creating a coagulant demand is also worthy of further investigation. For example, is charge density evenly distributed across the surface of algae cells such that as surface area increases a stoichiometric dose of coagulant is required to neutralise these point charges? Furthermore, if this were the case, is charge density a more appropriate method of establishing coagulant demand as opposed to surface area, given that charge density measurement of an algae system would also take into account the charge of associated charged AOM? Certainly, a reduction in the magnitude of the surface charge measured using the zeta potential was shown to be critical when achieving the minimum algae residual (Figure 2.1.3). Further work is therefore required to assess precisely how coagulant demand can be predicted. This would also involve a closer examination of the parameters of AOM that impact on coagulation. Our understanding of the role of AOM in coagulation lags behind that of other organic based systems. For example, tools have been developed to understand the coagulation mechanism and predict treatability for natural organic matter (NOM)

(Sharp *et al.*, 2006). Application of similar methods to improve knowledge of algae systems may help explain algae coagulation and thus reduce the knowledge gap.

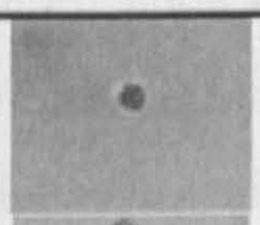
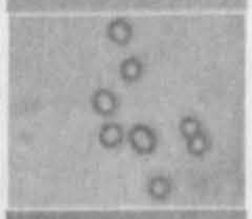
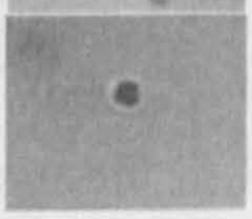
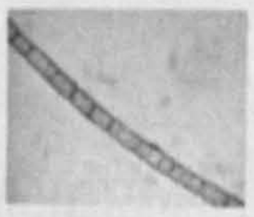
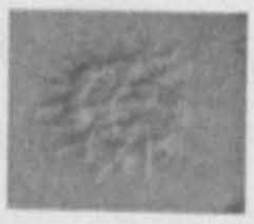
2.1.5 CONCLUSIONS

The key characteristics that impact on treatment processes are morphology, motility, surface charge, cell density and the AOM composition and concentration. With the exception of density, these are not phyla specific. The optimisation of coagulation has been identified as a key step for algae removal; however, this process is susceptible to changes in almost all the aforementioned algae characteristics. Furthermore, if coagulation is unsuccessful then downstream clarification in turn becomes vulnerable to algae character, particularly morphology and motility. DAF is the most robust clarification process in this regard. Overall, if pre-treatment and the DAF process are optimised, algae can be successfully managed and cell removal of 96-99.8 % or more is achievable.

Surface area has been identified as a useful preliminary indicator of coagulant demand required for optimum cell removal. This would therefore provide a relatively simple basis for regrouping algae such that the classification is informative with respect to water treatment. The character of the AOM will further alter the coagulant demand but to a lesser extent.

Further work is required to assess the influence of varying coagulation conditions on the correlation of surface area and coagulant demand, in addition to assessing the importance of charge density. Importantly, the influence of AOM character on coagulation mechanism requires addressing. Currently, there is a lack of understanding of the impact of AOM on coagulation in comparison to other organic systems such as NOM.

Table 2.1.7 *Calculation of approximate surface area for individual cells and associated the associated optimum coagulant demand, where CB = cyanobacteria; G = green algae and D = diatom.*

Species	Photo	Cell Size[1]	Cell Area	Cell Area (μm^2)	Coagulant Demand (ng Al $cell^{-1}$)	Reference
Chlorella (G)		3.5 µm diameter	$4\pi r^2$	38	0.0118	Liu *et al.* (1999)
Microcystis (CB)		5 µm diameter	$4\pi r^2$	78.5	0.0103	Drikas *et al.* (2001)
Chlorella (G)		5.3 µm diameter	$4\pi r^2$	88	0.0133	Edwald and Wingler (1990)
Cyclotella (D)		6.1 µm diameter	$4\pi r^2$	117	0.0320	Edzwald and Wingler (1990)
Anabaena (CB)		6-7 µm diameter	$4\pi r^2$	133	0.0270	Jiang *et al.* (1993)
Asterionella (D)		2 µm width; 40 µm length	$2\pi r^2 + 2\pi rh$	257	0.0270	Jiang *et al.* (1993)
Melosira (D)		15-20 µm diameter; 30-40 µm length	$2\pi r^2 + 2\pi rh$	2320	0.4750; 1.08	Jun *et al.* (2001)
Pediastrum[2] *(G)*		65-250 µm diameter (disc)	$2\pi r^2/15$	2598	0.4750	Mouchet and Bonnélye (1998)
Synedra[2] *(G)*		4.5-6 µm width; 100-300 µm length	$2\pi r^2 + 2\pi rh$	3068	1.08	Jun *et al.* (2001)

[1]Where possible cell dimensions referenced by the study were used to calculate the surface area, otherwise most likely dimensions were estimated (John *et al.*, 2002)

[2]Photos provided by Dr. Susanne Feist-Burkhardt, Dr. Eileen Cox and Prof. Elliot Shubert of the Natural History Museum.

2.1.6 ACKNOWLEDGEMENTS

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2.2 EXPERIENCES OF ALGAE IN THE UK – A TREATMENT PERSPECTIVE

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ABSTRACT

Algae blooms are a seasonal problem in UK waters and during these periods interferences with treatment plants are reported. This paper presents an analysis of data from 2000-2005 demonstrating UK experiences of algae at water treatment works. Cell populations are lower than those reported in the 1970s and 1980s, but reach concentrations that adversely affect treatment processes. Diatoms and cyanobacteria dominate in spring and autumn respectively. A treatment works including pre-oxidation, coagulation, flotation and filtration removes on average 96 % of influent cells, while rapid gravity filters alone remove 63-75 %. Cells present in the filtrate are typically either unicellular, micro-algae, or flagellated algae. Filter blockages in the spring and autumn are caused by large cells of complex morphology, including the diatoms *Melosira* and *Asterionella*. Overall, since the 1980s the key issue with respect to algae treatment has changed from one of treatability to that of process optimisation and economics.

Keywords: Algae; cell count; filtration; seasonal succession; speciation; treatment.

2.2.1 INTRODUCTION

Algae blooms are a seasonal problem in UK waters and are of particular concern to water companies with respect to the provision of drinking water to a satisfactory standard. Cell populations can vary widely in terms of cell count and diversity and this can impact on the treatability of the algae. In the 1980s a number of reports highlighted issues as follows: increased coagulant demand; difficulty in operation due to pH shifts; disturbances to flocculation, particularly by excreted organic matter; overloading of sedimentation tanks as a result of increased coagulant dose, leading to carry over of algae; filter penetration and filter clogging; trihalomethane (THM) precursor formation; and increased chlorine demand (Collingwood, 1979; Greene and Hayes, 1981; Bernhardt, 1984; Hutson *et al.*, 1987). The presence of algae in final supply was reported. For example, 156,000 cells mL^{-1} of *Anabaena* resulted in 3400 cells mL^{-1} in supply, a removal rate of 97.8 %, while a *Microcystis* bloom of 400,000 cells mL^{-1} could not be treated, resulting in the treatment plant being out of service for an 8 week period (Greene and Hayes, 1981). Algae can also affect the colour, taste and odour of drinking water and a limited number of species also excrete toxic metabolites which, if consumed in sufficient quantities, can cause health problems (Hutson *et al.*, 1987; WHO, 1998). Recommendations were therefore suggested for improving treatment processes to cope with influxes of algae, such as the inclusion of pre-oxidation to improve coagulation, flotation rather than sedimentation to improve floc removal, and granular activated carbon (GAC) to remove excreted organic matter (Greene and Hayes, 1981; Hutson *et al.*, 1987). Furthermore, various methods for control of eutrophication were advised. For example, the limiting of nutrients such as phosphate and nitrate, careful design of reservoirs and destratification (Greene and Hayes, 1981; Hutson *et al.*, 1987).

The current paper presents an examination of data relating to algae treatment obtained directly from several UK water companies since 2000. The aim was to compare data from various UK water companies in order to gain an understanding of the current

situation with respect to algae population diversity and abundance, process choice for reservoirs prone to algae blooms and associated treatment capabilities. Specifically, an assessment of today's key issues and the species associated with the identified issues will be provided.

2.2.2 METHOD

Data was collected from seven water treatment works (WTW) within the following UK water companies: Thames Water; Anglian Water; Northumbrian Water; and Yorkshire Water. The locations of the WTW were therefore in regions covered by the participating water companies and tended to lie in the north east of and south east of England (Figure 2.2.1).

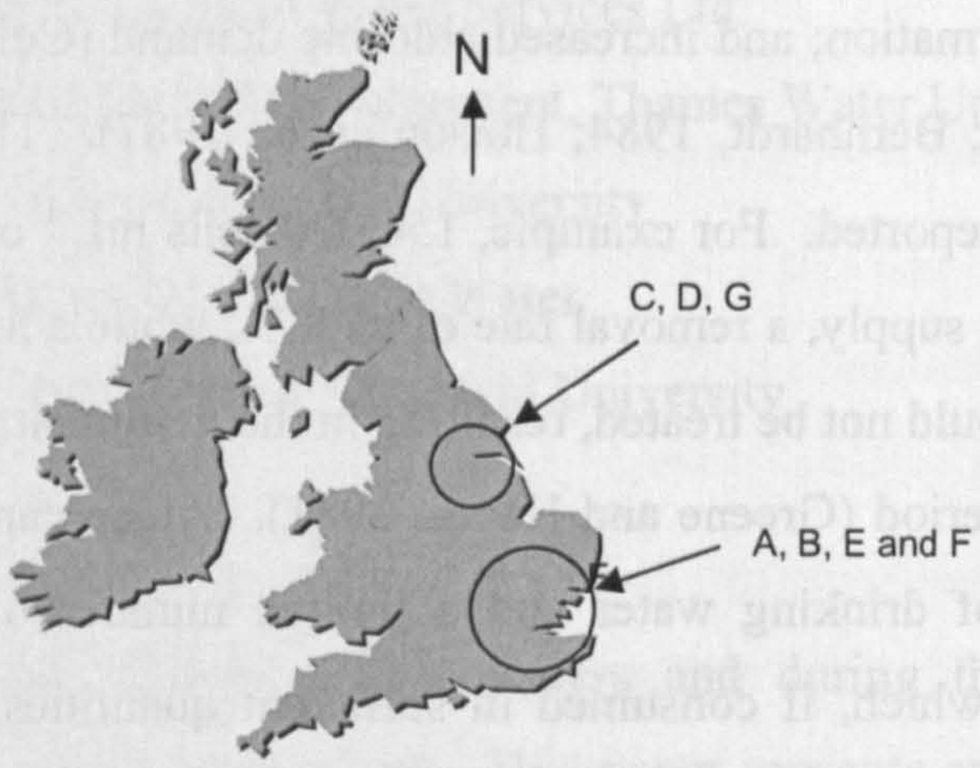


Figure 2.2.1 *Details of locations of the UK water treatment plants used in the study.*

Of the seven treatment plants under investigation (Figure 2.2.2), five included pre-treatment with ozone. Coagulation/flocculation using ferric or aluminium based coagulants was used in all but WTW E. Clarification post coagulation was mostly achieved by dissolved air flotation (DAF) not sedimentation, as would have historically been the case. In fact, WTW B and F use more advanced technologies of DAF Rapide and Counter Current Dissolved Air Flotation and Filtration (COCODAFF), respectively. The exceptions were WTW G which uses a Superpulsator ® clarifier, while WTW C and E rely more traditionally on roughing rapid gravity filtration (RGF) followed by slow sand filtration (SSF). Post-ozone and

granular activated carbon (GAC) filters have been installed in WTW A, B, and F, and all have final chlorination before delivery to the customer. It is of note that source control methods for reducing algae blooms were utilised in reservoirs supplying WTW A and B. This includes ferric sulphate dosing for phosphorus removal at a ratio of 15 Fe: 1 P in order to keep phosphate concentration below 0.05 mg L^{-1} as P and helixor air guns to destratify. WTW B also includes a bubble curtain.

The data analysed dated from 2000 to 2005 and typically included chlorophyll *a* concentrations, cell counts and observations on algae speciation, depending on the Water Company and season. Where available, data detailing process performance with respect to algae removal was examined. Specifically, WTW A had cell count data available over a number of years detailing cell removal post filtration, post ozonation and post GAC filtration as well as for final effluent. Additionally, WTW E had data demonstrating cell removal across its primary roughing filters for the years 2001-2004. Finally, filtration data corresponding to periods of high and low algae populations was obtained for WTW A and F, to assess the impact of algae using indicative parameters such as headloss and filter run time.

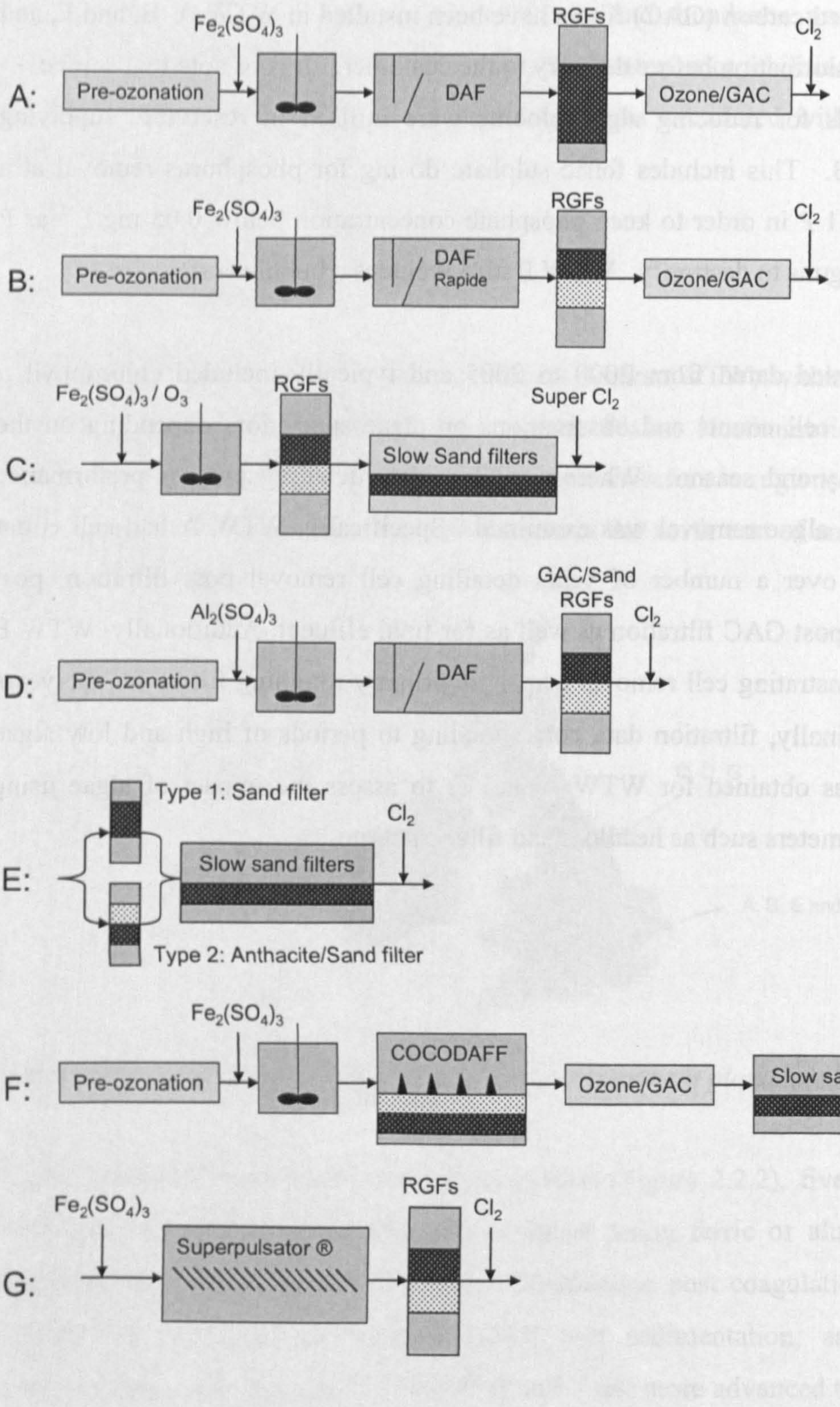


Figure 2.2.2 *Treatment processes utilised at each water treatment works labelled A-G*

2.2.3 RESULTS

2.2.3.1 Abundance and diversity at the source reservoir

Algae are present all year round, albeit at low population densities, with cell counts recorded between 10-177,000 cells mL^{-1} and 0.05-61.2 μg L^{-1} chlorophyll *a* concentration (Figure 2.2.3). Peaks in cell counts were principally observed across the study between February to April and July to October, indicating the key bloom periods to be spring and late summer (Figure 2.2.3). One exception was observed at WTW G where high cell counts of between 125,000-177,000 cells mL^{-1} occurred in November and December of 2003. While such similarities were common to all reservoirs examined, there were notable variations in cell abundances observed both between sites and year. To illustrate, cell counts at WTW E never rose above 10,000 cells mL^{-1} for the period 2000-2004, whereas cell counts of up to 177,000 cells mL^{-1} were observed at WTW G in 2003. However, cell populations for preceding years at the latter site were not greater than 100,000 cells mL^{-1}. In the case of WTW A, reservoir cell counts of up to 35,000 cells mL^{-1} were observed despite the source control measures in place to reduce algae population density. Hence, at no time for the period examined did the total cell counts reach peak cell densities such as those that were reported in the 1970s and 1980s in similar localities, for example, 400,000 cell mL^{-1} of *Microcystis* and 2.1 million cells mL^{-1} of *Aphanizomenon* (Greene and Hayes, 1981).

The cell population data was further analysed to provide an indication of the dominant groups during the identified bloom periods (Figure 2.2.4). Cells were classified primarily according to their phyla and it was observed that diatoms, green algae, and cyanobacteria were most prevalent in the reservoirs examined. This is typical of previous observations, particularly during periods of eutrophication when the diversity of algae is reduced (Shapiro, 1973; Murphy, 1976; Hutson, 1987). Flagellates were also common and while they are not an algal phylum, they were examined and grouped together due to their prevalence, similar character and ability to breach treatment processes.

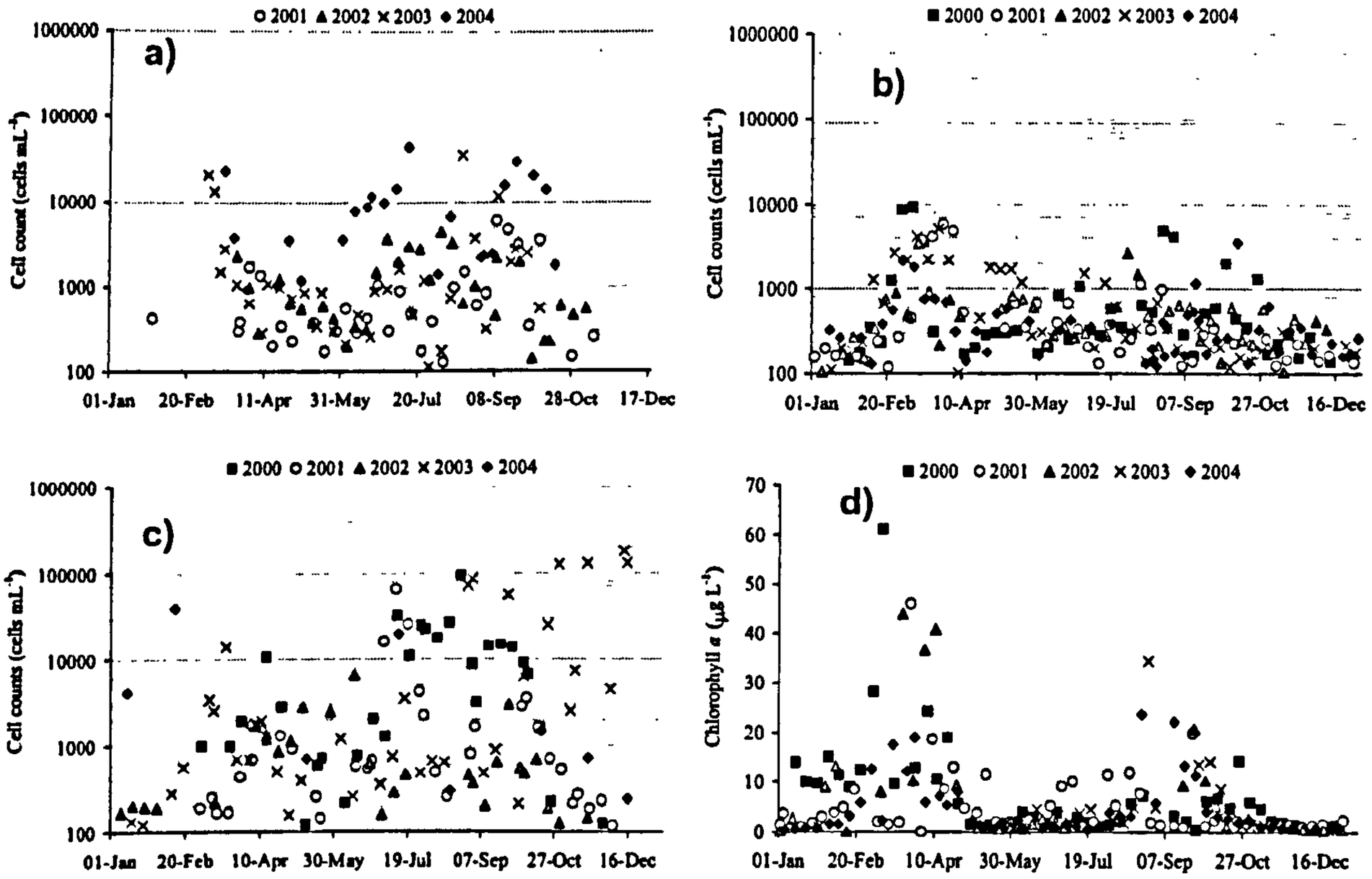
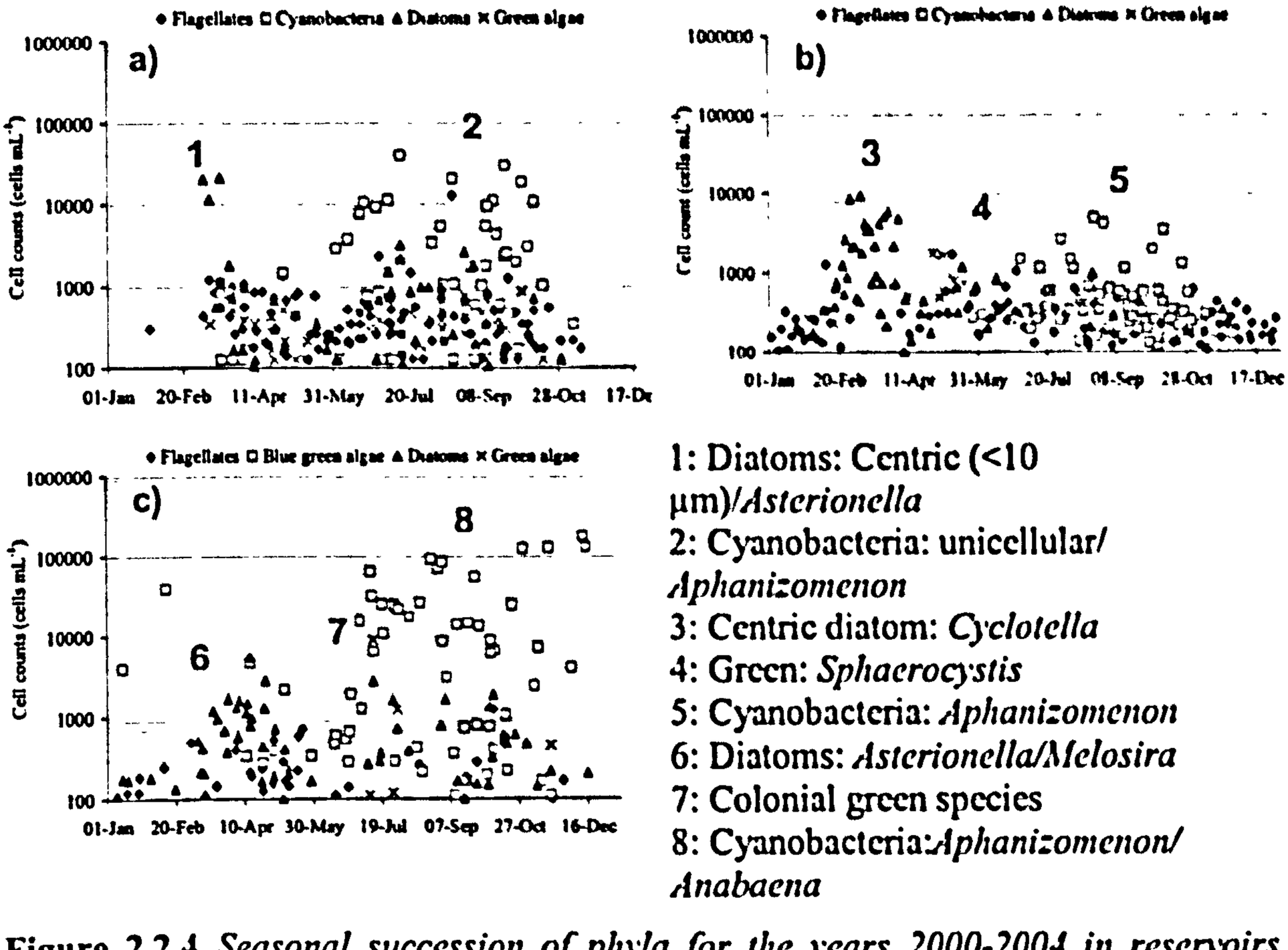


Figure 2.2.3 *Annual algae populations for WTW a) A; b) E; c) G; and d) F. Note only chlorophyll a data was available for WTW F.*

In general, it was observed that spring diatom blooms and late summer cyanobacteria blooms were very common among the reservoirs studied (Figure 2.2.4). For example, centric diatoms and colonial diatoms including *Asterionella* and *Melosira* were found in spring months and unicellular and colonial blue-green species including *Aphanizomenon* and *Anabaena* were observed in autumn. There was a background population of green algae at most times of year which increased in May and July for WTW E and G respectively due to increases in colonial species. Flagellated algae were present at relatively low concentrations all year round. The observations in terms of seasonal succession of specific phylum for the sites correspond well with the literature. For example, diatom counts of *Asterionella* in Llangorse Lake, South Wales, peaked in March, while green algae counts of the colonial species *Pediastrum, Scenedesmus and Coelastrum* peaked in April to June (Benson-Evans *et al.*, 1999). This is similar to an earlier study which found that diatoms dominated in winter and

spring, green algae in the summer and autumn with cyanobacteria succeeding in the later summer and autumn, leading into winter (Casterlin and Reynolds, 1977).



1: Diatoms: Centric (<10 µm)/*Asterionella*
2: Cyanobacteria: unicellular/ *Aphanizomenon*
3: Centric diatom: *Cyclotella*
4: Green: *Sphaerocystis*
5: Cyanobacteria: *Aphanizomenon*
6: Diatoms: *Asterionella/Melosira*
7: Colonial green species
8: Cyanobacteria:*Aphanizomenon/ Anabaena*

Figure 2.2.4 *Seasonal succession of phyla for the years 2000-2004 in reservoirs supplying WTW a) A, b) E and c) G. See adjacent key for details corresponding to 1-8*

Overall, it was observed that algae diversity became limited during spring and autumn blooms such that the population was dominated by one or two groups at the most (Figure 2.2.4). For example, the diatom blooms experienced by WTW E in spring were dominated by the centric diatom *Cyclotella*, while autumn cyanobacteria blooms comprised mainly *Aphanizomenon* and *Anabaena* at WTW E and G. Whilst cell counts do not appear to be reaching the extreme population density maxima observed in the 1970s and 80s, the blooms still reach concentrations that have previously been reported to interfere at treatment works. The *Asterionella/Melosira* bloom at WTW G for instance achieved cell counts of 5467 cells mL^{-1} which was double the size of the *Melosira* bloom that was reported at the Wahnbach Reservoir in Germany which

reduced the filter run time from 30 to 8 hours (Bernhardt, 1984). Another example is that of the *Aphanizomenon/Anabaena* bloom at WTW G that reached 177,000 cells mL^{-1} which is comparable with the *Anabaena* bloom of 156,000 cells mL^{-1} that resulted in 3400 cells mL^{-1} reaching supply (Greene and Hayes, 1981).

2.2.3.2 The treatability of algae

Overall, algae removal by WTW A was in the range of 91-100 % and 95-100 % for 2001 and 2002 respectively, with an average of 98.1 %. Notable exceptions in 2001 occurred at the beginning of August and July when 78 % and 85 % of algae were removed respectively, coinciding in each case with high populations of the motile *Rhodomonas*, other unicellular flagellates and unicellular cyanobacteria species, including *Microcystis*. These removal rates are comparable with those reported by Greene and Hayes (1981) such as 63.6% removal of *Microcystis*, 97.8 % removal of *Anabaena* and 99.9 % removal of *Aphanizomenon*. It is interesting that low removal efficiency was again reported for *Microcystis*, suggesting this species is particularly difficult to remove.

The majority of the influent algae to the works were removed by pre-oxidation/coagulation/DAF/RGF. This is illustrated in Figure 2.2.5 where percentile is plotted versus cumulative cell counts at various sampling points at WTW A, where the 50 %ile is the median of the cell count distribution throughout the year. For example, for the years 2001 and 2002, 96.1 % and 96.0 % removal had occurred by the post-filtration stage at the 50 %ile. More than 50 % additional removal was achieved by the downstream combination of ozone, GAC and chlorination processes such that 98.3 % and 99.4 % of influent cells were removed by the final water stage at the 0.5 percentile (Figure 2.2.5). This was also observed in another study at Grafham Water, Anglian Region, where it was suggested that secondary ozone sufficiently damaged the cells thus allowing the GAC to act as a filter (Daldorph, 1998).

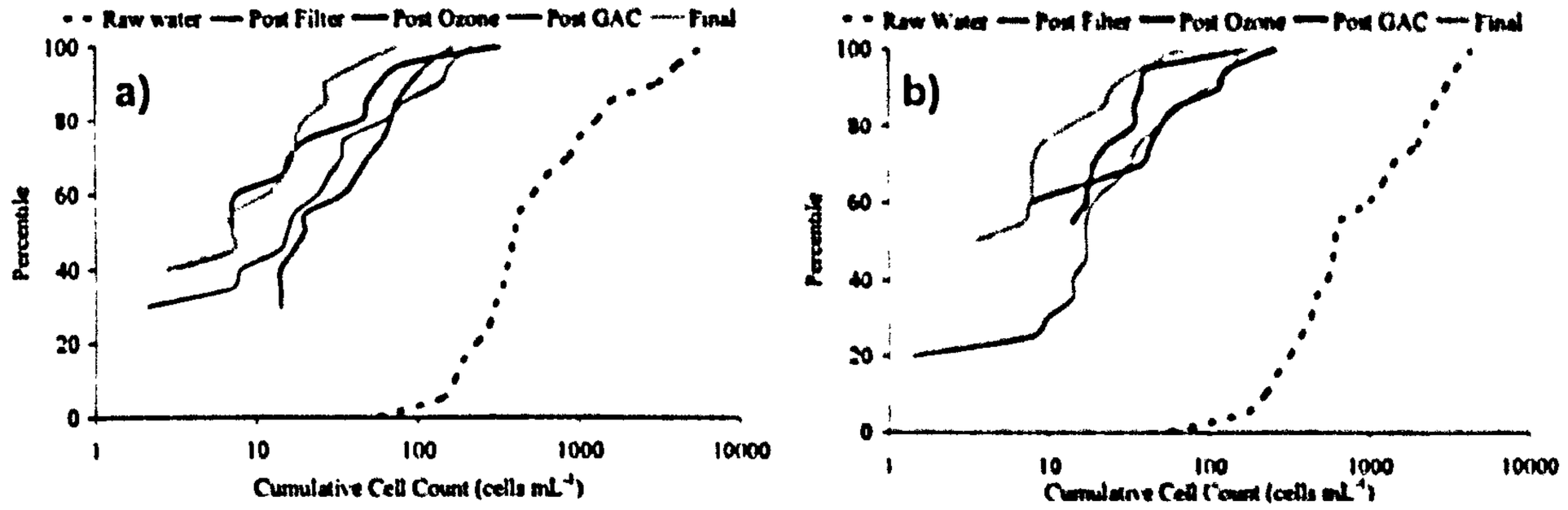


Figure 2.2.5 *Cumulative cell count after each treatment process following filtration for WTW A in a) 2001 and b) 2002.*

When roughing filters provided the initial barrier to influent algae, as opposed to a combination of coagulation/DAF/filtration, algae incursion across the filters was much greater. For example, an assessment of the cumulative cell counts that breached primary filters at WTW E (not illustrated graphically) showed that at the 50 %ile removal was 68.1 % and 63.8 % for Filters Type A (sand) and Type B (sand/anthracite) respectively in 2003 and 75 % and 63.6 % in 2004. However, while removals achieved by these RGFs were far lower than when DAF was utilised upstream, the inclusion of downstream SSFs by WTW E provide an effective secondary barrier to algae.

By examining all algal cells present in the filtrate, it was observed that algae of certain characteristics dominated over others in terms of the respective proportions of the total cell count. Specifically, micro-algae of less than 7 μm such as unicellular cyanobacteria (including *Microcystis*) and centric diatoms in addition to motile, flagellated algae (*Rhodomonas* and *Chlamydomonas* among others) were prevalent, while large, colonial and filamentous green algae, cyanobacteria and diatoms were not observed to penetrate the RGFs (Figure 2.2.6). For instance, at WTW A unicellular cyanobacteria were predominant comprising 58 % of all filtrate cells, while at WTW E centric diatoms were most prevalent downstream of filters at 42 % and 56 % for Filter Type A and B respectively. The lack of unicellular cyanobacteria and centric diatoms in the filtrate at WTW E and A respectively can in part be explained by the

influent cell speciation. For example, the proportion of influent unicellular cyanobacteria to WTW A and E were 36 % and 8 % respectively, whilst for centric diatoms the proportion was 12 % and 24 % respectively. Importantly, the presence of centric diatoms in the filtrate has not previously been reported. In contrast, the prevalence of unicellular cyanobacteria in the filtrate is a common observation. For example, one study noted that the proportion of cyanobacteria in raw water increased from approximately 10-50 % to 85-100 % of the algae population on filtration (Mouchet and Bonnélye, 1998). Filtrate flagellate proportions were more consistent at 16-31 % (Figure 2.2.6). As anticipated, the relative proportion of influent flagellates was also more consistent at 29 and 20 % for WTW A and E respectively. Flagellate filter incursion to form 15 % of the filtrate has previously been observed (Mouchet and Bonnélye, 1998). Furthermore, other studies reported that only 5 % of *Rhodomonas minuta* were removed by dual media filtration following coagulation (Petruševski *et al.*, 1993), and at best 50 % removal was observed for *Cryptomonas erosa* and *Rhodomonas minuta*, again following coagulation and filtration (Bernhardt and Clasen 1991).

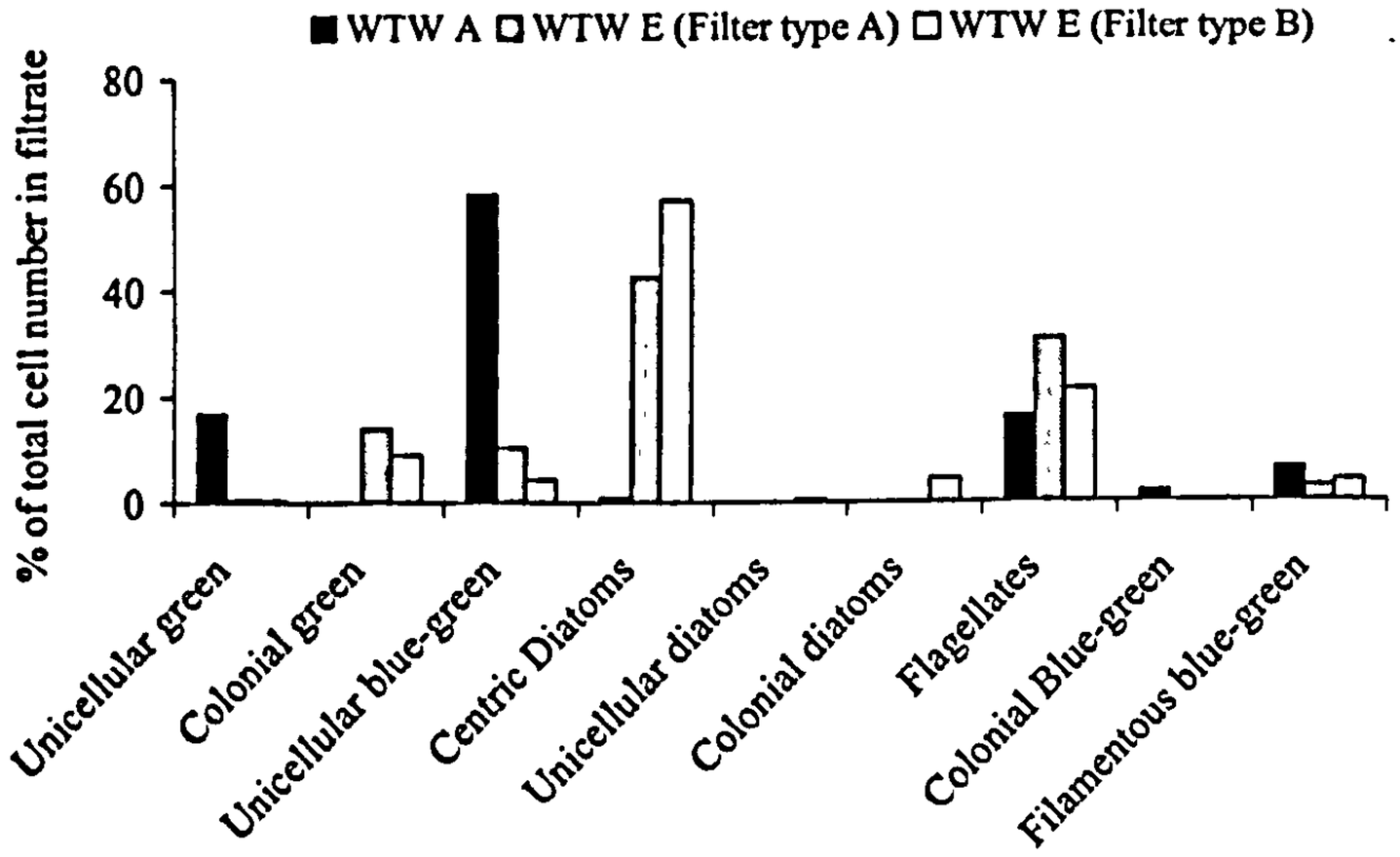


Figure 2.2.6 *Analysis of the groups represented after filtration during the period 2001-2003 for WTW A, WTW E (Filter Type A, single media – sand) and WTW E (Filter Type B, dual media – anthracite/sand).*

Further understanding of filter performance was obtained by examining available headloss and run time data for WTW A and F respectively. Overall, there seemed to be little disturbance to the filtration process while algae counts were low. For example, at WTW A, average headloss maxima tended to be at a minimum in the winter months at 0.6-0.7 m, increasing to an autumn maximum of 1.24 m in October, approximately double its winter value (Figure 2.2.7). Similarly, WTW F showed no deviation from a maximum run time of 45 hours in the winter (Figure 2.2.8), while run times decreased significantly during the spring and autumn months to 1.28 and 1.25 hours respectively. It should be noted however that due to the nature of the data collection system exact figures should be treated with caution and more important are overall trends.

The standard deviation of filter headloss maxima also increased markedly during the summer months suggesting that the filters were subject to sporadic variations in influent water quality and, by inference, frequent variations in algae population that resulted in spiking of headloss values (Figure 2.2.7). While algae sampling frequency did not allow for a direct comparison of headloss maxima with species, it was apparent that the presence of higher than usual concentrations of algae did impact distinctly on the filtration process. Specifically, algae spot samples show that large colonial species including the filamentous *Melosira* and colonial green algae species were present. To illustrate, Sample A comprised 83 % colonial green algae (12 % of which were *Scenedesmus*); Sample B had 33 % *Melosira* and 22 % *Scenedesmus*; whilst Sample C comprised 18 % *Melosira*.

A reduction in filter run times was observed when the diatoms *Melosira* and *Asterionella* increased to Frequent or Abundant (> 20 organisms present within 1 mm^2) (Figure 2.2.8, Sections 2 and 4). However, when only very low concentrations of *Melosira*, denoted as Rare (1-2 organisms present within 18 × 18 mm coverslip), and when Frequent abundancies of *Anabaena*, *Aphanizomenon* and colonial green species were present (21-100 organisms present within 18 × 18 mm coverslip) there was no reduction in run times (Figure 2.2.8, Section 3). This suggests that high population densities of *Melosira* and *Asterionella* were chiefly responsible for

decreasing filter run times at WTW F, similar to observations for WTW A (Figure 2.2.7). The filter blocking potential of *Melosira* has been reported previously, for example, in one study an influx of 2700 cells mL^{-1} of *Melosira* reduced filter run times from 30 to 8 hours (Bernhardt, 1984).

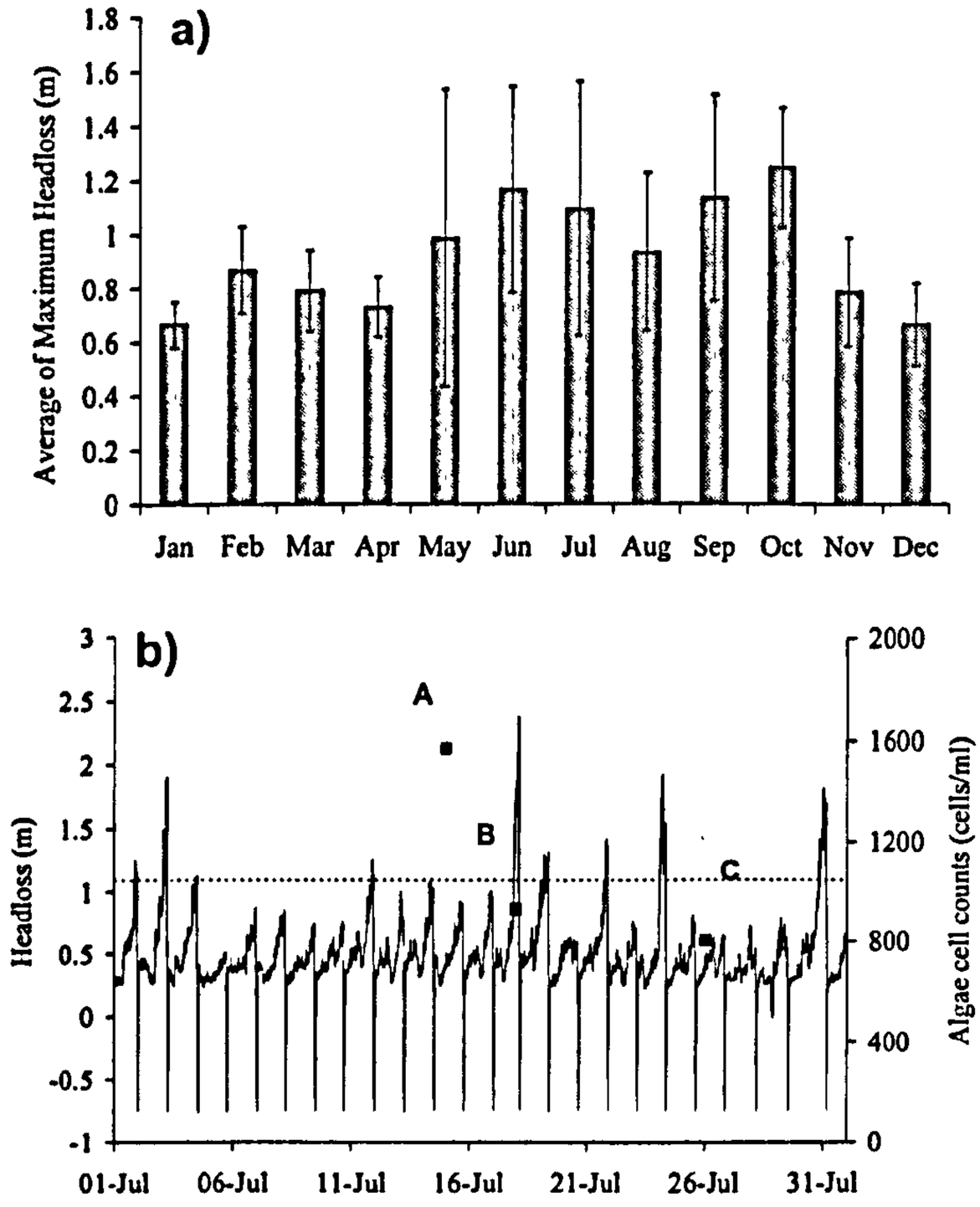
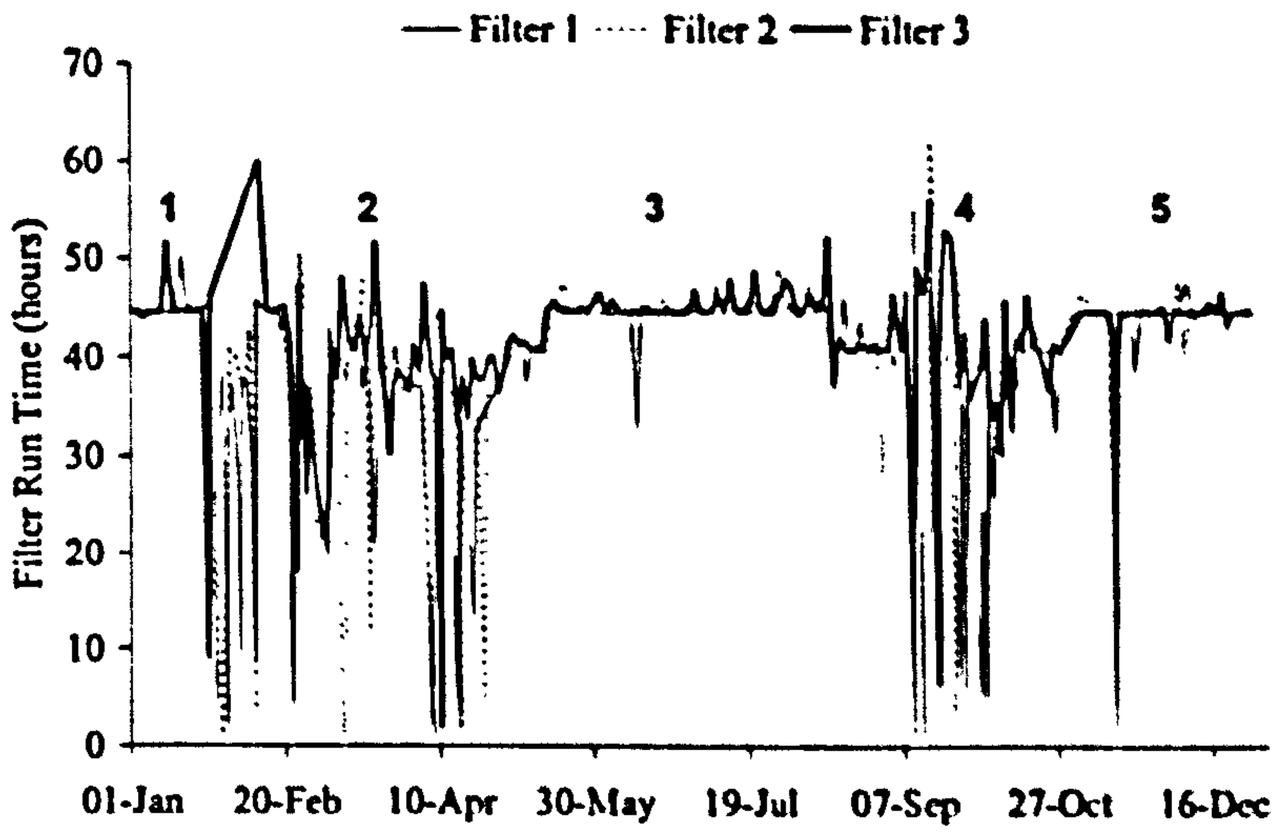


Figure 2.2.7 *Filtration data from WTW A in 2005 demonstrating: a) monthly average maximum headloss values and b) headloss data for July with associated algae cell counts at points A, B, and C.*

2.2.4 DISCUSSION

Seasonal algae blooms occur with a certain amount of predictability from year to year at all reservoirs examined. This is in spite of efforts to combat algae growth at the source, including the implementation of the Urban Wastewater Treatment Directive 1995 which seeks to reduce nutrient release to the environment and local measures

including phosphate removal by ferric dosing and reservoir destratification. Whilst reductions in nutrient levels have resulted in an overall decrease in algae concentrations, algae populations are still significant with respect to treatment. Algae abundance is not a function of nutrient concentration alone. Factors including physical variables such as the photoperiod and the wind activity as well as the presence of zooplankton which feed on the algae (Reynolds, 1973; Casterlin and Reynolds, 1977; Canovas *et al.*, 1996; Benson-Evans *et al.*, 1999) are also very important. Other solutions, such as the use of barley straw for algae control (Barrett, 1994; Martin and Ridge, 1999) are therefore required in addition to nutrient reduction to ensure algae concentrations remain low.



1. Diatoms most prevalent at 80 % of total algae observed, although generally algae abundancy is Rare.
2. Diatoms most prevalent at 72 % of total observed; however large diatoms including *Melosira* and *Asterionella* most dominant and now reported as Frequent and Abundant.
3. Diatoms now reduced to 23 % overall with *Melosira* and *Asterionella* much lower in concentration. Colonial green species now dominant at 40 % such as *Sphaerocystis* and *Scenedesmus*. Also prevalent are cyanobacteria including *Anabaena* and *Aphanizomenon* with abundancy noted as Frequent.
4. Diatoms increased to 53 % additionally with *Melosira* increasing in abundancy to Frequent. Also prevalent is cyanobacteria *Microcystis*.
5. Diatoms still prevalent however overall concentrations are much lower, reported as Rare.

Figure 2.2.8 *Filter run times for 3 filters at WTW F in 2004. Graph split into 5 sections as a result of spring and late summer run time decreases and algae population described.*

There are specific groups responsible for these predictable blooms, notably the colonial diatoms *Melosira*, *Asterionella*, and centric diatoms which tend to bloom in spring, and cyanobacteria including unicellular and filamentous algae such as *Microcystis* and *Aphanizomenon/Anabaena* respectively which all tend to reach peak concentrations in autumn. Seasonal successions observed in the current paper are similar to those observed in the 70s and 80s and for the most part well understood. For example, diatoms tend to prevail in spring for two reasons: firstly, they are able to grow in weaker light and lower temperatures than other types of algae; and secondly, diatoms are not very buoyant and require suspension in the photic zone by turbulent action (Reynolds, 1973; Casterlin and Reynolds, 1977). The summer maximum generally comprises large colonial green algae, for example *Eudorina* and *Pediastrum*, which are not susceptible to grazing (Canovas *et al.*, 1996). One study has suggested that a low N:P ratio is responsible for the on-set of cyanobacteria blooms in late summer and autumn (Pliński and Jóźwiak, 1999), although this does not explain cyanobacteria blooming for WTW studied in the current paper.

The principal barrier stopping algae entering water supply was the coagulation/DAF/filtration process. For example, 96 % removal efficiencies were achieved when using upstream pre-oxidation, coagulation/flocculation, DAF and filtration (WTW A), while use of roughing filtration alone removed 63-75 % of cells (WTW E). A key issue associated with the filtration process is therefore filter penetration. Algae observed downstream of the filters were of similar character in terms of shape and size irrespective of whether coagulation/clarification preceded filtration. For example, high proportions of unicellular cyanobacteria, including *Microcystis*, flagellated algae and centric diatoms were found in the filtrate. All of these species are small, less than 10 μm, relative to other species observed at the WTWs included in the study, explaining in part the ease at which they enter the filtrate. Importantly, influent concentrations of both unicellular cyanobacteria and

centric diatoms were both relatively high at the times when filter penetration by these groups occurred. Reducing the numbers of algae reaching the filters by ensuring optimisation of coagulation and clarification upstream is therefore required in order to minimise filter incursion. Despite the fact that there were no bloom periods of flagellates observed at any of the treatment works under investigation, flagellates still formed a large proportion of algae present in the filtrate. Explanations for the phenomenon have largely centred on the motility of the species enabling these species to liberate themselves from flocs (Bernhardt and Clasen, 1991). Such arguments are corroborated by studies that demonstrate that when cell motility is inhibited by pre-oxidation, removal by filtration of motile algae including *Euglena* and *Chlamydomonas* improves by 85-95 % (Steynberg *et al.*, 1993). Indeed, the data presented in the current paper shows that flagellates presented a larger proportion of the filtrate population for WTW E which did not have pre-oxidation compared to WTW A which did, although it is acknowledged that WTW A also had coagulation and flotation so the two cannot be compared directly.

The final issue with respect to treatment of algae was that of filter blockage by certain key groups, specifically the diatoms *Melosira*, *Asterionella* and on occasion colonial green algae including *Scenedesmus*. These species are all large, colony forming algae, indeed *Melosira* can reach filaments of 5 mm or more in length. As such these species are all prone to block filters, accounting for the peak headloss values and sharp decreases in run times that were observed at WTW A and F respectively. As a result, the major filtration mechanism switches from one of depth filtration to surface filtration. Complete removal of the large colony forming species *Asterionella formosa* and *Fragillaria crotonensis* by predominantly surface filtration has been found when using a low dosage of metal coagulants (Bernhardt and Clasen, 1991). They observed that colonies of these species were so large that if a flocculant was added, even RGFs with a grain size of 1.5 mm would completely retain the cells. While complete cell retention is desired, this should not be at the expense of economic filter operation.

Overall, there has been significant progression with respect to algae treatment in the UK since the 1980s. Previously, key issues related to very high algae densities which often resulted with algae in supply as a result of sedimentation processes becoming overloaded and inefficient flocculation. Treatment plants were sometimes closed for the period of the algae bloom due to the difficulty in treating such algae blooms. Problem algae in the 1980s were identified as cyanobacteria *Anabaena*, *Microcystis* and *Aphanizomenon* (Greene and Hayes, 1981) and diatoms *Asterionella*, *Fragillaria* and *Melosira* (Hutson, 1987). The current paper demonstrates that the very high algae concentrations previously observed are no longer commonly found, although the same problematic algae types still appear to attain significant abundances at certain times of the year. Lowering of algae concentrations in combination with improved technologies, notably DAF, has enabled algae blooms of all types to be treatable. However, frequent filter blockage and breach suggests that upstream DAF and coagulation processes are not optimised and thus high cell concentrations are reaching the filters decreasing run times. For example, coagulation of algae can be difficult and may require cationic flocculant for incorporation into flocs (Jun *et al.*, 2001). Furthermore, Markham *et al.* (1997) found that poor removal, 37 %, of filamentous cyanobacteria including *Aphanizomenon* and *Anabaena* was improved by increasing the DAF recycle ratio from 3% to 7-10 %. Both of these optimisation procedures will increase the cost of operating coagulation and DAF; however, these costs may be offset by savings made by more efficient filter operation. Hence, the overall lowering of cell concentrations and the inclusion of the more efficient DAF process and downstream ozone and GAC processes has resulted in the key issue changing from that of treatability to that of process optimisation and economics.

2.2.5 CONCLUSIONS

1. In general, UK waters demonstrate a seasonal succession of algae such that diatoms dominate in spring, green algae in early summer and cyanobacteria in late summer.

2. While algae population maxima are lower than those reported in the 1970s and 1980s, cell counts are still reaching levels that have been previously recorded to interfere with treatment works. It is suggested that measures in addition to nutrient limitation are implemented.
3. Evidence from the current study has demonstrated that a modern treatment works that includes pre-oxidation, coagulation, flotation, filtration, ozone and GAC can remove up to 98 % of influent cells.
4. Roughing filters alone can remove 63-75 % of influent cells.
5. The key challenges with respect to treatment are reducing filter penetration by motile and/or micro species including unicellular cyanobacteria, flagellates and centric diatoms and filter blockage by large colony forming species such as *Melosira* or *Asterionella*.
6. Since the 1980s, the key issue with respect to algae treatment has changed from one of treatability to that of process optimisation and economics.

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CHAPTER 3: CHARACTERISATION OF ALGAE

Paper 3 Identifying the linkage between particle characteristics and understanding coagulation performance

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Paper 4 Characterisation of algogenic organic matter extracted from cyanobacteria, diatoms and green algae

Submitted: *Water Research*

3. CHARACTERISATION OF ALGAE

3.1 IDENTIFYING THE LINKAGE BETWEEN PARTICLE CHARACTERISTICS AND UNDERSTANDING COAGULATION PERFORMANCE

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ABSTRACT

The coagulation/flocculation process is important for particle separation in water treatment. However, difficulties arise when coagulation is not optimised for the dominant particle. The current paper investigates the surface characteristics and floc properties of three common systems– natural organic matter (NOM), algae and clay – in order to aid understanding of the coagulation/flocculation process. It was demonstrated that charge density and specific surface area (SSA) are important parameters with respect to coagulant demand for charge neutralisation for all systems. However, algogenic organic matter (AOM) affected the coagulant demand of algae to the extent that it appears the presence of AOM could dominate the coagulation process. Controlling the zeta potential of the systems prompted improved particle aggregation and hence removal efficiency in all cases. Floc growth profiles revealed that algal flocs required 5 times the flocculation period to reach a steady state floc size compared to NOM and clay and on exposure to increased shear were much weaker. Despite similarities between algae and NOM in terms of organic content and coagulant demand, the fact that algae is a dynamic, biological system as opposed to an inert system creates numerous problems for the coagulation/flocculation process.

Keywords: Algae; natural organic matter; clay; coagulant demand; floc strength

3.1.1 INTRODUCTION

Coagulation/flocculation is an important and established process in water treatment for removing suspended particles. The nature of the contaminant load varies from source to source. For example, source water originating from rivers can have a high proportion of suspended clay colloids, whereas upland, peaty areas are generally dominated by natural organic matter (NOM). In all source waters algae are ubiquitous, although abundance differs depending on the extent of eutrophication. Seasonal algal growth can interfere extensively with a process that has been optimised for either clay or NOM systems. This typically results in algal and coagulant carry over, increased coagulant demand and filter clogging (Mouchet and Bonnélye, 1998).

The coagulation process is generally optimised for a particular system in terms of coagulant dose and pH, achieved through a series of bench scale jar tests. However, a limitation of such an approach is that particle characteristics are not linked to treatment optimisation. An understanding of the differences and similarities between systems could aid optimisation, for example, coagulation of NOM is thought to be principally driven by charge neutralisation mechanisms and as such, monitoring of the charge properties of the raw waters is extremely useful (Jefferson *et al.*, 2004).

The current paper presents a comparison the surface characteristics and floc properties of three systems – kaolin, algae and NOM. The aim of this work is to determine similarities and differences between the fundamental characteristics of these systems in order to improve current understanding of the coagulation/flocculation process.

3.1.2 METHOD

3.1.2.1 Materials

Preparation of kaolin suspension. 200 g of lab grade kaolin (acid rinsed) was blended at high speed with 500 mL of deionised water for 20 minutes at pH 7.5, adjusted using 0.1 M NaOH. The resultant suspension was made up to 1 L in a measuring cylinder using deionised water and left overnight. Subsequently, 500 mL was decanted and the concentration was determined gravimetrically. A stock solution of 50 g L^{-1} was prepared from which further dilute suspensions were made as required using tap water (hardness – 104 mg L^{-1} as $CaCO_3$; alkalinity – 55 mg L^{-1} as $CaCO_3$).

Cultivation of *Chlorella vulgaris*. *C. vulgaris* (211/11B) culture was obtained from the Culture Collection of Algae and Protozoa (CCAP), Oban, Scotland. Cultures were grown using 50 mL of autoclaved Jaworski Medium, prepared as advised by CCAP, in 250 mL conical flasks. Samples were grown on a Patterson Scientific Bibby Stuart SO1 shaker under 24 hour radiation using a Sun-glo 30W aquatic light. Samples were taken at the end of the log growth phase and diluted with tap water to a population concentration of approximately 5×10^5 cells mL^{-1}.

Natural Organic Matter (NOM). NOM rich water was obtained from a reservoir fed by an upland peat catchment system, situated in Halifax, UK. It was stored at 4 °C until use.

The characteristics of each of the above systems are summarised (Table 3.1.1).

3.1.2.2 Experimental Procedures

Isoelectric Point. Varying doses of aluminium sulphate (alum), $Al_2(SO_4)_3.18\ H_2O$, were added to 100 ml aliquots of kaolin, *C. vulgaris* or NOM suspensions and mixed thoroughly. The pH adjusted accordingly to give a range of pH 2-10 with 0.1 M HCl and 0.1 M NaOH. The zeta potential was subsequently measured using a Malvern

ZetaSizer 2000HSA (Malvern Instruments, UK) and the isoelectric point (i.e.p.) was plotted.

Table 3.1.1 *Characteristics of NOM, algal and kaolin systems.*

	NOM	Algae	Kaolin
Concentration	8.8-14 mg L^{-1}	5×10^5 cells mL^{-1}	50 mg L^{-1}
Turbidity (NTU)	5.9-7	3.2	50
Particle Size (μm)[1]	0.15 ± 0.02	4.5	0.2
Density (g cm^{-3})	1.00 (dissolved)	1.07	2.67
Specific surface area (m^2 g^{-1})	40	1.09	9.09
Charge Density (meq g^{-1})	10-15 [2]	Variable[3]	0.1-1 [2]
Zeta Potential (mV)	-18 at > pH 3	-15 at > pH 3	-50 at > pH 6
i.e.p.	1.5	1.5	2

[1]NOM particle size was obtained using a Malvern Zetasizer 3000HSA (Malvern Instruments, UK) and that of algae and kaolin using a Malvern Mastersizer 2000 (Malvern Instruments, UK)
[2]Edzwald (1993)
[3]Gregor *et al.* (1996) determined that alginate, a typical cell component, consumes twice the coagulant as NOM on a weight basis.

Removal Efficiency. Jar testing of all three systems was conducted using a PB-900 variable speed jar tester (Phipps and Bird, Camlab, UK) with flat paddle impellers (76 × 25 mm) with 800 ml suspensions in cylindrical jars. The procedure comprised a 3 minute rapid mix period at 200 rpm for addition of varying coagulant doses and subsequent pH adjustment using 0.1 M HCl to pH 5 for algae, pH 5.5 for NOM and pH 7 for kaolin. This was followed by a 15 minute flocculation period at 30 rpm and settling period of 20 minutes. The treated suspensions were analysed for zeta potential and turbidity (using a HACH 2100N Turbidimeter, Camlab, UK) for all systems in addition to DOC for NOM (using a Shimadzu TOC-5000A analyser).

Floc size and strength. Jar testing was conducted under optimum conditions of pH and coagulant dose, which were checked by measurement of the zeta potential. A feed pump connected the suspension from the jar tester to a dynamic laser diffraction

instrument (Malvern Mastersizer 2000, Malvern, UK) to measure the average floc size every minute. Suspensions were flocculated at 30 rpm after rapid mix as for standard jar tests for 15 minutes (NOM and kaolin) and 25 minutes (algae) before the shear was increased by adjusting the paddle speed. In all cases flocs were subjected to 30 rpm, 40 rpm, 50 rpm, 75 rpm, 100 rpm, 150 rpm and 200 rpm paddle speeds, equivalent to mean velocity gradient, G, values of 7.4 s^{-1}, 11.4 s^{-1}, 15.9 s^{-1}, 29.3 s^{-1}, 45.2 s^{-1}, 82.9 s^{-1}, 128 s^{-1} respectively, for a further 15 minutes.

3.1.3 RESULTS AND DISCUSSION

3.1.3.1 Coagulant demand

The organic particles required a colloid to coagulant weight ratio of between 1 mg mg^{-1} to 10 mg mg^{-1} to reach the i.e.p. (Figure 3.1.1). By comparison, the colloid to coagulant ratio required for kaolin was 100 times greater. Theoretically, the difference between kaolin and NOM can be explained in terms of the specific surface area (SSA) and charge density as both parameters are far greater for NOM (Table 3.3.1). For example, based on values given in Table 3.1.1, the total charge load for 1 L of NOM will be in the range 0.088 meq L^{-1} to 0.21 meq L^{-1}, while that of kaolin will be less at 0.005 meq L^{-1} to 0.05 meq L^{-1}. This observation is in accordance with conclusions drawn by a similar study as DOC was demonstrated to control coagulation as opposed to turbidity (Edzwald, 1993). However, with respect to algae, the relatively low SSA (1.09 m^2 g^{-1}) does not fit the observed data despite the high charge density. The observed difference is likely to be due to the excretion of algogenic organic matter (AOM). EOM comprises neutral and acidic components such as polysaccharides and uronic acids (Hoyer *et al.*, 1985) and is known to interfere significantly with the coagulation process (Bernhardt *et al.*, 1985). If it is assumed that the charge density of the cells and associated AOM is similar (Gregor *et al.*, 1996) and that stoichiometry exists between neutralisation and coagulant addition, the AOM must be significantly contributing to the coagulant demand, possessing a larger SSA. This has been similarly demonstrated in a study where AOM concentrations by mass were determined to be 3 times greater than that of the cells

(Leppard, 1997). This was attributed to the increased surface area of the AOM, as it was assumed that the surface area per volume (SA/V) of 0.005 μm fibrils must be greater than the SA/V of the particles >0.45 μm. This infers that the presence of AOM in algae laden water could dominate water quality changes as opposed to the cells themselves. This suggests that algae laden water should be treated similarly to water with high NOM concentrations rather than water with a high turbidity. The implications are that monitoring algal abundance would be insufficient as an indication of coagulant demand and DOC monitoring is equally, if not more, important.

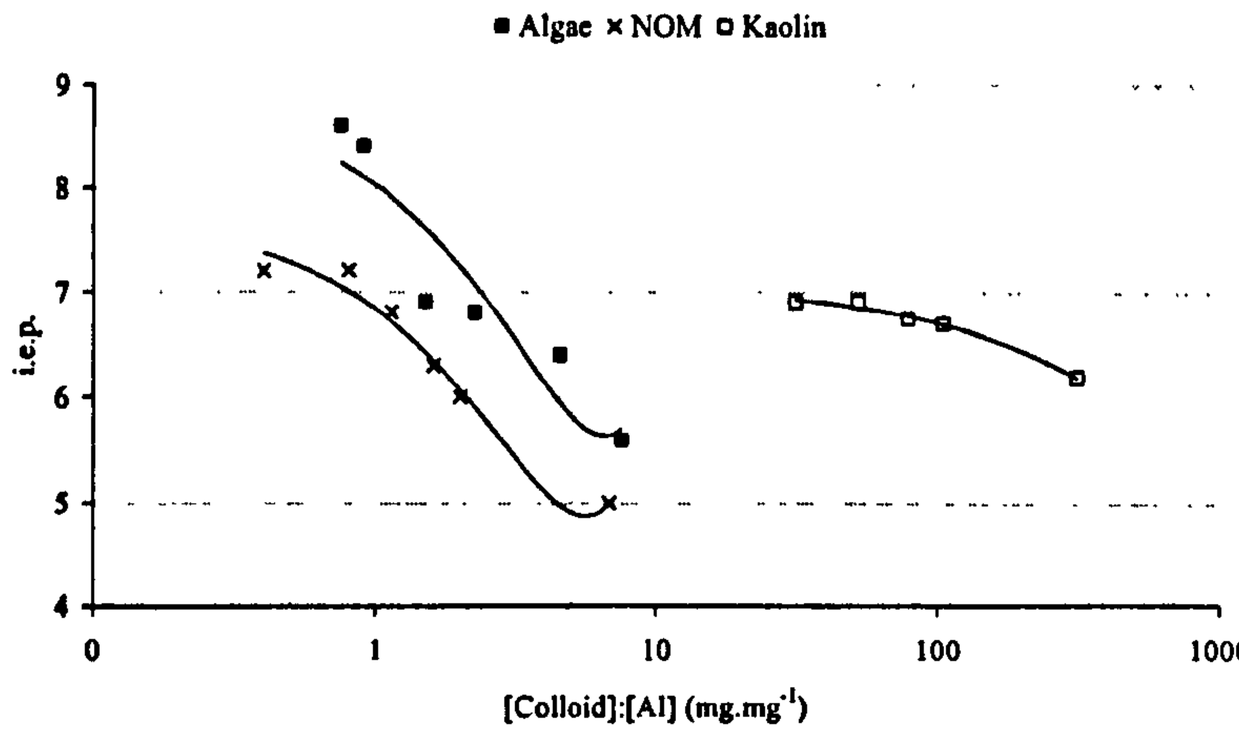

Figure 3.1.1 *The isoelectric point (i.e.p.) vs the colloid to coagulant weight ratio for kaolin, algae and NOM systems.*

3.1.3.2 Control using zeta potential

The zeta potential of each of the systems was monitored and correlated with removal efficiency. In each instance the removal efficiency was greater as the magnitude of the zeta potential was reduced; resulting in optimum operational zeta potential ranges (Figure 3.1.2). These operational ranges were different depending on the system, for example, NOM was successfully removed between -10 mV and +5 mV, whereas the zeta potential band for kaolin was much wider at -20 mV to +5 mV. The algae had a symmetrical optimal removal band of -12 mV to +12 mV, which was more like the removal band for NOM than for kaolin. This implies that the organic particles are

much more reliant on charge neutralisation for removal than inorganic particles. This observation could be related to differing coagulation mechanisms. Optimum conditions for organic particles required a low pH (~pH 5-6) where the dominant removal mechanism is charge neutralisation by complexation reactions between the cationic coagulant and anionic organic ligands (Stumm and Morgan, 1968). However, for inorganic particles coagulation was conducted at pH 7. At this pH, not only would charge neutralisation occur to a degree, considered to be a result of physical adsorption of the cationic amorphous hydroxide onto the surface of the inorganic particle (Duan and Gregory, 2003), but sweep flocculation would also occur increasing the density of the flocs. Hence, organic particles are much more dependent on the decrease in the magnitude of the zeta potential and subsequent reduction in the electrostatic barrier to contact.

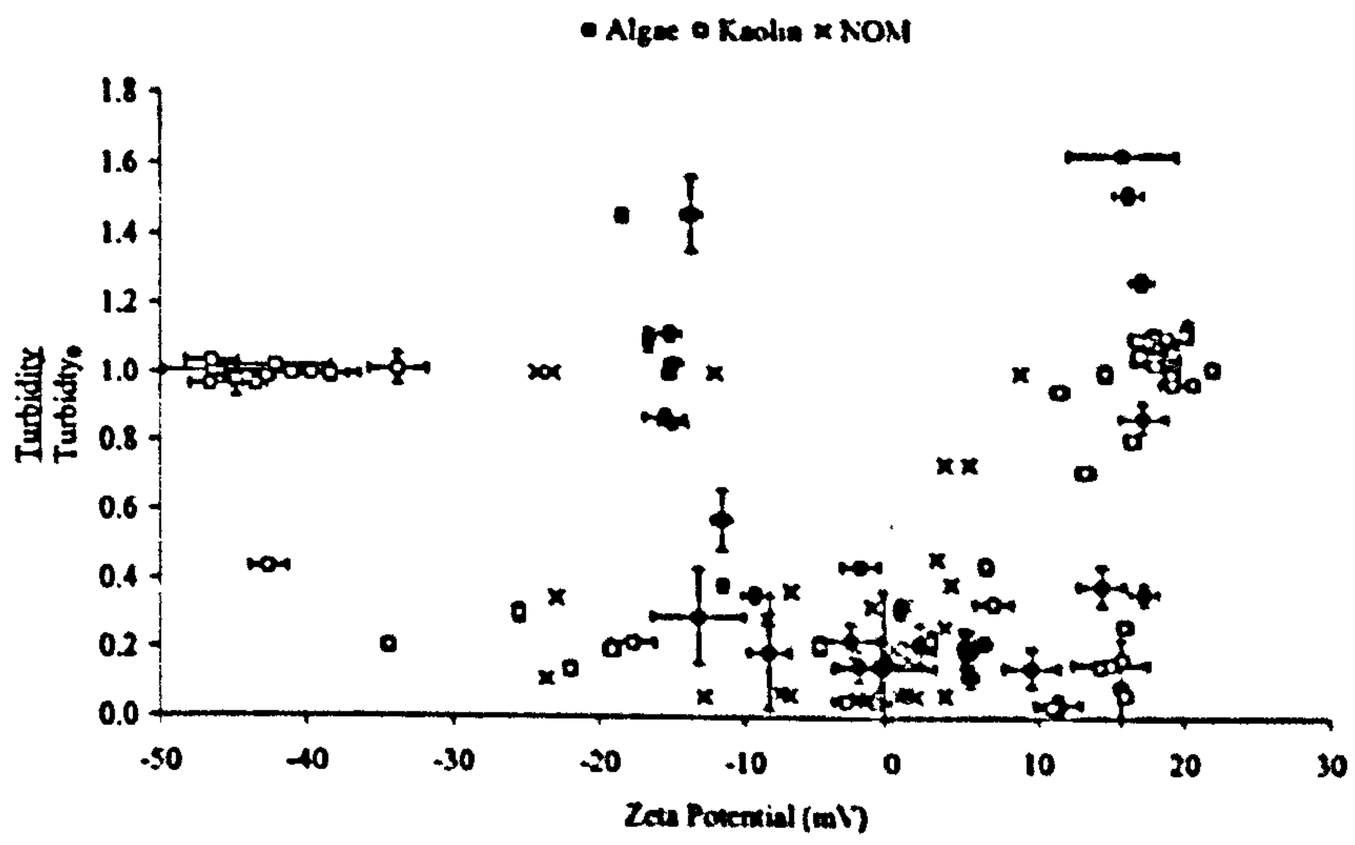

Figure 3.1.2 *The correlation between zeta potential and removal efficiency or impurity particulates kaolin, algae and NOM systems.*

Despite the differing coagulation mechanisms dominating, so long as the appropriate coagulation pH is maintained, measuring zeta potential can be used to determine appropriate coagulant dose without having to complete numerous jar tests. In this way the fundamental surface characteristics of the particles, in terms of the negative zeta potential, could be linked to treatment optimisation.

3.1.3.3 Floc growth profiles

Despite the similarities observed between organic particles with respect to their coagulant demand and removal mechanisms, the respective floc growth profiles were very different (Figure 3.1.3). Steady state floc size was achieved after 4 minutes for NOM and 6 minutes for kaolin. The growth rate observed for NOM was double that for kaolin (565 μm min^{-1} and 290 μm min^{-1} for NOM and kaolin respectively), perhaps due to the requirement of a lag time for kaolin in order to allow larger hydroxide precipitates to form (Duan and Gregory, 2003). By comparison, algal flocs required 25 minutes to achieve steady state floc size and during the first 6-7 minutes no agglomeration was observable. The algal floc growth rate was also extremely variable, ranging from 30 μm min^{-1} to 290 μm min^{-1} (taken between 10-20 minutes), and far slower than the initial growth rate for the inert particles, especially that of NOM. These observations indicate the dynamic nature of the algal population in that population abundance and AOM concentrations can change in relatively short time spans affecting the growth rate of the flocs. The varying growth rate can be attributed to steric interactions by loosely bound AOM which has been demonstrated to interfere with coagulation (Bernhardt *et al.*, 1985). The time-lag observed prior to floc growth was attributed to the biological systems ability to react to changes in the immediate environment. Such an observation is supported by the work of Clasen *et al.* (2000) who reported that a 7 minute stabilisation time was required to prior to measuring post-coagulation algae zeta potential. It was postulated that algae exude stored negative molecules to maintain negative charge when cationic hydrolysis products interact with the cell surface, thus remaining stable in suspension.

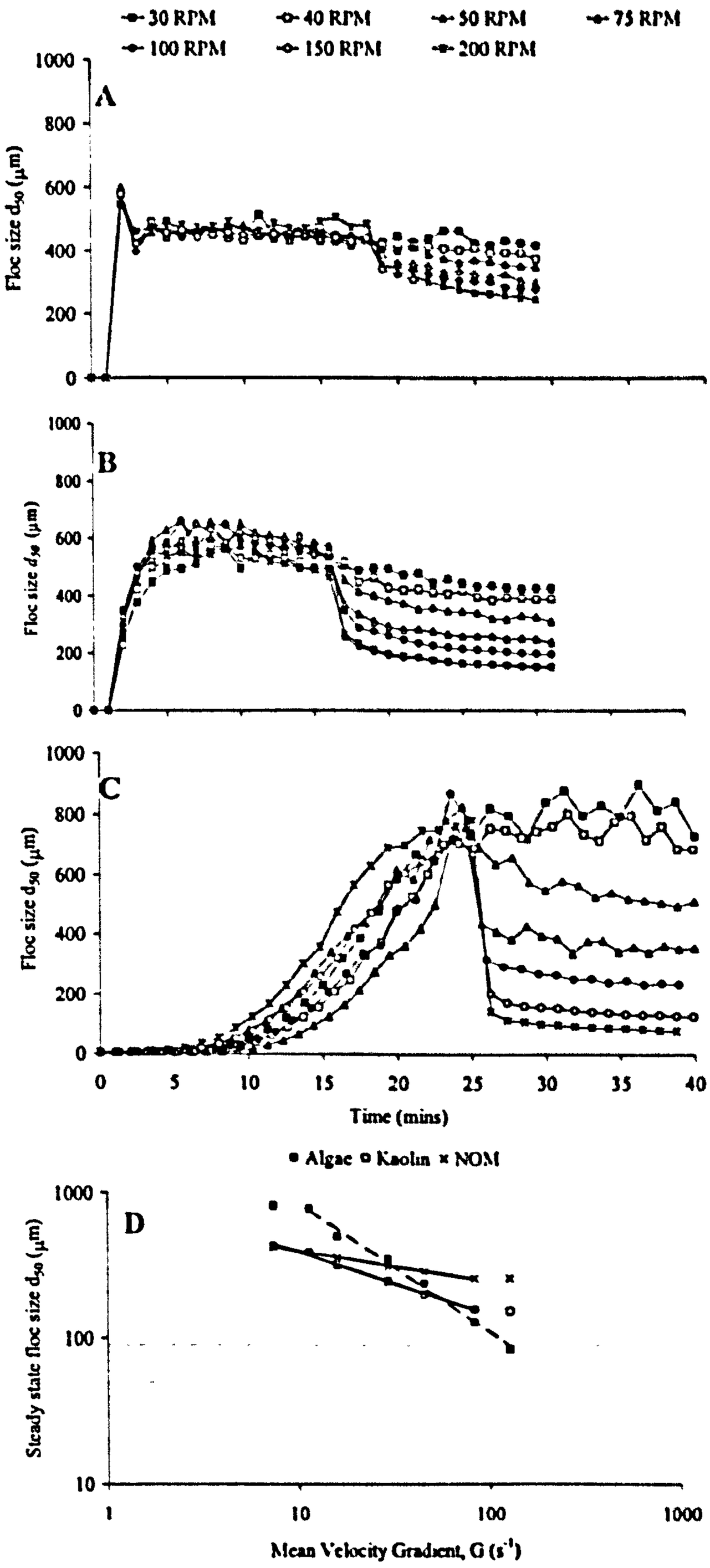

Figure 3.1.3 *Floc growth profiles for A. NOM+alum, B. kaolin+alum, C. C. vulgaris +alum and D. Log log plot of steady state floc size vs increasing shear rates.*

Initial floc strength was in the following order: algae>kaolin>NOM, as the steady state floc size indicates the ability to withstand shear rate in the flocculator (Figure 3.1.3). Exposure to increased shear rate, which in this work was simply inferred using increasing rpm values (Jarvis *et al.*, 2004), altered the floc size of the algae by the largest proportion as flocs were reduced to 11 % of their original size compared to the NOM and kaolin flocs which were reduced to 50 % and 27 % of their original size respectively on an increase to 200 rpm. Interestingly, the strength of the algal flocs resembled similar profiles obtained for polymer and NOM flocs, in terms of the initial size and floc breakage on exposure to increased shear rate, as they too reached approximately 800 μm and decreased to 23 % of their original size (Parsons *et al.*, 2004). This infers that polysaccharide exudates from the algae play an important role in their flocculation. As such, it is likely that algal cells and any hydroxide precipitate are bridged together by polymeric substances forming large flocs at low shear rate, similar to that experienced while using a polymer.

A comparison of the strength between the three systems (Figure 3.1.3d) shows that two distinct zones exist for the algal flocs as at 40 rpm and below the steady state floc size does not change greatly, implying resistance to these shear rates and thus a high floc strength. At shear rates greater than 40 rpm however the degradation rate increased at a constant rate with increasing rpm. However, for NOM and kaolin flocs there was constant degradation until reaching 150 rpm upon which no further degradation occurred. This demonstrates that although the algae formed much stronger flocs initially at low shear rate, on exposure to higher shear rates they were more prone to breakage compared with NOM and kaolin flocs. These observations can be quantified in terms of the floc strength coefficient, log C (the y-axis intercept) and floc strength constant, σ (the gradient of the slope) (Jarvis *et al.*, 2005a), provided rpm values are plotted as G. The larger the value of log C at a fixed shear rate the stronger the floc, while the response to increasing shear rate is quantified by the magnitude of σ. The floc strength constant can also be used as an indicator of the dominant floc breakage mechanism, where σ values of 0.5 are indicative of floc fragmentation while σ values of 1-2 suggest erosion mechanisms dominate (Bache *et al.*. 1997). Comparisons of log C and σ values extrapolated from Figure 3d, indicate

that while algae flocs are initially much stronger than NOM and kaolin flocs, NOM flocs are far more resistant on exposure to increased shear rate (Table 3.1.2).

The floc strength values suggest that fragmentation is the likely breakage mechanisms for NOM, kaolin and algae. This is supported by examination of the breakage profile of algae which shows floc size reduction from 800 μm to ~100 μm after the initial exposure to increased shear rate of 200 rpm (Figure 3.1.4). The only evidence of surface erosion was a small increase in the volume of 5 μm, the size of the algal cell, after exposure to 15 minutes of increased shear rate. The level of fragmentation was not so distinct for NOM and alum flocs, and it was observed that algal floc breakage profiles resembled those of NOM + polymer flocs (Jarvis *et al.*, 2005b). Hence, the suggestion that polymeric algal AOM has a significant influence on algal flocculation is reinforced.

Table 3.1.2 *The value of floc strength constants and flocs strength coefficients for the three systems and literature values of other aluminium-based flocs for comparison.*

Type of Floc	Log C	σ	Reference
C. vulgaris	3.82	0.89	Current study
NOM	2.81	0.21	Current Study
Kaolin	3.02	0.43	Current study
High alkalinity and NOM	4.1	0.81	Bache and Rasool (2001)
Commercial humic acid	3.1	0.44	Bache *et al.* (1999)

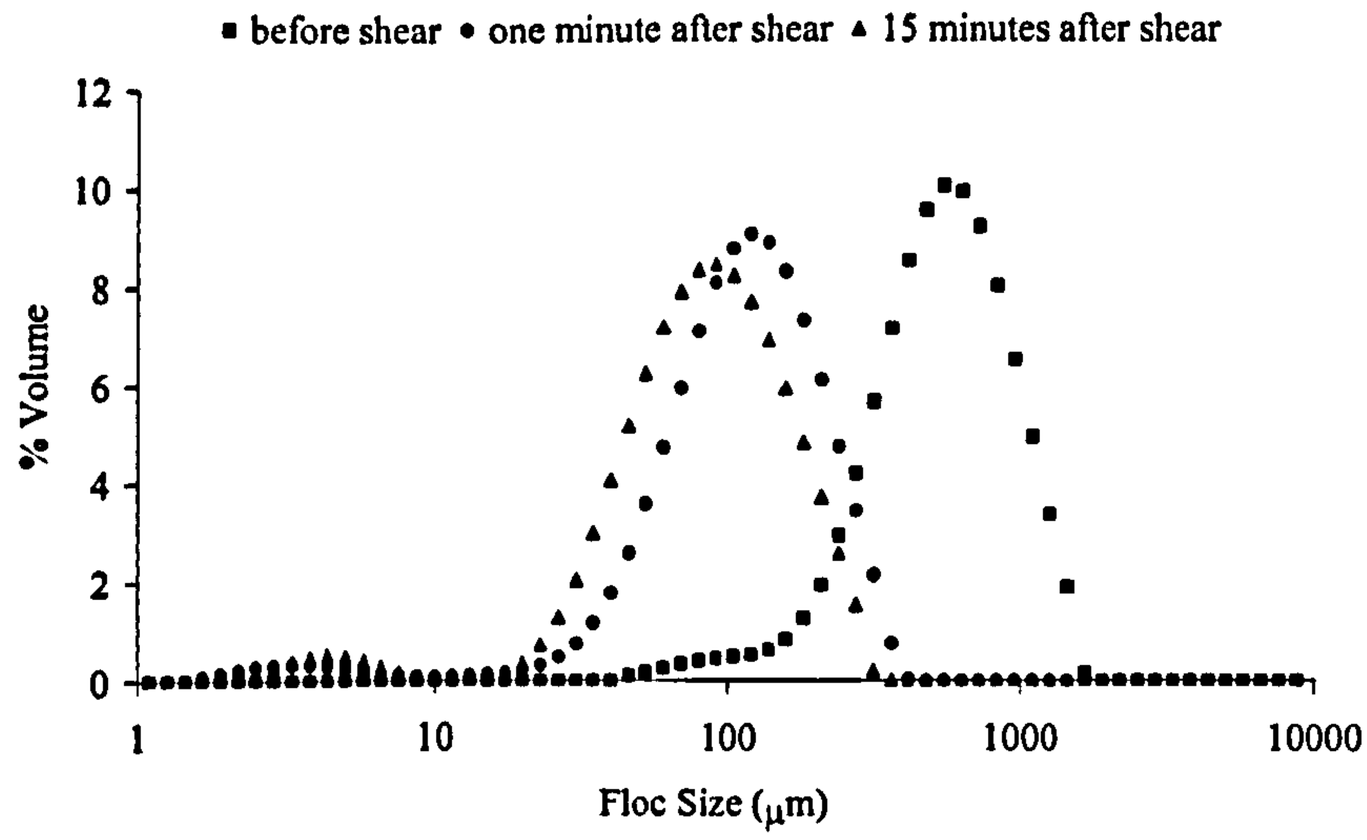

Figure 3.1.4 *Floc breakage profile for algae + alum flocs before and after exposure to a shear rate of 200 rpm.*

3.1.4 CONCLUSIONS

The overall picture indicates that organic surface layers dominate coagulation processes such that NOM and algae systems have similar responses and will thus control coagulation if present in a turbidity based system. Similarly, AOM in an algal based system may control coagulation as opposed to the algal cells themselves. Zeta potential monitoring will provide useful insights in the optimisation and control of all of these systems. The results indicate that the relatively high levels of AOM (carbohydrates) on the surface of the algae produce floc networks of a very different nature to NOM and kaolin. As such algal flocs are initially much stronger compared to kaolin and NOM flocs but on exposure to increased shear are much weaker. In short, knowledge transfer between different coagulating systems is difficult and optimisation should be considered in terms of both removal and physical properties.

3.1.5 ACKNOWLEDGEMENTS

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3.2 CHARACTERISATION OF ALGOGENIC ORGANIC MATTER EXTRACTED FROM CYANOBACTERIA, DIATOMS AND GREEN ALGAE

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ABSTRACT

Algogenic organic matter (AOM) can interfere with drinking water treatment processes and comprehensive characterisation of AOM will be informative with respect to treatability. This paper characterises the AOM originating from four algae species (*Chlorella vulgaris*, *Microcystis aeruginosa*, *Asterionella formosa* and *Melosira* sp.) using techniques including DOC, SUVA, zeta potential, charge density, hydrophobicity, protein and carbohydrate content, molecular weight and fluorescence. All AOM was predominantly hydrophilic with a low SUVA. AOM had negative zeta potential values in the range pH 2-10. The stationary phase charge density of AOM from *C. vulgaris* was greatest at 3.2 meq g^{-1} while that of *M. aeruginosa* and *Melosira* sp. was negligible. Lower charge density was related to higher hydrophobicity, which was related in turn to increasing proteins >500 kDa:carbohydrate ratio. This demonstrates that AOM is of a very different character to natural organic matter (NOM). AOM originating from *M. aeruginosa* and *C. vulgaris* is likely have a greater impact on coagulation in comparison to that of *A. formosa* and *Melosira* sp., due to increased size, ability of the AOM to complex with the coagulant, steric hindrance due to high surface coverage and, with respect to *C. vulgaris* AOM, increased charge density.

Keywords: algae; organic matter; carbohydrates; charge density; hydrophobicity; protein

3.2.1 INTRODUCTION

Algae are ubiquitous in rivers and reservoirs supplying drinking water treatment facilities. When these populations increase, treatment processes can be adversely affected. For example, coagulant demand is increased and floc formation is poor (Bernhardt *et al.*, 1984) or membrane fouling is increased (Her *et al.*, 2004). This is a result of not only increased cell concentration but also associated algogenic organic matter (AOM) which can form a substantial component of the algae system. AOM arises extracellularly via metabolic excretion or intracellularly due to cell lysis and is known to comprise proteins, neutral and charged polysaccharides, nucleic acids, lipids and small molecules (Fogg, 1983), of which polysaccharides can comprise up to 80-90 % of the total release (Myklestad, 1995). Hence, AOM provides a significant contribution to the heterogeneous mixture of compounds that forms dissolved organic matter (DOM) in algal systems. Understanding the character of DOM, and therefore AOM, is essential in order to determine the level of process interference and treatability that may be anticipated.

The first major investigation into AOM character from a treatment perspective was undertaken by Bernhardt and team (Bernhardt *et al.*, 1985; Hoyer *et al.*, 1985; Lüsse *et al.*, 1985). It was demonstrated that molecular weight and the concentration of dissolved organic carbon (DOC), carbohydrates and uronic acid was highly variable, depending on both species and culture age. Molecular weight (MW) is particularly important as high MW AOM can act as a flocculant aid while low MW AOM can increase the negative charge at the surface of particles (Bernhardt *et al.*, 1985). A later study showed that alginate, which is frequently used as a model for AOM and comprises two uronic acids, has a similar capacity for aluminium ions as fulvic acid (Gregor *et al.*, 1996). More recent investigations have demonstrated that proteins can interfere with coagulation (Tirado-Miranda *et al.*, 2003), while both proteins and polysaccharides have been shown to foul membranes (Her *et al.*, 2004).

In related fields involving treatment of organic systems, including that of natural organic matter (NOM) and wastewater biomass, additional parameters have been shown to be useful for linking organic matter character to treatment. For example, NOM is frequently characterised in terms of Specific UV Absorbance (SUVA), hydrophobicity, charge density and zeta potential (Edzwald, 1993; Sharp *et al.*, 2005). Furthermore, the character of soluble microbial products (SMP) and extracellular polymeric substances (EPS) in biomass, in terms of protein:carbohydrate ratios, charge density, hydrophobicity, and MW distribution, has been linked to flocculation and membrane fouling potential (Brookes *et al.*, 2003). Additionally, information on organic matter character has been gained from fluorescence excitation-emission matrices (EEMs) which can provide information specifically on protein and humic/fulvic-like substances in DOM and sewage effluent (Baker, 2002; Her *et al.*, 2004; Nguyen *et al.*, 2005).

The current paper aims to characterise AOM originating from algae that are commonly found in water sources using techniques that have not only been used by previous algae studies (Hoyer *et al.*, 1985), but have also been used by NOM and wastewater biomass studies. Specifically, the AOM character from four algae – the cyanobacteria, *Microcystis aeruginosa*; the green *Chlorella vulgaris*; and the diatoms, *Asterionella formosa* and *Melosira* sp – is compared. Additionally, the overall character is compared to well characterised and understood NOM and biomass systems. Consequently, the implications of the AOM character with respect to treatment are assessed.

3.2.2 MATERIALS AND METHODS

3.2.2.1 Algae cultivation procedure

The freshwater algae cultures of *Chlorella vulgaris* (211/11B), *Microcystis aeruginosa* (1450/3) and *Asterionella formosa* (1005/9), were obtained from the Culture Collection of Algae and Protozoa (CCAP), (Oban, Scotland), while *Melosira*

sp. (JA386) was obtained from Sciento, Manchester, UK. Average cell surface areas for these species are 55, 95, 370 and 6000 μm^2 respectively. All algae were grown in aquarium tanks at 50 litre volumes. The *C. vulgaris* and *M. aeruginosa* were grown at 20 °C using Jaworski Media, under 24 hour radiation and mixed using a pump. *Melosira* sp. and *A. formosa* were grown using Diatom Media at 15 °C and a 14/10 hour light/dark cycle, with daily mixing by hand. Sun-glo 30 W aquatic lights were used for lighting. AOM was extracted from all algae during the middle of the exponential and at the onset of stationary phases of growth, with the exception of *Melosira* sp. for which AOM was extracted during only the stationary phase. Daily checks were undertaken to ensure contamination had not occurred and to determine cell concentrations. Similar to previous observations during cultivation of algae on a comparable scale, if cultures were invaded by other organisms, this only occurred in the late stationary/decline phase (Lüsse *et al.*, 1985). It is acknowledged that experiments are not based on sterile cultures; however, from a practical perspective this reflects natural environment algae blooms. Cell populations were measured by counting at least 100 cells in triplicate using a light microscope and haemocytometer or Sedgewick Rafter cells.

3.2.2.2 AOM extraction procedure

AOM was extracted by centrifuging the cell suspension at 10,000 G for 15 minutes and subsequently filtering the supernatant (0.7 μm Whatman GF/F glass microfibre). When the DOC content of the filtered AOM solution was low, specifically for diatoms, concentration was undertaken using rotary evaporation at 70 mb and 40 °C followed by hardness ion removal using cation exchange resin (Dowex 50-WX-8, 200 mesh, Na^+ form) (Hoyer *et al.*, 1985). This method extracts dissolved organic material as well as organics loosely bound to the cell surface. No loss of DOC was observed on concentration and SUVA remained consistent. Furthermore, *C. vulgaris* AOM was used as a control as characterisation results from concentrated AOM were compared to those without concentration and no difference was observed. AOM extracted from *M. aeruginosa, C. vulgaris*, *A. formosa* and *Melosira* sp. will be denoted as MA-AOM, CV-AOM, AF-AOM and Msp-AOM respectively from here forth.

3.2.2.3 AOM characterisation procedures

All characterisation was undertaken within 4 days of extraction and conducted at pH 7 unless stated otherwise.

DOC and SUVA analysis: DOC was measured using a Shimadzu TOC-5000A analyser as the difference between total carbon (TC) and inorganic carbon (IC). Each sample was analysed in triplicate with errors less than 2 %. Furthermore, the DOC was measured for a minimum of duplicate AOM samples grown on separate occasions. UV_{254} absorbance was measured using a Jenway 6505 UV/Vis spectrophotometer and SUVA was calculated as UV_{254}/DOC.

Zeta potential: Zeta potential measurements were obtained using a Malvern ZetaSizer 2000 (Malvern, UK) which measures the electrophoretic mobility (EM) and converts this to zeta potential based on the Smoluchowski Approximation which is valid when $\kappa a >> 1$. The AOM solution zeta potential was measured for concentrated solutions (5 times), which correspondingly had 5 times the ionic strength. Hence, $\kappa a >> 1$ for AOM greater than 4 nm. Zeta potential was measured across a pH range of 1-10. All analyses were obtained in triplicate.

Charge Density: A solution containing a known amount of AOM, 1 mM NaH_2PO_4/Na_2HPO_4 pH 7 buffer, excess 6.2 meq L^{-1} low molecular weight PolyDADMAC (Sigma, UK) and the indicator ortho-Toluidene blue was back titrated against -1 meq L^{-1} poly (vinylsulphonic acid) sodium salt (PVSA) (Sigma, UK) to measure charge density (Kam and Gregory, 2001). Solutions were standardised using +1 meq L^{-1} cationic cetyltrimethylammonium bromide (CTAB) (Sigma, UK). The point of neutralisation was observed by measuring UV_{635} absorbance using a Jenway 6505 UV/Vis spectrophotometer (coinciding with a colour change from blue to purple). The measurement was repeated for three different volumes of AOM.

Carbohydrate and protein analysis: The carbohydrate content was determined using the phenol-sulphuric acid method (Zhang *et al.*, 1999). The modified Lowry

method was used for protein analysis (Frølund *et al.*, 1995). Glucose and bovine serum albumin (BSA) were used for calibration respectively at UV_{480} and UV_{750} absorbance using a Jenway 6505 UV/Vis spectrophotometer. Carbohydrate and protein measurements were performed on triplicate samples.

XAD resin fractionation: An XAD-7HP/XAD-4 column pair was used to fractionate AOM into hydrophobic and hydrophilic components as described by Malcolm and MacCarthy (1992). A 2 L AOM sample of approximately 10 mg L^{-1}, acidified to pH 2, was passed consecutively through the XAD-7HP and XAD-4 resins (resin volume was 60 mL in each 15 mm column). The non-retained sample comprised the hydrophilic fraction (HPI). Each column was back eluted with NaOH (0.1 M, 120 mL) such that the XAD-7HP and XAD-4 resin back-effluent comprised the hydrophobic fraction (HPO) and transphilic fraction (TPI) respectively. Each fractionation was completed in duplicate. The DOC and carbohydrate content of all fractions was measured as previously described.

Molecular weight fractionation: Nitrogen gas at a constant pressure of 1 Bar was used to drive the AOM solution through Biomax 500, 100, 30, and 10 and Ultracell PL-3 and PL-1 ultrafiltration membranes (Millipore, Billerica, MA, USA) using the Amicon Stirred Cell (Model 8400) in series such that AOM was fractionated into portions of >500, 100-500, 30-100, 10-30, 3-10, 1-3 and <1 kDa. The stirred cell was operated at 75 RPM and 60 % permeate, with the exception of initial filtration through the 500 kDa membrane for which only 40 % permeate was obtained. This was due to a gelatinous layer developing on the membrane surface at a throughput >50 % specifically for MA-AOM. Each MW fractionation was conducted only in the stationary phase and repeated in triplicate.

Fluorescence Spectroscopy: Excitation-Emission Matrices (EEMs) were obtained using a Cary Eclipse Fluorescence Spectrophotometer (Varian, Surry, UK) and a 4 mL, 1 cm path length cuvette. Emission spectra were scanned from 300 to 500 nm at 0.5 nm increments and excitation spectra scanned from 250 to 400 nm with 5 nm increments (Baker, 2002). The slits for excitation and emission were 5 nm and the

PMT voltage was set at 725 V. Deionised water blanks were run every 4 analyses and the intensity of the Raman line of water at 350 nm excitation wavelength measured to monitor instrument stability. However, EEMs have been used to provide a qualitative rather than quantitative insight to the investigation, as has previously been shown to be useful (Her *et al.*, 2004).

3.2.3 RESULTS

3.2.3.1 DOC

Stationary phase DOC averaged 27 ± 9.7, 18.0 ± 2.3, 7.5 ± 2.3 and 3.6 ± 1 mg L^{-1} as C for CV-, MA-, AF- and Msp-AOM respectively. However, given the widely varying cell concentrations results were primarily normalised for cell number. This resulted in AOM concentration varying in orders of magnitude with species (Figure 3.2.1a). For example, in the stationary phase the DOC released per cell increased in the order MA-<CV-<AF-<Msp-AOM with values 0.00095<0.0029<0.019<0.65 ng $cell^{-1}$ as C respectively. The DOC results were reported additionally in terms of DOC per cell surface area (Figure 3.2.1b). In this instance, DOC released per cell surface area at the stationary phase was 0.00002<0.000051<0.000053<0.0001 ng μm^{-2} for MA-, AF-, CV- and Msp-AOM respectively. Hence, although *Melosira* sp. had the lowest absolute concentration of DOC, on a per cell and per surface area basis it released more AOM than the other species. Additionally, DOC associated with the green algae, *C. vulgaris*, was consistently higher in concentration than that of the cyanobacteria, *M. aeruginosa*, despite having a smaller surface area, as was found in a previous study (Hoyer *et al.* 1985).

Stationary growth phase DOC concentrations per cell were consistently higher than those observed for the exponential phase. For example, DOC measured in MA- and CV-AOM increased from 0.00071 ± 5 × 10^{-5} and 0.00088 ± 0.00037 ng $cell^{-1}$ to 0.0015 ± 0.0009 and 0.0029 ± 0.0013 ng $cell^{-1}$ respectively, although DOC of AF-AOM only increased marginally from 0.018 to 0.019 ng $cell^{-1}$. Absolute DOC values for CV-AOM increased by 18 mg L^{-1} from exponential to stationary phase which is

consistent with the literature where a 20 mg L^{-1} increase was observed (Hoyer *et al.*, 1985). It has been postulated that while organic matter is actively excreted during the exponential growth phase, much of the material remains bound at the surface of the cell, to be worn away during the stationary phase (Konno, 1993).

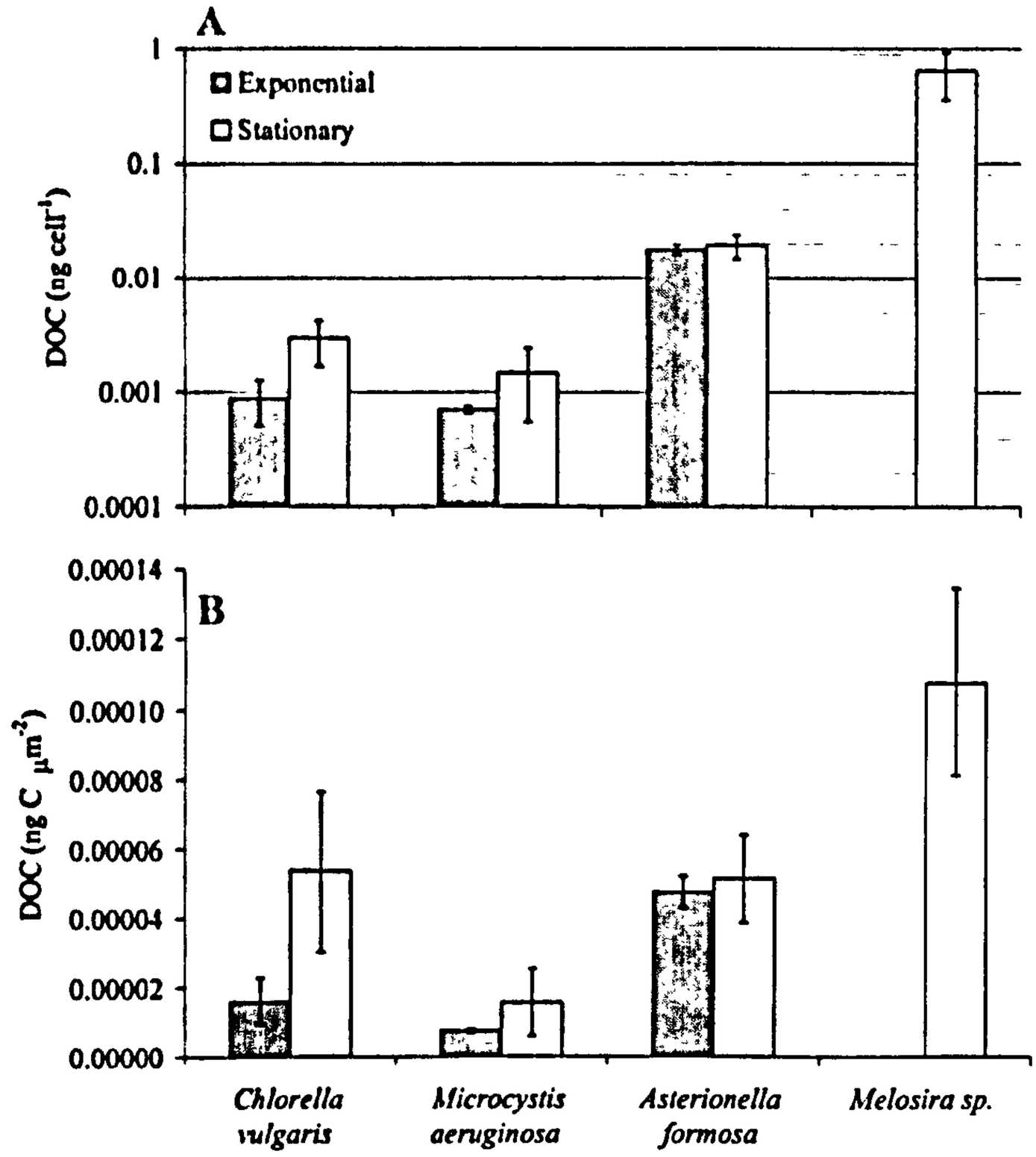

Figure 3.2.1 *The DOC concentration for CV-AOM, MA-AOM, AF-AOM and Msp-AOM normalised A. by cell and B. by surface area.*

3.2.3.2 Zeta potential and charge density

The zeta potential (ZP) of stationary phase AOM was demonstrated to decrease sharply between pH 1-3, reaching a plateau between pH 4-10. Isoelectric points (i.e.p.) for the AOM were 1.0, 1.8, 0.8, and 1.6 and the ZP stabilised at values of -21.5 ± 0.94 mV, -23.7 ± 2.1mV, -33.3 ± 3.5 mV and -15.9 ± 1.7 mV for CV-, MA-, AF-

and Msp-AOM respectively. The AF-AOM and MA-AOM exponential growth phase ZP curves were similar to the stationary phase curves in that i.e.p. values were 0.9 and 1.9, while average ZP values for pH 4-10 were -33.7 ± 4.7 mV and -21.5 ± 3.9 mV respectively. However, the exponential phase ZP curve for CV-AOM was significantly different. From an i.e.p. of 1.0, the ZP stabilised between pH 5-6 at -18.9 mV, before decreasing steeply from pH 6-10 to reach -35.3 mV. Overall, ZP values reported in the current study are comparable with the literature. For example, *Chlorella* had a relatively low ZP of between -17.4 mV and 19.8 mV (reported as -1.4 to -1.6 $\mu mV\ s^{-1}\ cm^{-1}$) independent of pH 4-10 (Edzwald and Wingler, 1990) and the ZP of the diatom *Nitzschia* was -28 mV in the stationary phase (Konno, 1993). The shape of the ZP curves relates to the ionisation of functional groups in the colloidal organics. In the case of the AOM reported here, the steep decrease observed between pH 1 and 4 can be attributed to the ionisation of carboxylic groups that are present in charged polysaccharides and proteins. Decreases at high pH values, such as that observed for the CV-AOM in the exponential growth phase, can similarly be attributed to the amino groups that have pKa values of 9-11 (Lehninger, 1970).

During the exponential growth phase, the charge density of CV-AOM and MA-AOM were 0.9 meq g^{-1} and 0.2 meq g^{-1} of C, whilst that of AF-AOM was negligible. An increase was observed for CV- and AF-AOM on transition to the stationary phase to 3.2 and 1.0 meq g^{-1} C respectively. However, the charge density of MA-AOM decreased to 0.1 meq g^{-1} C (Figure 3.2.2). Msp-AOM had a negligible charge density in the stationary phase. Charge density values obtained in the current study were comparable with literature values, for example, 3.1 and 2.3 meq g C^{-1} for AOM from *Dictyosphaerium* and *Pseudanabaena* respectively (Bernhardt *et al.*, 1985) and 0.5-1.8 meq g C^{-1} for *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Cyclotella* sp. (Paralkar and Edzwald, 1996). The charge density obtained for CV-AOM is comparable with those typical observed for NOM of 2.7-3.8 meq g^{-1} C (Sharp *et al.*, 2005).

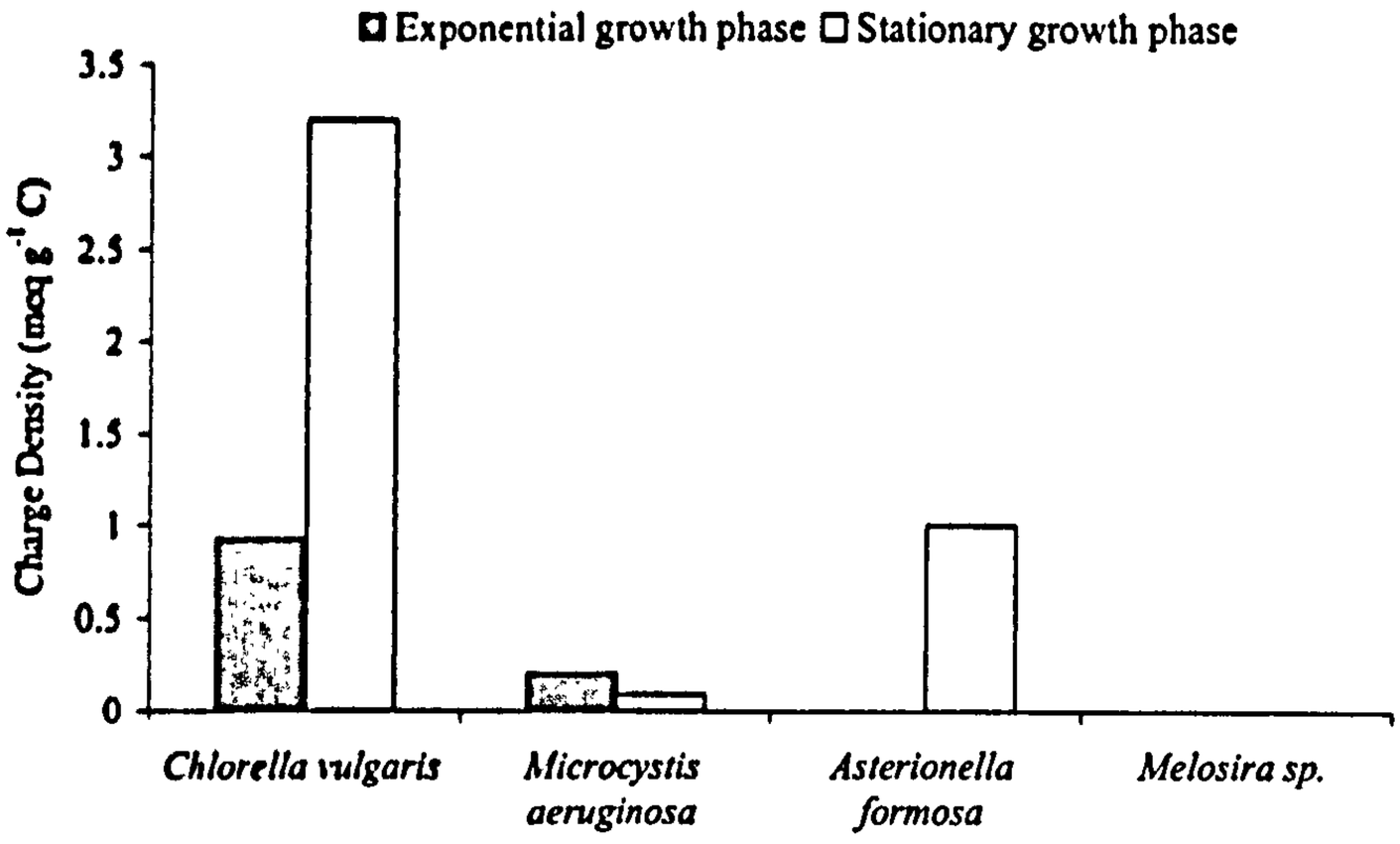

Figure 3.2.2 *The charge density for each system in the stationary phase at pH 7.*

3.2.3.3 Hydrophobicity – XAD resin fractionation and SUVA

The AOM was largely hydrophilic (57 % or more) for all species examined (Figure 3.2.3), consistent with the presence of polysaccharides, sugars and hydroxy acids (Edzwald, 1993). Specifically, stationary phase MA- and Msp-AOM contained significantly more hydrophobic material at 30 % and 32 % in comparison to CV- and AF-AOM at 11 % and 15 % respectively. On comparison of exponential and stationary growth phases, it was observed that the hydrophobic/hydrophilic proportions of MA- and AF-AOM remained relatively consistent, while the hydrophobic material of CV-AOM decreased from 24 % to 11 % respectively. The transphilic proportion of the AOM varied from a minimum of 8 % for Msp-AOM to a maximum of 17 % for stationary phase CV-AOM. MA-AOM fractionation results compare well with a study investigating cyanobacteria AOM character (species not reported), where HPI and HPO results were 57 % and 26 % respectively (Her *et al.*, 2004).

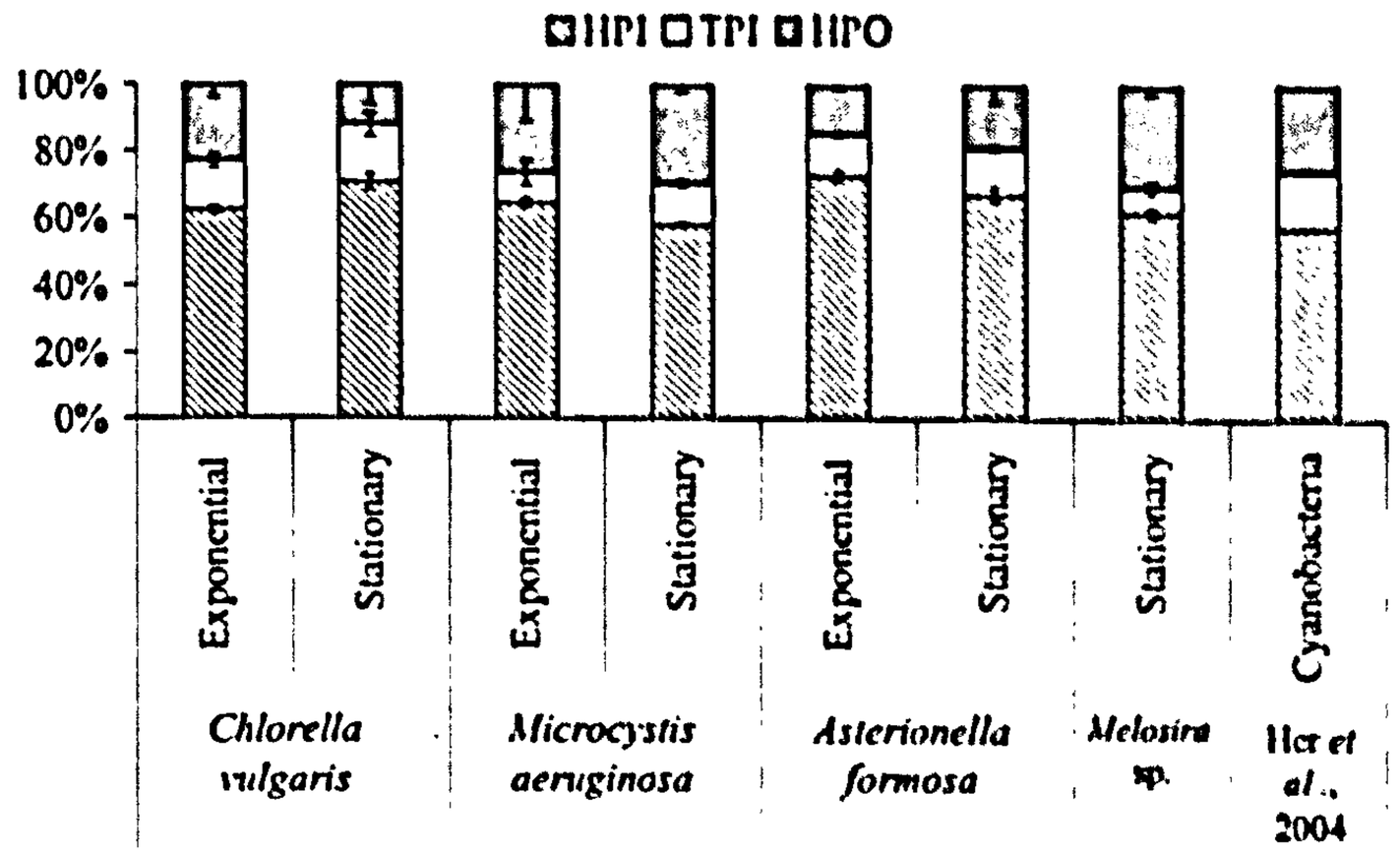

Figure 3.2.3 *The proportion of AOM contained within hydrophilic (HPI), transphilic (TPI) and hydrophobic (HPO) fractions for Chlorella vulgaris, Microcystis aeruginosa, Asterionella formosa and Melosira sp. compared with that of cyanobacteria obtained using the same method in a separate study (Her et al., 2004).*

The high hydrophilicity of the AOM as measured by the XAD-resin fractionation procedure was supported by SUVA results which similarly indicated the AOM was of a highly hydrophilic nature. SUVA values during the exponential growth phase were 1.29, 1.65 and 1.7 l m^{-1} mg^{-1} for CV-, MA- and AF-AOM respectively while in stationary growth phases, it decreased to 0.54, 0.48 and 0.54 l m^{-1} mg^{-1} for the same species and 0.58 l m^{-1} mg^{-1} for Msp-AOM. Hence, regardless of the growth phase, the SUVA was consistent with a material of a very hydrophilic nature. The low SUVA is a result of the relatively low aromaticity associated with AOM such that it would not be expected to be highly absorbing (Hoyer *et al.*, 1985). This correlates with larger scale studies where increasing eutrophication and consequent DOC concentration coincided with a decrease in the SUVA (Cheng and Chi, 2003).

3.2.3.4 Carbohydrates and proteins

The stationary phase carbohydrate:DOC weight ratio were relatively similar at 1.1, 0.7, 1.0 and 0.8 mg mg^{-1} as glucose:C for CV-, MA-, AF- and Msp-AOM

respectively; however, the protein:DOC ratio was more varied with values of 0.4, 0.64, 0.19 and 0.16 mg mg^{-1} as BSA:C for the same systems (Figure 3.2.4). Hence, it was protein concentration that was responsible for the variability observed in the protein:carbohydrate ratio of 0.4, 0.6, 0.2 and 0.2 mg mg^{-1} as BSA:glucose for CV-, MA-, AF- and Msp-AOM. The exponential phase protein:carbohydrate ratios (not depicted here) were 0.58 and 0.31 mg mg^{-1} for CV- and MA-AOM respectively, demonstrating that while the amount of protein relative to carbohydrate decreased with age for CV-AOM, that of MA-AOM doubled. Protein:carbohydrate ratios for activated sludge were determined as 0.59 and 0.85 for a full scale and lab process respectively (Morgan *et al.*, 1990), and are therefore similar to values obtained for MA-AOM in this study.

The proportion of total carbohydrates found in the HPO fraction was consistent irrespective of growth phase and species at between 9 and 17 %, demonstrating that carbohydrates were predominantly hydrophilic or transphilic (Figure 3.2.5). Proportions of carbohydrates found in the HPI fraction were 52 %, 61%, and 49 % in the exponential growth phase and 82 %, 69 % and 80% in the stationary phase for CV-, MA- and AF-AOM respectively. The recovery of carbohydrates across the fractionation procedure varied from 72-82 % in the exponential phase and 93-104 % in the stationary phase, which suggests that some carbohydrate material in the exponential growth phase becomes irreversibly associated with the resin. This accounts for the difference observed between exponential and stationary phase carbohydrates. Hydrophilic compounds are described as neutral polysaccharides, low MW mono- and di-carboxylic carboxylic acids and acidic sugars (Edzwald, 1993), thus supporting observations in the current study.

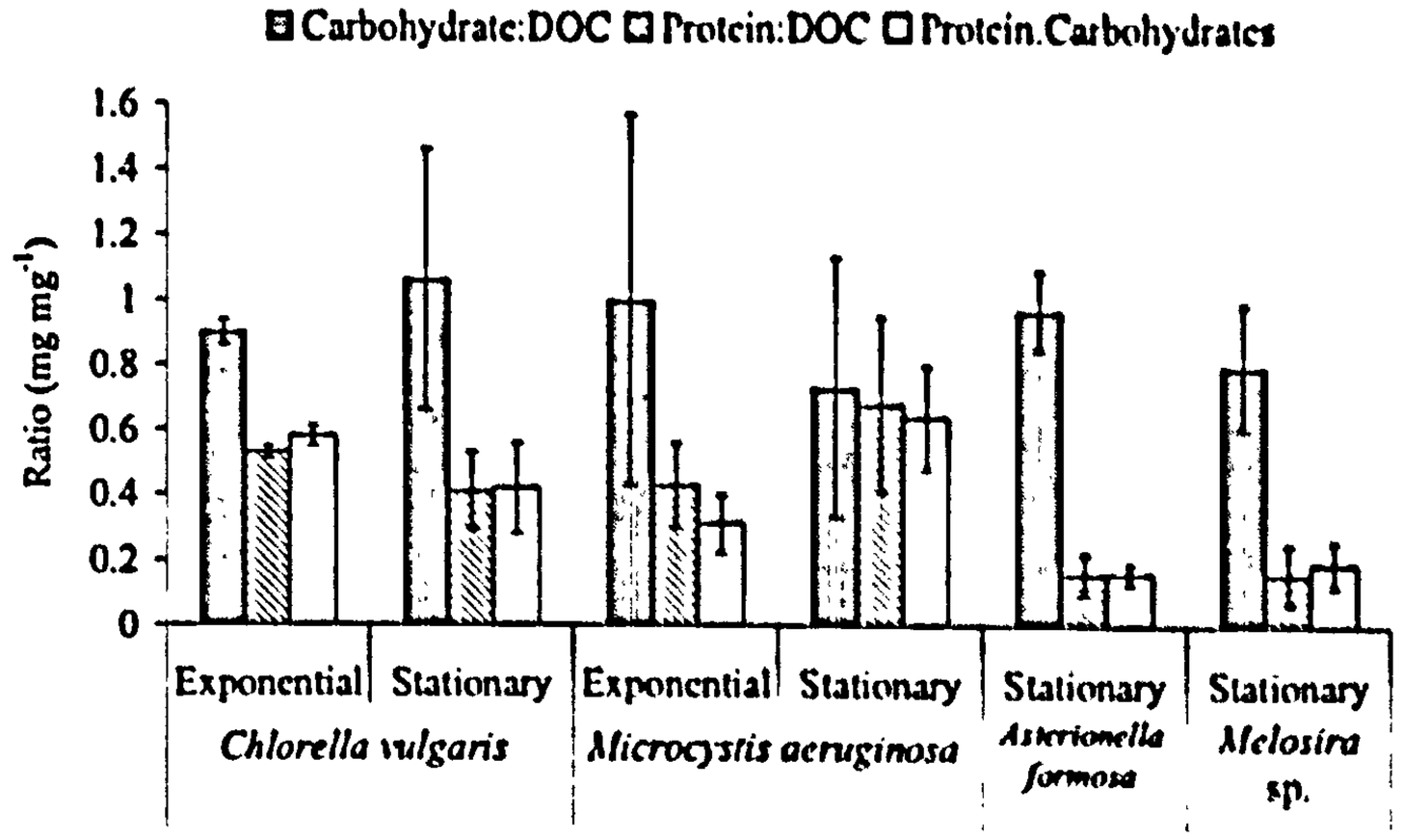

Figure 3.2.4 *Carbohydrate:DOC, protein:DOC and protein:carbohydrate ratios for stationary phase CV-, MA-, AF-, and Msp-AOM.*

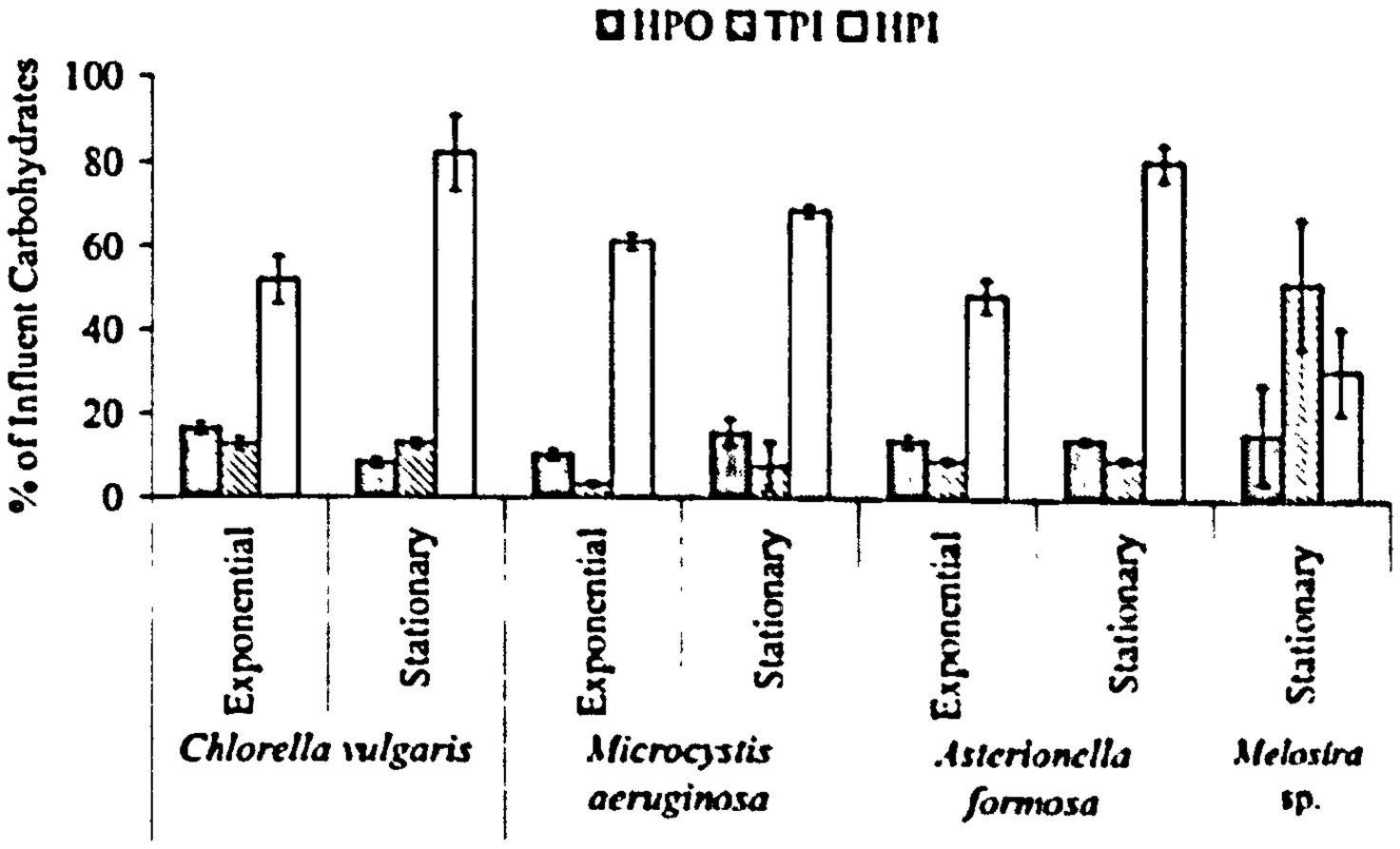

Figure 3.2.5 *% of total carbohydrates in the AOM present in the HPO, TPI and HPI fractions.*

3.2.3.5 Fluorescence EEM

Fluorescence in the current study has been designated according to Coble (1996) as follows: Peak T_1 and T_2 is tryptophan-like (protein-like); Peak A is humic-like; Peak B is tyrosine-like (protein-like); and Peak C is also humic-like. Tryptophan-like

rather than humic/fulvic acid-like fluorescence dominated in all EEMs, with the exception of exponential phase CV-AOM (Figure 3.2.6). This is common for micro-organisms and has previously been observed for algae, such as the diatom *Nitzschia* (Determann *et al.*, 1998), the green algae, *Scendesmus quadricauda* (Nguyen *et al.*, 2005) and planktonic bacteria including *Pseudomonas aeruginosa* at culture ages of 168 hours (Elliott *et al.*, 2006). MA- and AF-AOM had comparable EEMs (Figure 3.2.6). For example, in the exponential phase both had Peak T_1 and T_2 maxima at $\lambda_{emission}$ = 340 nm and $\lambda_{exitation}$ = 305 nm (T_1) and 240 nm (T_2). On transition to the stationary phase the excitation wavelengths of Peaks T_1 and T_2 maxima decreased to 285 nm and 225 nm for MA-AOM and similarly to 280 nm and 230 nm for AF-AOM. Given that the solution environment remained stable in terms of composition, temperature and pH, this is attributable to a change in protein structure. Furthermore, in both cases the ratio of relative peak intensities decreased with age by six (T_1) and two (T_2) times for MA-AOM and 87 (T_1) and 55 (T_2) times for AF-AOM. This indicates that tryptophan-like substances were present in lower quantities during the stationary phase. The EEM of MA-AOM was similar to that obtained for cyanobacteria in previous studies as tryptophan-like fluorescence was detected; however, additional fluorescence was also detected in locations attributable to humic/fulvic-like substances (Her *et al.* 2003; Nguyen *et al.*, 2005). Similarly to AF-AOM, there was only very low intensity fluorescence at $\lambda_{emission}$ = 335 nm and $\lambda_{exitation}$ = 225 (T_2) for Msp-AOM, demonstrating that only low levels of tryptophan-like fluorescence was detected (Figure 3.2.6g).

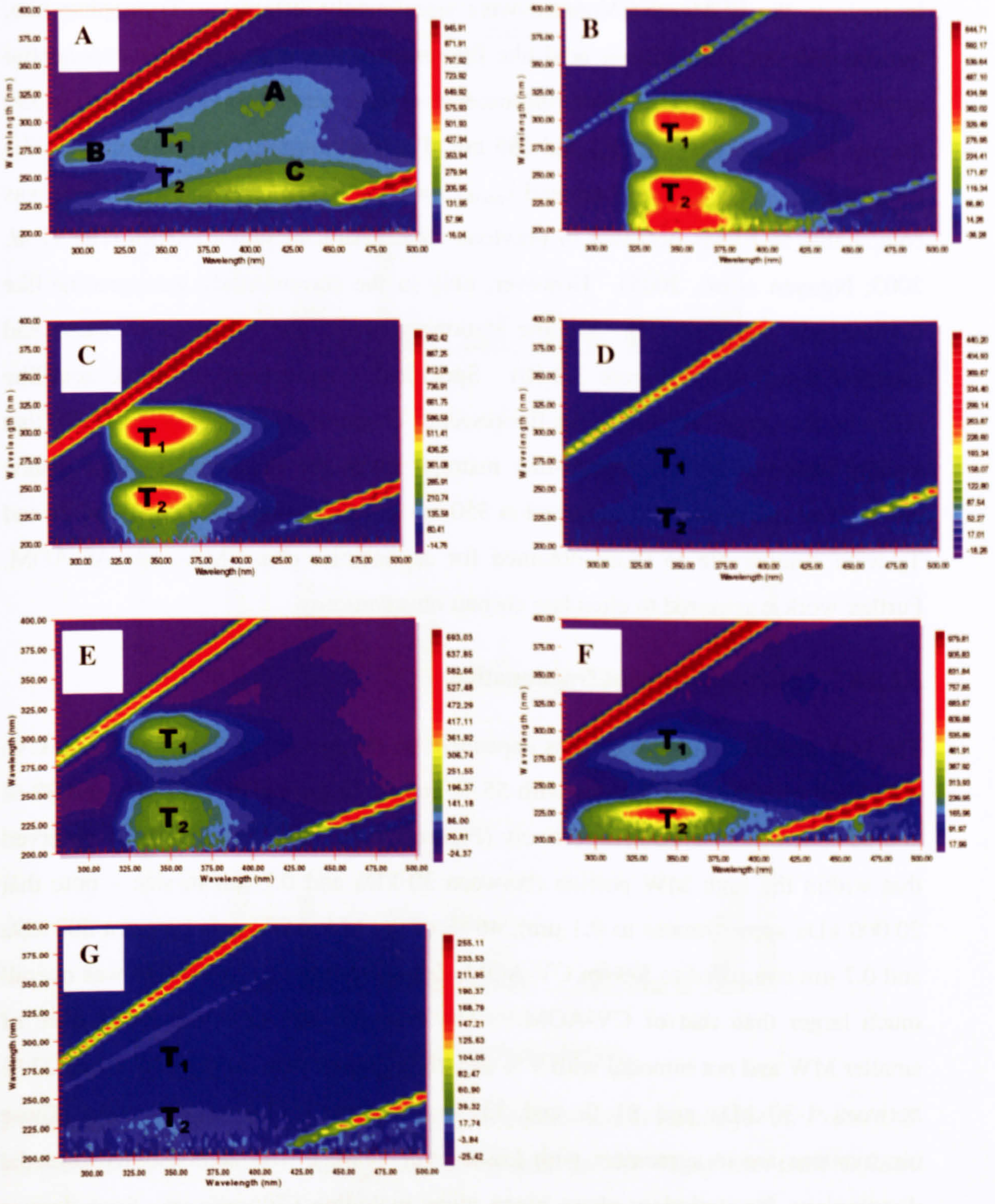

Figure 3.2.6 *Examples of fluorescence Excitation-Emission Matrix (EEM) Spectra for A. Exponential CV-AOM; B. Stationary CV-AOM;* **C.** *Exponential AF-AOM; D. Stationary AF-AOM; E. Exponential MA-AOM; F. Stationary MA-AOM; and G. Stationary Msp-AOM. Z-axis = Excitation (nm); X-axis = Emission (nm); and Y-axis = Intensity.*

In contrast, the EEMs of CV-AOM were significantly different. Tryptophan-like, tyrosine-like and humic/fulvic acid-like fluorescence was detected in the exponential growth phase (Figure 6a), where fluorescence centres were observed at $\lambda_{emission}$ = 350 nm and $\lambda_{excitation}$ = 230 nm (T_2) and 285 nm (T_1) in addition to humic/fulvic acid-like fluorescence at $\lambda_{emission}$ = 410 nm and $\lambda_{excitation}$ = 240nm (C) and 320 nm (A). This was comparable to EEMs obtained in previous algae characterisation studies (Her *et al.* 2003; Nguyen *et al.*, 2005). However, only in the current study was tyrosine-like fluorescence observed (B). By the stationary phase, the fluorescence EEM had changed significantly (Figure 3.2.6b). Specifically, there were no fulvic acid-like peaks by this stage. However, the fluorescence observed was not consistent with that usually observed for tryptophan-like material given the detection of three peaks; although emission was still observed at 350 nm and excitation wavelengths of T_1 and T_2 were comparable to those obtained for exponential phase MA- and AF-AOM. Further work is required to elucidate current observations.

3.2.3.6 Molecular weight fractionation

The MW distribution of AOM was dependent on the species of algae. MA- and CV-AOM had bimodal distributions with 55 % and 62 % greater than 30 kDa and 38 % and 30 % less than 1 kDa respectively (Figure 3.2.7). Additionally, it was observed that within the high MW portion (between 30 kDa and 0.7 μm in size – note that 20,000 kDa approximates to 0.1 μm), 46 % of the MA-AOM was between 500 kDa and 0.7 μm compared to 5 % of CV-AOM, demonstrating that MA-AOM was overall much larger than that of CV-AOM. The diatomic AF- and Msp-AOM were of smaller MW and not bimodal with 9 % and 28 % greater than 30 kDa, 16 % and 22 % between 1-30 kDa and 81 % and 53 % less than 1 kDa respectively. These observations are in agreement with Lüsse *et al.* (1985), who demonstrated bimodal distributions for stationary phase green algae including *Chlorella* sp., *Scenedesmus obliquus* and *Dictyosphaerium* sp. and the cyanobacteria, *Pseudanabaena catenata*, using 0.2 μm and <1.1 nm (~2 kDa) membranes. Similarly, the same study showed that AOM of MW <1.1 nm dominated for the diatom *Melosira granulata*.

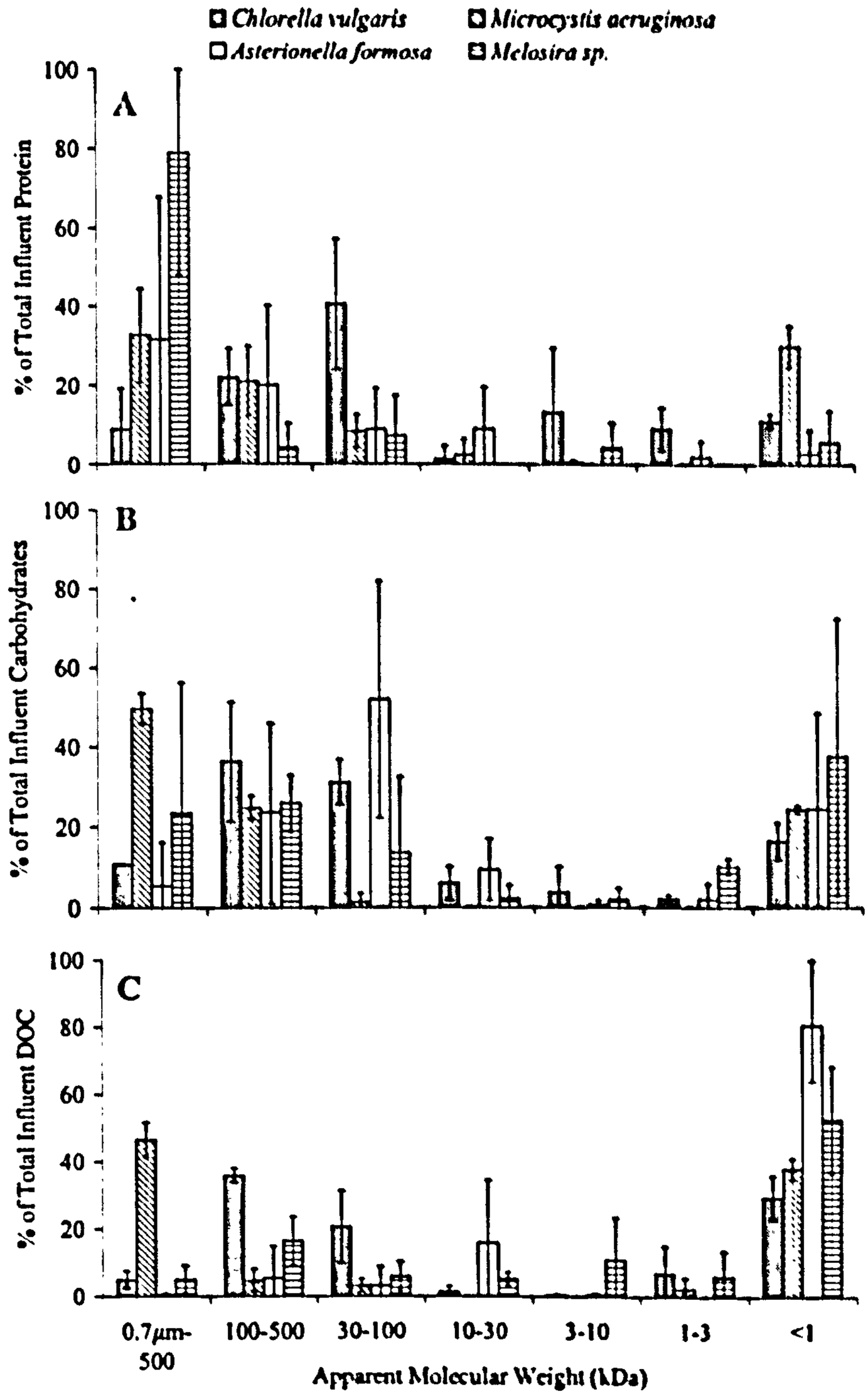

Figure 3.2.7 *Molecular weight UF membrane fractionation results for A. Protein, B. Carbohydrate, C. DOC. Results are presented as the % of the total influent respective parameter.*

Carbohydrate and protein analysis of the membrane permeate demonstrated that 78 %, 77 %, 81% and 62 % of the total carbohydrates and 72 %, 62 %, 60.3 % and 90.3 % of the total proteins were in the range 30 kDa to 0.7 µm for CV-, MA-, AF- and Msp-AOM (Figure 3.2.7). This demonstrates that much of the high MW AOM is

carbohydrates and proteins, suggesting that lower MW material was dominated by smaller molecules not measured by either the carbohydrate or protein methods.

3.2.4 DISCUSSION

3.2.4.1 Comparisons of AOM from different species with NOM and SMP

Irrespective of growth phase or species AOM comprised more than 57 % hydrophilic compounds, SUVA values of less than 2.0 l m^{-1} mg^{-1}, tryptophan-like fluorescence and similar carbohydrate to DOC ratios (Table 3.2.1) indicating that all AOM was dominated by compounds with low absorbance at 254 nm, including both hydrophobic proteins and hydrophilic polysaccharides (Edzwald, 1993). Interestingly, aromatic tryptophan-like proteins were not detected by SUVA. Hence, SUVA is only indicative of humic/fulvic type aromaticity. All AOM was negatively charged for pH 2-10 and with the exception of exponential phase CV-AOM, shared a similar zeta potential profile across the range pH 1-10 that was typical of systems dominated by carboxylic acid functional groups.

Comparisons of the different AOM revealed the major differences in character to be associated with charge density, hydrophobicity, protein:carbohydrate ratios and molecular weight fractionation. Analysis across stationary phase AOM samples showed that increased charge density was related to lower hydrophobicity (Table 3.2.1). For example, CV-AOM had a charge density of 3.2 meq g^{-1} with a hydrophobicity of 11 % whereas MA-AOM had a charge density of 0.1 with a hydrophobicity of 30 %. This observation is inconsistent with previous knowledge concerning NOM where highly charged material has been associated with the hydrophobic humic acid fraction (HAF) and fulvic acid fraction (FAF) (Sharp *et al.*, 2005). To illustrate, 8.8 meq g^{-1} and 1.0 meq g^{-1} were associated with the hydrophobic and hydrophilic components of NOM respectively. However, the lack of humic/fulvic acid-like fluorescence (Table 3.2.1), with the exception of exponential phase CV-AOM, demonstrates that these compounds were not present in the AOM. In fact, the AOM was dominated by hydrophobic proteins and hydrophilic

polysaccharides. The charge density of AOM occurs as a result of hydrophilic, charged polysaccharides including acetylamino sugars, sulphated sugars and carboxylated sugars (uronic acids) (Leppard, 1995) where the latter has been directly related to metal complexation capacity (Kaplan *et al.*, 1998).

Table 3.2.1 *Summary table of characterisation for each species in the exponential growth phase (EG) and stationary growth phase (SG). (Neg. = Negligible).*

	CV-AOM		MA-AOM		AF-AOM		Msp-AOM
	EG	SG	EG	SG	EG	SG	SG
SUVA ($l\ m^{-1}\ mg^{-1}$)	1.29	0.54	1.65	0.48	1.7	0.54	0.58
Isoelectric Point	1.0	1.0	1.9	1.8	0.9	0.8	1.6
Hydrophilicity (%)	60	71	59	57	73	70	64
Hydrophobicity (%)	22	11	24	30	15	20	32
Charge Density (meq g^{-1})	0.9	3.2	0.2	0.1	Neg.	1.0	Neg.
Fluorescence EEMs Peaks	T_1, T_2, A, B, C	T_1, T_2	T_1, T_2	T_1, T_2	T_1, T_2	T_1, T_2	T_1, T_2
Carbohydrate: DOC ($mg\ mg^{-1}$)	0.9	1.1	1.0	0.7	-	1.0	0.8
Trans/hydrophilic carbohydrates (%)	65	95	64	77	58	90	83
Protein:DOC ($mg\ mg^{-1}$)	0.53	0.40	0.40	0.64	-	0.19	0.16
Protein: carbohydrate ($mg\ mg^{-1}$)	0.58	0.40	0.30	0.60	-	0.20	0.20
>500 kDa proteins: carbohydrate ($mg\ mg^{-1}$)	-	0.04	-	0.20	-	0.05	0.15
>500 kDa proteins:carbs/ hydrophobicity	-	0.0034	-	0.0067	-	0.0025	0.0047
AOM >30 kDa (%)	-	62	-	55	-	9	30
AOM <1 kDa (%)	-	30	-	38	-	81	53

The lack of humic/fulvic material implies that proteins govern hydrophobicity in AOM systems. Indeed, one study determined that the hydrophobic fraction of EPS comprised predominantly protein and not carbohydrate components, as a result of amino acids with hydrophobic side groups (Jorand *et al.* (1998). Furthermore, it has been proposed that as the protein:carbohydrate ratio of EPS/SMP in biomass solutions increases the charge density decreases (Morgan *et al.*, 1990) and hydrophobicity increases (Jorand *et al.*, 1998). In this study, MA-AOM and Msp-AOM had high hydrophobicity and low charge; however, their protein:carbohydrate ratios were very high and low respectively. Hence, MA-AOM adheres to the correlation observed for EPS/SMP, while that of Msp-AOM does not, suggesting that the relationship is only valid for proteins excreted by bacteria, including cyanobacteria such as *M. aeruginosa*. In fact, increases in AOM hydrophobicity were more closely related to an increase in the ratio of high MW proteins (>500 kDa) to carbohydrates, rather than the bulk protein content (Table 3.2.1). This observation is likely a result of more hydrophobic proteins tending to associate to form agglomerates as a result of their hydrophobicity. Furthermore, proteins have been implicated as charge neutralisers as amino groups in some proteins carry positively charged groups that neutralise anionic functional groups e.g. carboxylic acids, hence decreasing the nett surface charge (Liao *et al.*, 2001).

3.2.4.2 Implications for water treatment

Water that is characterised by a large hydrophilic portion and low SUVA (less than 3), such as the AOM in the current study, is generally assumed to have a low coagulant demand due to its low charge density and relatively low DOC removals can be anticipated on coagulation (Edzwald, 1993). One study demonstrated that the concentration of the hydrophilic component for NOM dominated water could be used as a good indicator for the proportion of DOC that was untreatable (Sharp *et al.*, 2005). However, as shown AOM and NOM exhibit very different properties. Crucially, the hydrophilic material in NOM tends to be uncharged whilst that of AOM can carry a significant proportion of the total charged load. The importance of such differences is that previously found predictive relationships are unlikely to hold for systems that contain AOM.

AOM levels from all algal species tested exceeded 2 mg L^{-1} at the cell concentrations commonly reported during blooms (Henderson *et al.*, 2007) suggesting that AOM should have a significant influence on the coagulation process. CV- and MA-AOM was predominately of a large MW with concentrations of high MW (>100 kDa) protein that equated to a coverage of 9-20 mg m^{-2}. Such levels are significantly higher than the 2.5 mg L^{-1} reported to provide high coverage of BSA on latex and thus inhibited coagulation (Tirado-Miranda *et al.*, 2003). In contrast, the Msp- and AF-AOM was predominately below 10 kDa which is unlikely to extend beyond the double layer and so should mainly influence charge density and hence coagulant demand but not effect removal potential (Bernhardt *et al.*, 1985).

Comparison with previous studies on algae related treatment indicate that MA- and CV-AOM should produce the higher THM levels up on chlorination due to there higher protein contents (Scully *et al.*, 1988). Similar studies on nanofiltraton of algae have demonstrated higher MW AOM to be the predominant foulant and as such greater operational problems could be expected than when treating the diatoms. Importantly comparing the different treatment stages indicates that the varying character of AOM from the different algae means that each species will cause a unique set of challenges restricting the ability to generalise about the treatment of algae.

3.2.5 CONCLUSIONS

1. The following similarities were observed for all samples: a) AOM was dominated by hydrophilic polysaccharides and hydrophobic proteins; b) low SUVA values were exhibited for all species at both growth phases, signifying a lack of UV_{254} absorbing compounds, and indicating that aromatic tryptophan-like proteins were not detected by SUVA; and c) all AOM had a negative zeta potential of between -15 and -35 mV for pH 4-10 with i.e.p. values of 1-2.

2. Two relationships were observed for AOM: a) Charge density was observed to decrease as hydrophobicity increased; and b) increasing hydrophobicity was related to increasing proteins >500 kDa:carbohydrate ratio. The first was inconsistent with previous knowledge for NOM; while the second was similar to that reported for EPS/SMP systems. These observations are explained as follows: a) Charge density of AOM is attributable to hydrophilic, acidic carbohydrates and not hydrophobic, fulvic and humic acids as it is for NOM; b) Proteins of MW greater than 500 kDa govern hydrophobicity in the absence of humic/fulvic acids; and c) Hydrophobic proteins may neutralise some of the acidic groups thus reducing the charge density.

3.2.6 ACKNOWLEDGEMENTS

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CHAPTER 4: REMOVAL OF ALGAE BY CONVENTIONAL METHODS

Paper 5 An investigation into the relationship between coagulant demand and algae system characteristics

Submitted: *Environmental Science and Technology*

Paper 6 Successful removal of algae using zeta potential

Submitted: *Separation Science and Technology*

4. REMOVAL OF ALGAE BY CONVENTIONAL METHODS

4.1 COAGULATION AND FLOTATION OF ALGAE: IMPACT OF DIFFERING CELL AND ALGOGENIC ORGANIC MATTER (AOM) CHARACTER

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ABSTRACT

The aim of this study was to compare the coagulation and flotation of different algae species with varying morphology and algogenic organic matter (AOM) composition in order to link algae character to treatment. Specifically, *Microcystis aeruginosa* (a cyanobacterium), *Chlorella vulgaris* (a green alga), *Asterionella formosa* and *Melosira* sp. (diatoms) were treated by coagulation with aluminium sulphate and flotation. The AOM was extracted and treated separately. Subsequent analyses included residual cell counts, dissolved organic carbon, residual aluminium and zeta potential. Good removal efficiencies were obtained for each species, but the coagulant demand was species dependent and was related not to taxonomic groupings but to character. Cells, AOM and aluminium were concurrently removed at a coagulant dose that was related on a log-log basis to both cell surface area and charge density, although it was much stronger in the case of the latter. This was attributed to a significant proportion of the coagulant demand being generated by the AOM. The implications of such findings are that relatively simple charge measurement can be used to understand and control coagulation and flotation of algae.

4.1.1 INTRODUCTION

Algae are ubiquitous in reservoirs and rivers that supply drinking water treatment works. Seasonally, algae population densities can soar to very high concentrations which can result in the production of unwholesome water. For example, in addition to elevated residual cell counts in treated water, algae may also impart toxic metabolites or offensive taste and odour compounds to the water (Rosen *et al.*, 1992; Haider *et al.*, 2003). Furthermore, algogenic organic matter (AOM) can form trihalomethane (THM) precursors (Nguyen *et al.*, 2005) and has been shown to complex with metal coagulants, raising residual coagulant concentration (Bernhardt *et al.*, 1985).

Currently, the most common treatment combination for algae removal is coagulation-flotation-filtration. Successful flotation is reliant upon influent particles having both a minimum particle diameter of approximately 30 μm, although this depends on the operating conditions, and a minimum particle zeta potential of -10 mV to ensure effective particle-bubble collision and attachment efficiencies respectively (Han *et al.*, 2001). Well-executed coagulation will achieve both and therefore, if coagulation is unsuccessful, poor flotation can occur, resulting in high coagulant residuals and downstream filter blockage or breach, depending on influent cell character (Bernhardt, 1984). Coagulation of algae cells can be difficult as a result of their widely variable character, including: complex cell morphologies, such as spinal appendages preventing close contact of cells (Bernhardt and Clasen, 1991); cell motility, enabling liberation from flocs (Pieterse and Cloot, 1997); and AOM sterically interfering to prevent agglomeration, increasing the negative charge at the cell surface and chelating metal coagulant (Bernhardt *et al.*, 1985). These characteristics generally act to increase coagulant demand and furthermore have been suggested to complicate the coagulation process such that coagulant cannot be added on a stoichiometric basis (Bernahrdt and Clasen, 1991) as discussed by Stumm and O'Melia (1968). It has been suggested that only microscopic, spherical cells can be coagulated according to charge neutralisation mechanisms (Tilton *et al.*, 1972; Bernhardt and Clasen, 1994) thus allowing optimum coagulant dosage to be estimated stoichiometrically. However, these conclusions have resulted from studies

investigating coagulation followed by direct filtration (Tilton *et al.*, 1972; bernhardt and Clasen, 1991; Bernhardt and Clasen, 1994) and may not apply for flotation processes. A greater understanding of coagulating algae systems for removal by flotation is thus required. Specifically, the impact of variable morphology and AOM composition requires investigation.

A recent study examined the AOM composition of four algae species – specifically *Chlorella vulgaris* (micro, spherical, green algae), *Microcystis aeruginosa* (micro, spherical cyanobacteria), *Asterionella formosa* (large, elongated, colonial diatom), and *Melosira* sp. (large, filamentous diatom) (Henderson *et al.*, 2007a). Key differences were associated with charge density, molecular weight (MW), protein content and hydrophobicity of the AOM systems. It is anticipated that this may impact considerably on coagulant demand for optimum removal. For example, increases in charge density may increase the coagulant demand while the presence of high MW material could aid or hinder flocculation at low or high concentrations respectively, similar to a polymer aid (Bernhardt *et al.*, 1985). Hence, the major objective of this study was to link the coagulation of these algae to both their morphological and AOM characteristics. Specifically, the potential for knowledge transfer between species was examined. To achieve this objective, the coagulation and flotation of the four aforementioned algae species was undertaken. Furthermore, the treatability of each algae system was investigated to assess how the system characteristics influenced cell and AOM removal efficiencies.

4.1.2 EXPERIMENTAL PROCEDURES

Algae Cultivation. The following freshwater algae cultures were obtained from the Culture Collection of Algae and Protozoa (CCAP), (Oban, Scotland): *Chlorella vulgaris* (211/11B – Delft, Holland); *Microcystis aeruginosa* (1450/3 – Esthwaite Water, Cumbria, England); *Asterionella formosa* (1005/9 – Esthwaite Water, Cumbria, England), while *Melosira* sp. (JA386 – Redesmere, Cheshire, England) was obtained from Sciento, Manchester, UK. Growth conditions have previously been

described (Henderson *et al.*, 2007a). Algae were harvested for experiments in the early growth phase.

AOM Extraction. AOM was extracted from bulk algae by centrifuging at 10,000 G for 15 minutes and subsequently filtering through a 0.7 μm filter (Whatman GF/F glass microfibre). If required, concentration of the AOM solution was achieved by rotary evaporation at 70 mb and 40 °C and hardness was removed by ion-exchange using cation exchange resin (Dowex 50-WX-8, 200 mesh, Na^+ form) (adapted from established method (Hoyer *et al.*, 1985)).

Algae System Characterisation. The algae systems were assessed using the following procedures due to the influence of each characteristic on coagulant demand:

a) Cell concentration – a haemocytometer and Sedgewick Rafter cell were used with a light microscope to manually count cells. Samples were left to settle onto the grids for 15 minutes. At least 100 cells were counted in triplicate.
b) Cell surface area – images of cells were obtained microscopically and sized using a scale generated using a graticule. The dimensions were used to produce surface areas using basic geometric shapes as follows: *C. vulgaris* and *M. aeruginosa* were sized using a spherical surface area = $4\pi r^2$; *A. formosa* and *Melosira* sp. were sized using a cylindrical surface area = $2\pi r2 + 2\pi rh$. In each case the dimensions of 100 cells were measured.
c) Charge density – A back titration method was utilised that was adapted from an established method (Kam and Gregory, 2001). The specific procedure has been previously reported (Henderson *et al.*, 2007a). Additionally, due to the interfering effects of suspended cells on UV absorbance, centrifuging of the sample for 2 minutes at 5,000 rpm was undertaken prior to absorbance measurements. Three different volumes of algae were analysed.

Coagulation and Dissolved Air Flotation. Algae were diluted prior to treatment to concentrations more often observed in supply reservoirs using deionised water to which 0.5 mM $NaHC0_3$ and 1.8 mM NaCl had been added. Bench scale coagulation and flotation was undertaken using an EC Engineering Dissolved Air Flotation Batch

Tester, Model DBT6 (Alberta, Canada). An aluminium sulphate coagulant was added to 1 litre of algae suspension at the beginning of a 2 minute rapid mix (200 rpm) during which pH was adjusted to either pH 5 or 7. After 15 minutes of slow mixing (30 rpm), the paddles were gently removed and air saturated deionised water, buffered as previously described, at a pressure of 450 kPa and recycle ratio of 12 % was supplied and the algae flocs were allowed to float for 10 minutes. Residual samples were obtained from sampling ports located 5 cm from the vessel base for analyses as follows: cell count as previously described; zeta potential (ZP) using a Malvern Zetasizer 2000HSA (Malvern, UK); and DOC using a Shimadzu TOC-5000A analyser. All analyses were performed in triplicate. The experiment was also repeated for AOM extracted from *C. vulgaris*, *M. aeruginosa* and *A. formosa* at pH 7. Samples were adjusted to approximately 5 mg L^{-1} by dilution using DI water buffered as previously described. Subsequent analyses included DOC and zeta potential.

4.1.3 RESULTS AND DISCUSSION

4.1.3.1 Algae-coagulant interactions

The addition of coagulant on a mass of coagulant to charge equivalence of algae resulted in a log linear relationship between dose and zeta potential for all four algae systems tested at pH 7 (Figure 4.1.1). The gradients of the ZP curves were 22.8, 13.6, 9.5 and 11.0 mV for *C. vulgaris*, *M. aeruginosa*, *A. formosa* and *Melosira* sp respectively, indicating that the coagulant was significantly more effective at neutralising the charge of *C. vulgaris* in comparison to the other three species. Furthermore, the onset of neutralisation occurred at much lower dose:charge ratios for *Melosira* sp., *A. formosa* and *C. vulgaris* in comparison to *M. aeruginosa*. A combination of both the gradient and the ratio required for onset of neutralisation resulted in very different ratios required to achieve the isoelectric point (i.e.p.) of 183 and 456 ng neq^{-1} for *Melosira* sp. and *C. vulgaris* respectively, which were almost an order of magnitude lower than those of *A. formosa* and *M. aeruginosa* at 1290 and 2781 ng neq^{-1} respectively. These ratios compared well with the literature as at pH 6

the spherical cyanobacteria, *Synechocystis minuscular*, required a Al:charge ratio of 1400 ng neq^{-1} for complete neutralisation (Bernhardt and Clasen, 1994).

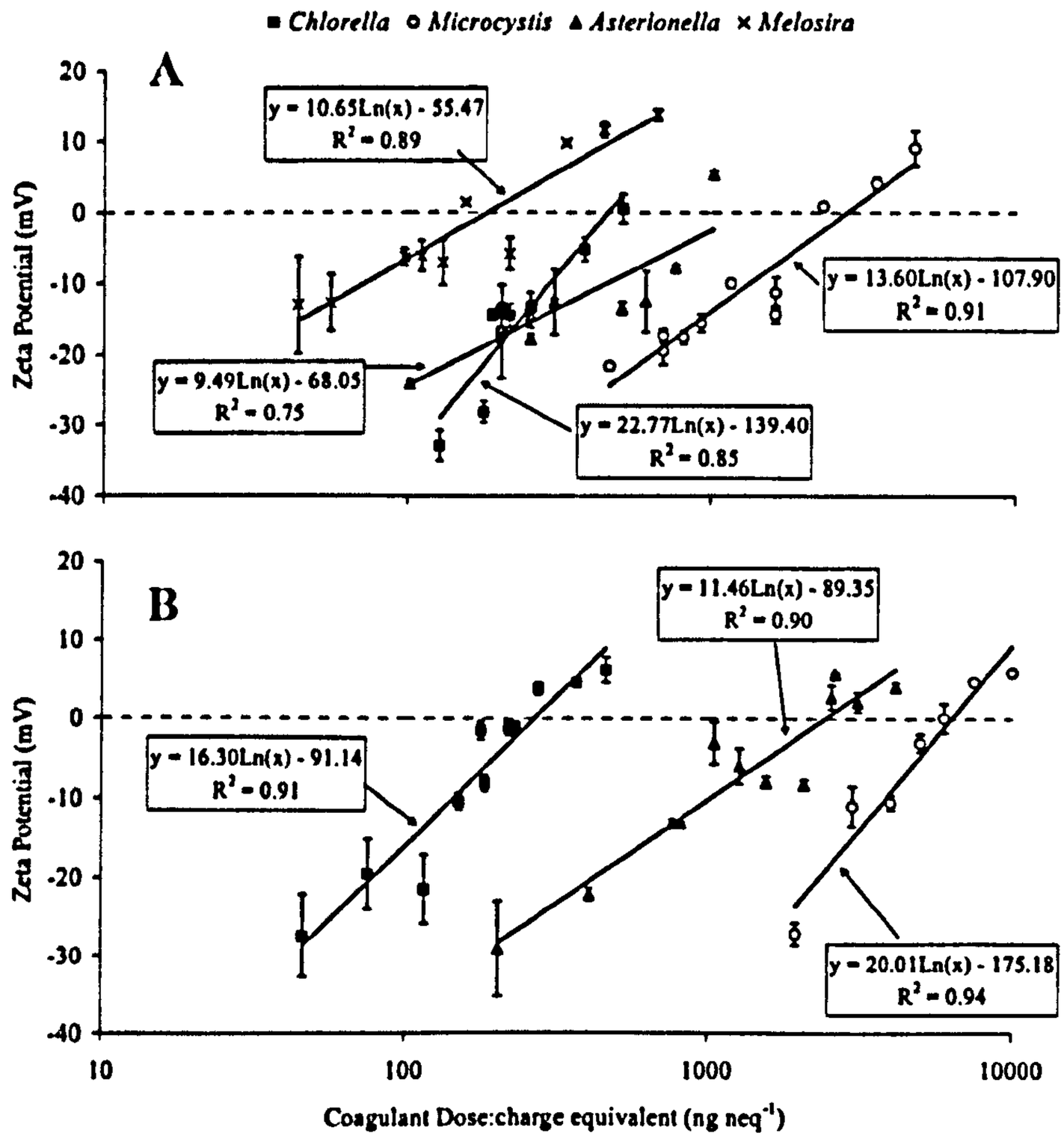

Figure 4.1.1 *The relationship between the coagulant dose:charge equivalent ratio and zeta potential for A. the entire system and B. the AOM.*

The ZP curves obtained for AOM were also examined in the same way (Figure 4.1.1). Gradients were 16.3, 20 and 11.5 mV *C. vulgaris*, *M. aeruginosa* and *A. formosa* respectively while the coagulant:charge ratio required to achieve the i.e.p. for *C. vulgaris* was 263 ng neq^{-1}, as opposed to the much larger values of 2426 and 6017 ng neq^{-1} for *A. formosa* and *M. aeruginosa* respectively. The gradient of *A. formosa* was very similar to that observed for the entire system while those of *C. vulgaris* and *M. aeruginosa* were less than and greater than that for the entire system respectively. However, the dose:charge ratio for complete neutralisation of the AOM alone was 1.7 times less than that required for the entire *C. vulgaris* system, that of *A. formosa* and

M. aeruginosa required 1.9 and 2.2 times more. This suggests that there were significant differences between the AOM of a system and the system including the cells. It was calculated for example, that 85, 5 and 31 % for *C. vulgaris*, *M. aeruginosa* and *A. formosa* of the total system charge was associated with the AOM component (Henderson *et al.*, 2007a) demonstrating that the character of the two components – cells and AOM – were very different in each case.

In general, differences observed in ZP curves can be a result of varying pH, charge density or complexation of coagulant. However, each experiment was conducted at the same pH and the coagulant dose has been normalised against the charge of the algae or AOM. Hence, the different doses required to achieve a neutral zeta potential and gradient reflect a difference in how the coagulant interacts with the cells and AOM, particularly with respect to complexation. It is known that at pH 7 the concentrations of dissolved cationic hydrolysis products are relatively low and the system is dominated by the negatively charged aluminate ion ($Al(OH)_4^-$) and by amorphous $Al(OH)_3$ precipitate (Duan and Gregory, 2003). This precipitate has an i.e.p. of 8 (Duan and Gregory, 2003) as a result of surface $\equiv Al\text{-}OH^+$ groups and is thus positively charged at pH 7. Surface complexation is likely to occur between these cationic sites and dissociated –COOH groups which are generally attributed to charge in an algae system (Bernhardt *et al.*, 1985). Adsorption of negatively charged AOM and cells would also occur such that a net decrease in charge results (Duan and Gregory, 2003). A low gradient such as that exhibited by *A. formosa* indicates that this neutralisation mechanism is relatively inefficient in comparison to larger gradients, such as that exhibited by *C. vulgaris*.

Explanation for the differences in efficiency of neutralisation lies in the system character and particularly that of the AOM as it will be closely associated with the cells. For example, the *M. aeruginosa* system required approximately 6 times the coagulant:charge ratio of *C. vulgaris* for complete neutralisation. This increased to 22 times in the case of the AOM. The AOM of *M. aeruginosa* has a very low charge but a significant protein concentration of 0.64 mg protein mg^{-1} DOC (Henderson *et al.*, 2007a) and, while that of *C. vulgaris* is also significant at 0.40 mg mg^{-1}, previous

studies have demonstrated that only the cyanobacteria protein and not green algae proteins have the appropriate characteristics for protein complexation (Takaara et al., 2004; Pivokonsky *et al.*, 2006). The fact that the gradient of the AOM curve for *M. aeruginosa* was relatively steep but that charge neutralisation was not instigated until a much larger coagulant:charge ratio had been achieved suggests that initially aluminium coagulant was consumed by protein complexation such that it was unavailable for charge neutralisation. The relatively high coagulant:charge ratio that achieved the i.e.p. of *A. formosa* was the result of the low gradient rather than the point of onset of neutralisation. This low gradient can be attributed to the prevalence of carbohydrate compounds of very low molecular weight, 80 % less than 1 kDa, (Henderson *et al.*, 2007a) in *A. formosa* AOM when compared to the other two species. Low MW, non-ionic, carbohydrate type compounds do not exhibit a coagulant demand or complex with coagulant (Bernhardt *et al.*, 1985) and thus may explain why coagulant complexation was more inefficient for this system.

4.1.3.2 Removal efficiencies of cells, AOM and aluminium

4.1.3.2.1 Cell Removal

There were four coagulation regions for *C. vulgaris* at pH 5 (Figure 4.1.2): Zone 1 – a region of no removal at low doses; Zone 2 - an initial zone of removal at low dose that coincided with a reduction in the magnitude of the ZP to +3.8 mV; Zone 3 - a restabilisation zone where ZP values were highly positive at +15 mV; and Zone 4 – a secondary removal zone at high coagulant doses. Optimum coagulant doses for Zone 2 and Zone 4 removal were 0.0195 pg μm^{-2} (289 ng neq^{-1}) and 0.742 pg μm^{-2} (11,027 ng neq^{-1}) respectively. This sequence of zones is commonly observed for both organic and inorganic systems at pH 5, attributed in the case of the former to charge neutralisation mechanisms and for the latter to sweep flocculation mechanisms (Duan and Gregory, 2003). In contrast, no restabilisation zone was observed for *M. aeruginosa* at pH 5, even upon reaching highly positive ZP values of +18.9 mV (Figure 4.1.2). Furthermore, a far lower dose of 0.00866 ng μm^{-2} in comparison to *C. vulgaris* was required to obtain good removal, corresponding to a ZP of -4.2 mV. This can be attributed to large MW proteins and carbohydrates acting as polymer aids, which is likely at pH 5 as they are only partially deprotonated (Bernhardt *et al.*, 1985).

It is suggested that this was not observed for *C. vulgaris* as the AOM was smaller with ~ 5 % larger than 500 kDa compared to ~45 % for *M. aeruginosa* (Henderson *et al.*, 2007a) and therefore would not be as efficient a polymer aid.

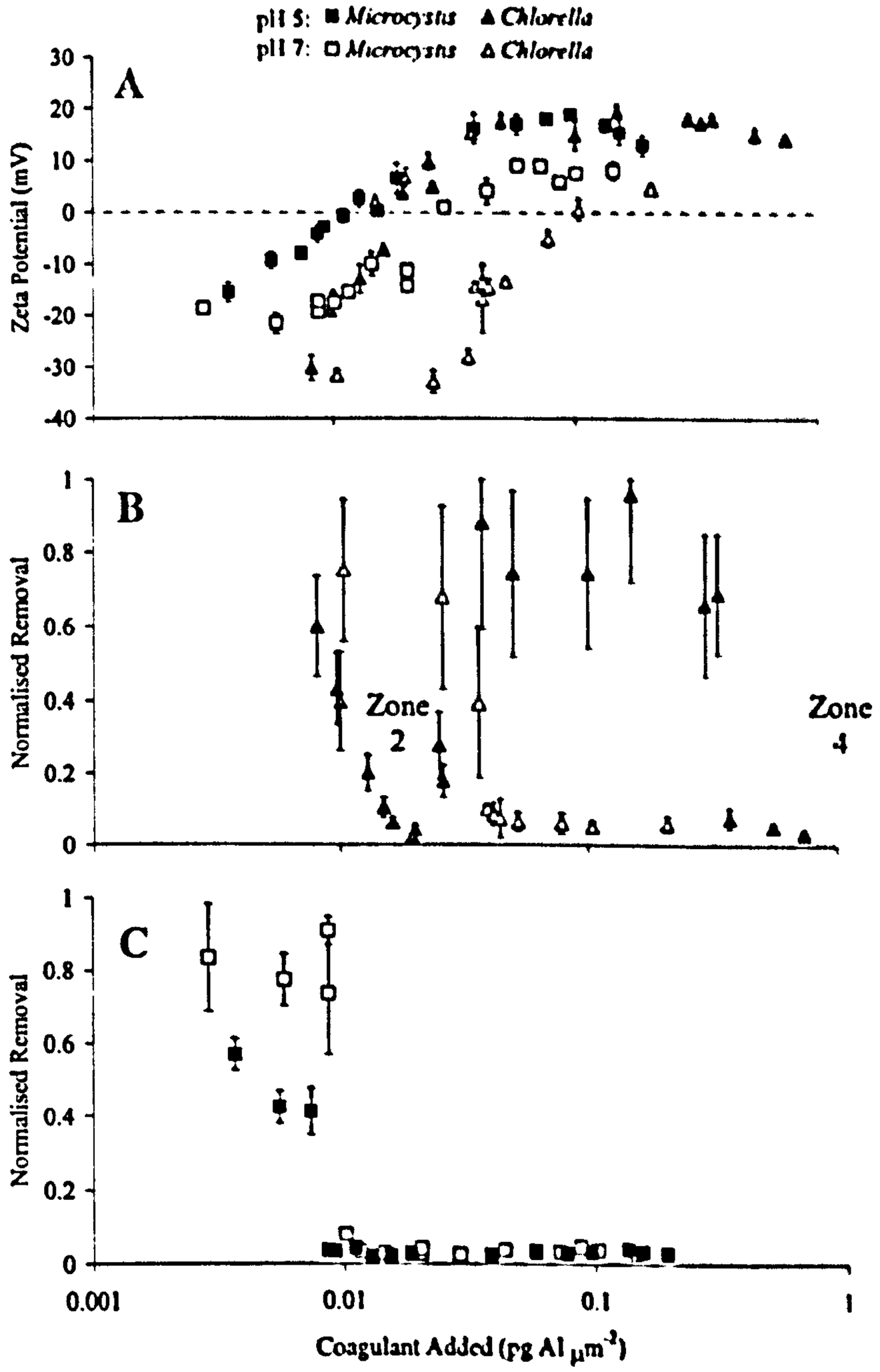

Figure 4.1.2 *Dose response curves depicting coagulant demand in terms of surface area at pH 5 and 7 for A. zeta potential, and for normalised removal based on cell count for B. Chlorella vulgaris and C. Microcystis aeruginosa.*

At pH 7, only one zone of removal was observed for each of the algae systems. Optimum removal occurred at a dose of 0.7 to 1.36 mg L^{-1} as Al, which when normalised for cell count was in the order 1.1 < 4.3 < 31.4 < 290 pg $cell^{-1}$ for *M. aeruginosa*, *C. vulgaris*, *A. formosa* and *Melosira* sp. The value obtained for *M. aeruginosa* is comparable with that obtained for the similar organism, *Synechocystis minuscula,* which had a coagulant demand at pH 6 of 1 pg Al $cell^{-1}$ (Bernhardt and Clasen, 1994). In contrast, values reported in another study were larger than those presented here, where *Chlorella* and *Cyclotella* required 13 and 32 pg $cell^{-1}$ respectively (Edzwald and Wingler, 1990). However, flocculation times were shorter at 5 minutes which may have resulted in larger doses as more recent studies have shown that algae flocs may not begin growing for low coagulant doses until after more than 7 minutes of slow stirring (Clasen *et al.*, 2000; Henderson *et al.*, 2006). Doses per charge and surface area were also calculated as 383, 927, 508 and 154 ng Al neq^{-1} and 0.078, 0.012, 0.085 and 0.053 pg μm^{-2} for *C. vulgaris*, *M. aeruginosa*, *A. formosa* and *Melosira* sp. respectively. Corresponding ZPs at optimum removal were -14.5 ± 1.6, -10 ± 2.2, -13.5 ± 0.4 and 1.4 ± 0.3 mV for the same species. Optimum removal was similar for all species at between 94.8 and 99.7 % cells removed.

The dose required for *C. vulgaris* at pH 7 in terms of surface area was four times higher than at pH 5 (Figure 4.1.2). This is a reflection of both the decrease in charge density of the system, which was 3 times lower at pH 5, and of the change of speciation of aluminium such that dissolved cationic hydrolysis species, which are more effective neutralisers, dominate. Interestingly, the corresponding coagulant:charge ratio decreased by only 1.3 times, as it only reflected the difference in alum speciation having already been normalised for charge. In contrast, the optimum coagulant demand of *M. aeruginosa* at pH 5 was 0.00866 pg μm^{-2}, only 1.4 times less than that required at pH 7. This is primarily a result of the change in alum speciation as the charge density of these algae was much lower than that of *C. vulgaris* and therefore less significant.

4.1.3.2.2 AOM Removal

Optimum removal for AOM occurred for doses in the order of 0.8 <1.2 < 1.5 mg Al mg^{-1} DOC for *C. vulgaris*, *M. aeruginosa* and *A. formosa* respectively (Figure 4.1.3) at ZP values of 3.8 ± 0.8, 1.0 ± 0.3 and -7.9 ± 0.7 mV for the same species. Additionally, AOM was relatively treatable with removal efficiencies of 71 %, 55 % and 46 % for *C. vulgaris*, *M. aeruginosa* and *A. formosa* respectively. Optimum doses are consistent with literature values for NOM which has a coagulant demand of approximately 1 mg Al mg^{-1} C (Duan and Gregory, 2003). The removal and dose requirements of the AOM can be related to the characteristics of the AOM. For instance, the fact that *M. aeruginosa* required a larger dose than *C. vulgaris*, despite having a lower charge density of 0.1 compared with 3.2 meq g^{-1} (Henderson *et al.*, 2007a), can again be attributed to protein complexation increasing the coagulant required to neutralise the charge of the AOM and consequently destabilise the system (Figure 4.1.1). The high coagulant demand of *A. formosa* can again be attributed to the relatively low molecular weight (MW) of the AOM in comparison to that of *M. aeruginosa* and *C. vulgaris* (Henderson *et al.*, 2007a). It is suggested that more coagulant is therefore required to build flocs by cross linking of the small MW AOM-aluminium compounds. Similarly, the high removal efficiency of *C. vulgaris* AOM is a result of the material being both highly charged and of relatively large MW such that flocculation is efficient. The lower removal efficiencies of *M. aeruginosa* and *A. formosa* are a consequence of lower charge density and lower MW respectively, limiting AOM-coagulant interactions. The material remained relatively treatable with comparable coagulant demands to NOM in spite of relatively high hydrophilicities of 71 %, 57 % and 70 % for *C. vulgaris*, *M. aeruginosa* and *A. formosa* respectively (Henderson *et al.*, 2007a). This is in contrast to studies of NOM where a relationship between decreased removal efficiency and increased hydrophilicity of the system has been observed (Sharp *et al.*, 2006).

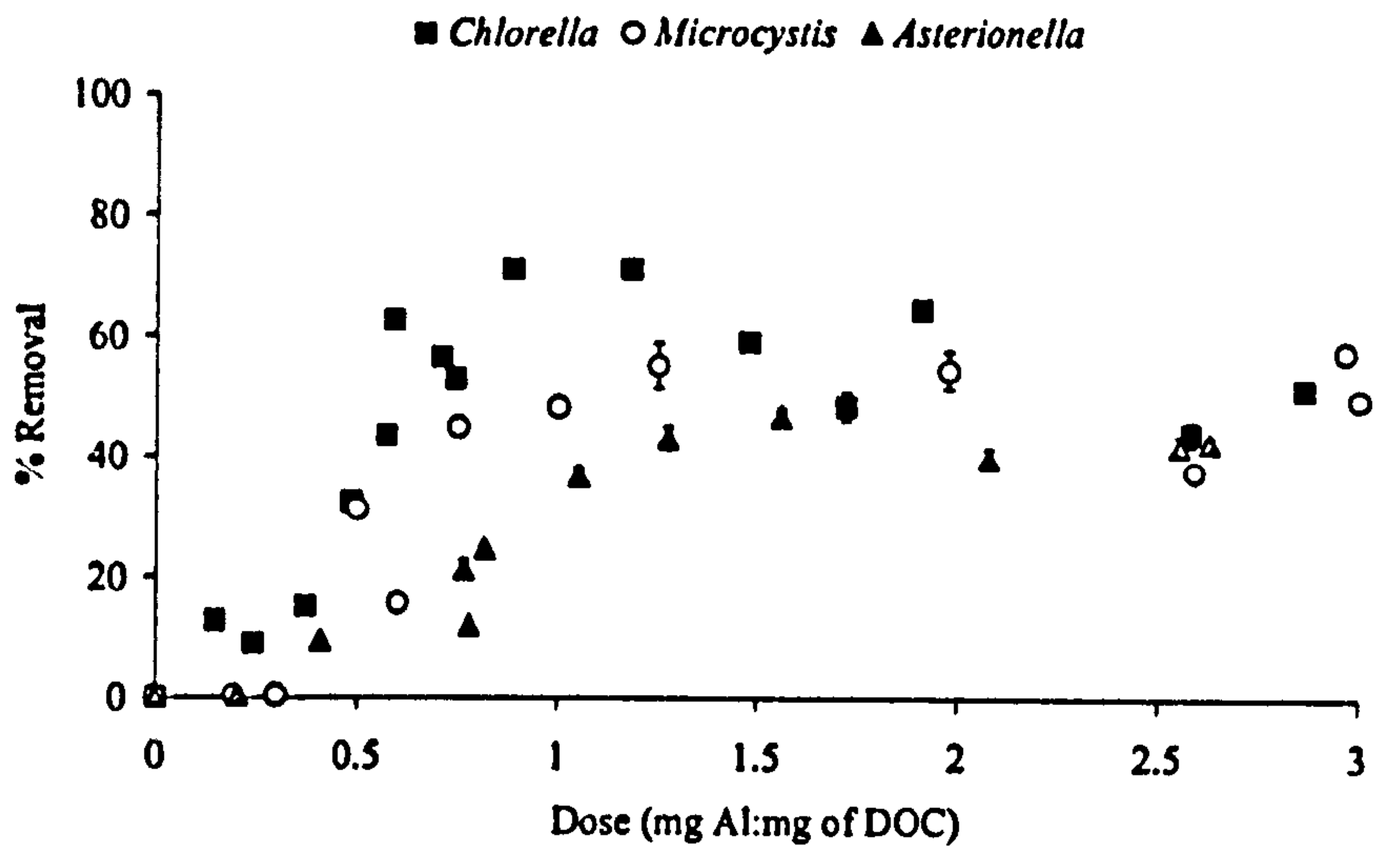

Figure 4.1.3 *AOM coagulation at pH 7*

4.1.3.3.3 Aluminium Removal

Residual aluminium data revealed that high Al residuals of up to 65 % could be anticipated for aluminium doses of less than 0.5 mg Al mg^{-1} DOC (Figure 4.1.4). The lowest aluminium residuals of 0.4 %, equating to a residual of 10-35 µg L^{-1}, were achieved for doses of greater than 0.8 mg Al mg^{-1} C (Figure 4.1.4), which is concurrent with optimum AOM removal (Figure 4.1.3). This high initial residual and subsequent lowering of aluminium at higher aluminium:DOC ratios has been previously observed for humic acid systems, whereby a dose of 0.54 mg Al:mg C was required to ensure low aluminium residuals (Jekel and Heinzmann, 1989). Similarly, a study examining the coagulation of *Chlorella* AOM with iron determined that residual iron was always found in the filtrate for doses of <0.2 mg Fe mg C^{-1} but never at doses of 1 mg Fe mg C^{-1} (Bernhardt *et al.*, 1985). This trend has been attributed to the coordination to AOM to Me-hydroxide polymers at low concentrations thus preventing the cross linking and clustering of Al-hydroxide polymers which consequently only becomes possible at higher doses (Bernhardt *et al.*, 1985; Jekel and Heinzmann, 1989), when simultaneous removal of both AOM (Figure 4.1.3) and aluminium (Figure 4.1.4) occurs. The fact that residual aluminium in *M. aeruginosa* systems was similar to those of *C. vulgaris* and *A. formosa* indicates that protein-Al complexates did not remain dissolved in solution and were bound into flocs by the aforementioned mechanisms.

While the treatability of cells, AOM and Al has been demonstrated, their concurrent removal must also be considered. If cells were removed preferentially, then high residual AOM and consequently high aluminium levels could result. At optimum dosages, the ratios of coagulant:DOC were calculated to be 0.93, 1.4 and 1.7 mg mg^{-1} for *C. vulgaris*, *M. aeruginosa* and *A. formosa* respectively, such that each was greater than the 0.8 mg mg^{-1} required for low residuals of both aluminium and AOM. Hence, for the optimum coagulant doses, removal of all three components would result.

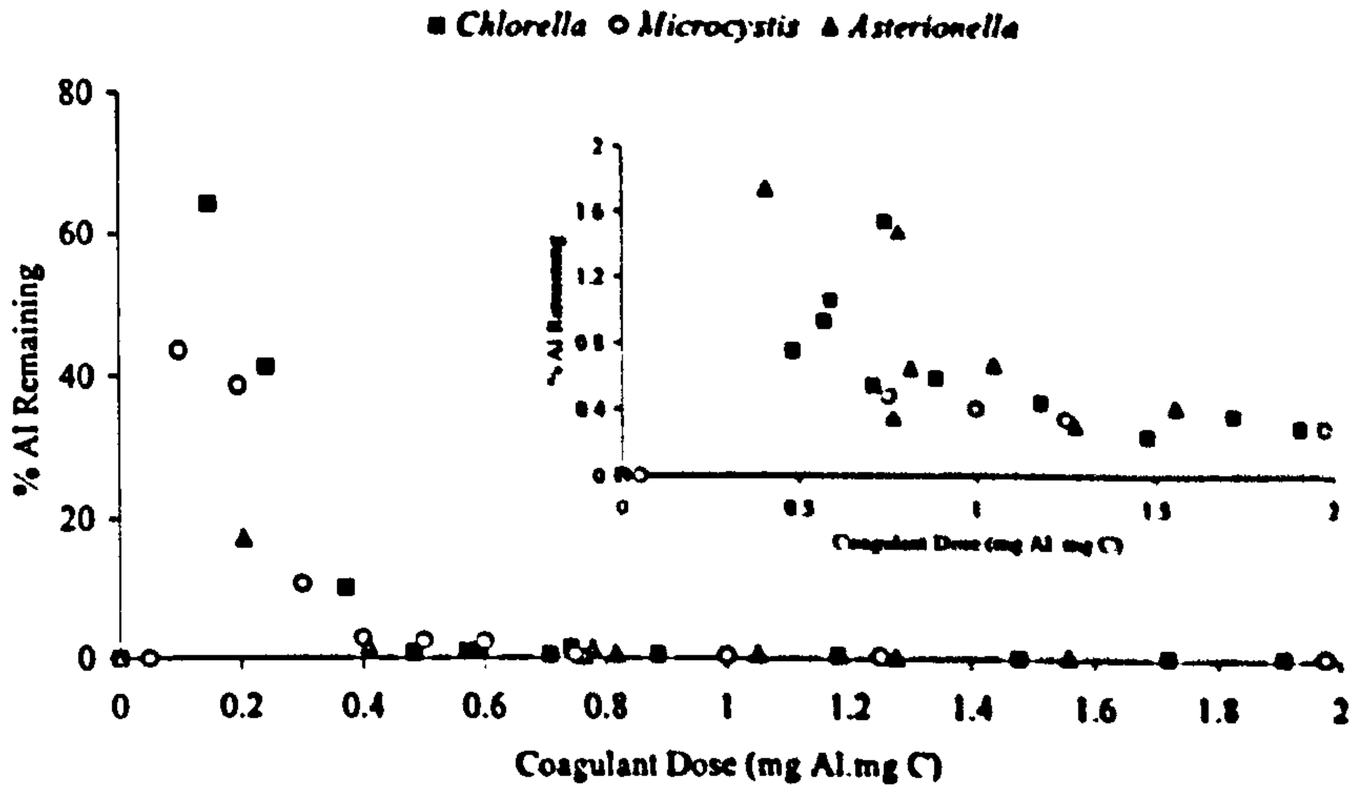

Figure 4.1.4 *Aluminium removal*

4.1.3.3 Implications for water treatment

The study demonstrated that, with appropriate application of coagulant, good removal could be anticipated for all of three system components – cells, AOM and aluminium, irrespective of algae species. The key difference between the systems was in the coagulant demand required to achieve optimum removal. Analysis of the presented data in combination with the available literature reveals a log-log relationship between optimum dose and both cell surface area and charge per cell (Figure 4.1.5). Note that there was a paucity of data relating the charge density of algae systems with coagulant demand. The relationship between coagulant demand and charge per cell

appears stronger than that of surface area, attributable to the fact that the dissolved organic component is also taken into account in the former. This is most important as the charge demand generated by some species is predominantly associated with the AOM component. For instance, in the case of *C. vulgaris*, 83 % of the charge is associated with the AOM. Other studies have reported a relationship between the concentration of algae and coagulant demand and have attributed this to increases in surface area and thus charge density (Tilton *et al.*, 1972; Bernhardt and Clasen, 1994); however they have been concerned with only one type of microscopic, spherical algae. This study further demonstrates that the optimum dose is dependent on the charge of the system irrespective of algae type. This is in contrast to a previous report which indicated that the relationship deteriorated for species with complex morphologies (Bernhardt and Clasen, 1991), such as *A. formosa* and *Melosira* sp. examined in the current study; however, this study was concerned with direct filtration as opposed to flotation, which is less susceptible to changing algae morphology (Petruševski *et al.*, 1993).

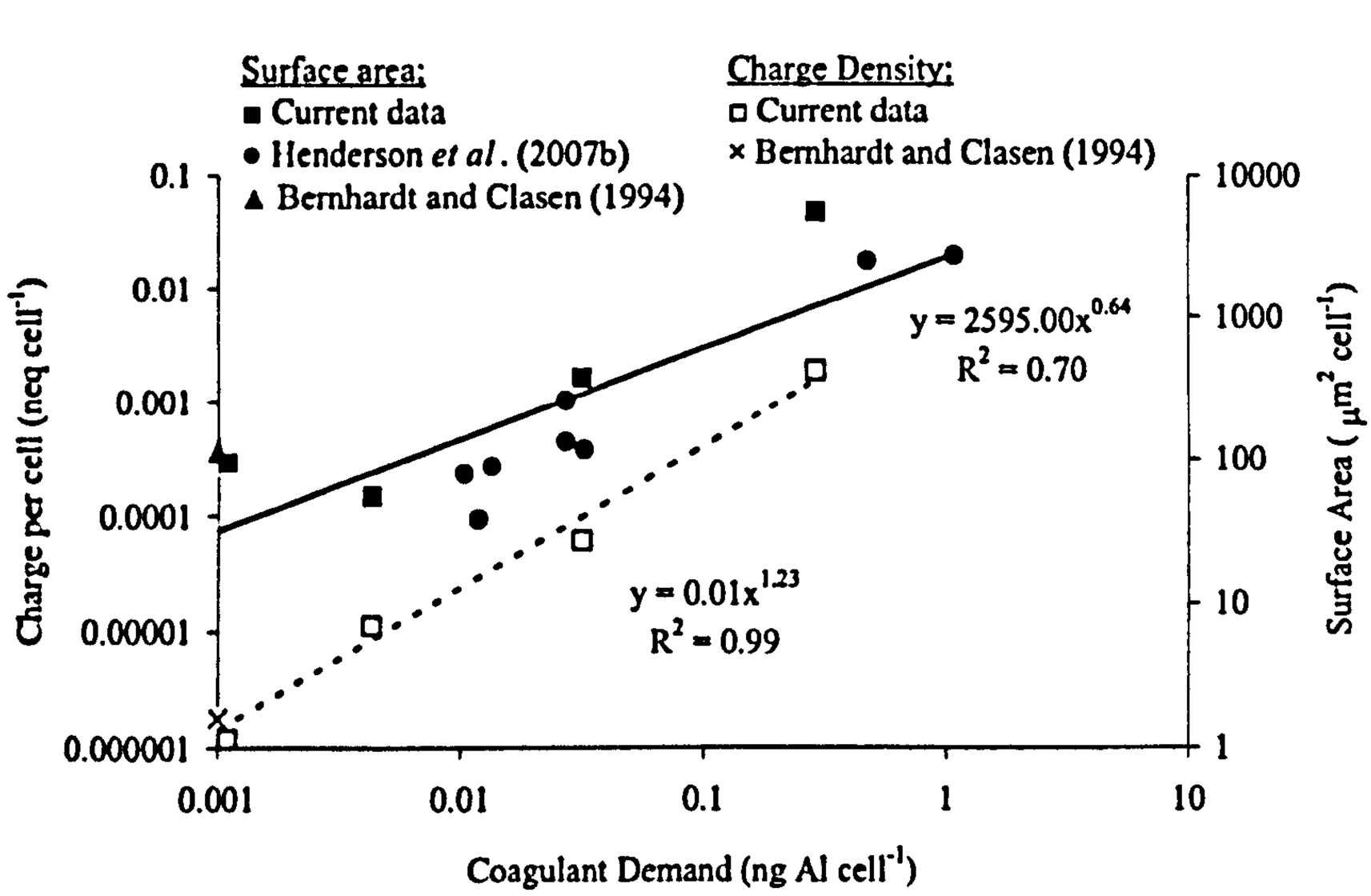

Figure 4.1.5 *The relationship between coagulant demand and both charge density and surface area of the algae systems with comparisons from the literature (Bernhardt and Clasen, 1994; Henderson et al., 2007b).*

The findings outlined here indicate a similar relationship to that observed for NOM, where coagulant demand was closely related to the hydrophobic components in the water (Sharp *et al.*, 2006). The implications of such findings are that relatively simple charge measurement, through either streaming current or zeta potential, can be used to understand and control coagulation and flotation of algae, irrespective of morphological differences. The former has been successfully implemented in a eutrophic lake in Germany (Bernhardt and Schell, 1993) while the latter is now being used in understanding practical issues related to the coagulation of NOM rich waters within a region of the UK (Sharp *et al.*, 2007) and has resulted in lower residuals, more stable systems and lower coagulant demands in certain sites. Surface area also provided a relationship with coagulant demand which could be utilised to understand changes in dose requirements as different species predominate in feed reservoirs. In contrast, monitoring cell counts without reference to species, will not give an indication of coagulant demand as, on a per cell basis, the coagulant demand required for optimum removal varied between species by orders of magnitude. Similarly, monitoring algae with respect to taxonomic grouping will not give any indication as to the coagulant demand.

4.1.4 ACKNOWLEDGEMENTS

The authors would like to thank the EPSRC, Anglian Water, Northumbrian Water, Thames Water, and Yorkshire Water for their financial support.

4.1.5 SUPPLEMENTARY INFORMATION

Table 4.1.1 *Summary of coagulation data for algae.*

	C. vulgaris		*M. aeruginosa*		*A. formosa*	*Melosira*
	pH 5	**pH 7**	**pH 5**	**pH 7**	**pH 7**	**pH 7**
Initial Cell concentration (cells mL^{-1})	$5.0 \times 10^5 \pm 5 \times 10^4$		$6.0 \times 10^5 \pm 1.5 \times 10^4$		$5.0 \times 10^4 \pm 1.2 \times 10^4$	$1.9 \times 10^3 \pm 550$
Surface area (μm^2 $cell^{-1}$)	55 ± 30		95 ± 34		370 ± 95	5500 ± 850
Charge Density (peq $cell^{-1}$)	0.0037	0.011	~0	0.0019	0.062	1.9
Optimum Coagulant Demand (*Zone 2/Zone 4 as depicted in Figure 4)						
mg L^{-1}	1.2 /20*	1.36	0.45	0.80	1.6	0.70
pg $cell^{-1}$	1.1/ 41*	4.3	0. 82	1.1	31	290
pg μm^{-2}	0.020/ 0. 74*	0.078	0.0087	0.012	0.085	0.053
ng neq^{-1}	290/ 11,000*	380	-	930	510	150
Zeta Potential at Optimum removal (mV)	3.8 ± 1.3	-14.5 ± 1.6	-4.2 ± 1.7	-10 ± 2.2	-13.5 ± 0.4	1.4 ± 0.3
% Cell Count Removal prior to Coagulation	22	3.5	13	9	33	98
Optimum % Cell Count Removal	97.7/ 96.8*	94.8	97.7	97.3	98.8	99.7

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4.2 SUCCESSFUL REMOVAL OF ALGAE USING ZETA POTENTIAL

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ABSTRACT

Algae can interfere with treatment processes at a water treatment works. Coagulation control is critical to reduce the impact of algae on downstream processes. This paper investigates the coagulation and flotation of four species of algae – *Asterionella formosa*, *Melosira* sp., *Microcystis aeruginosa* and *Chlorella vulgaris*. The zeta potential at optimum removal was measured and it was observed that when the zeta potential was reduced to between -8 mV and +2 mV, removal of algae and associated organic material was optimised, irrespective of coagulant dose or pH. Process control using zeta potential is therefore a viable tool for algae removal.

Keywords: Algae, coagulation, dissolved air flotation (DAF), zeta potential

4.2.1 INTRODUCTION

Many drinking water source reservoirs are subject to algae blooms which tend to occur on a seasonal basis. During such periods, carry over of algal cells and coagulant from the coagulation/clarification process to downstream filters can occur which can result in either filter blockage or penetration. Key to remedying the problem is better coagulation control such that the likelihood of its failure is

minimized. Particles or colloids that enter a water treatment works, including algal cells, are negatively charged (Ives, 1959; Edzwald, 1993) either as a result of dissociation or ionization of surface functional groups (for organic particles), adsorption of ions originating from organic matter, or lattice imperfections in inorganic particles. Consequently, there is electrostatic repulsion between adjacent particles if they come into contact at close separation distances and thus colloidal stability of the system is maintained (Gregory, 2006). Successful removal of influent particles relies on disrupting the stability of the system which is the objective of the coagulation process. Destabilisation is usually achieved at a water treatment works by the addition of cationic chemicals, including trivalent metal salts (Al^{3+} or Fe^{3+}) or cationic polymers, which interact with the particle surface to induce neutralization affects, although the mechanism by which this occurs is still disputed (Dentel, 1988; Licskó, 1997; Duan and Gregory, 2003). However, there is consensus that in destabilizing the system, the electrostatic barrier to contact between two adjacent components is minimized such that attractive van der Waals forces dominate over repulsive electrostatic forces (Gregory, 2006).

Surface charge is therefore an important parameter in coagulation experiments. The zeta potential is a measure of the electric potential at the plane of shear of the electrical double layer. The shear plane forms the boundary between the charged particle surface with adsorbed counter-ions and the diffuse region. Zeta potential therefore gives a measurement of the apparent surface charge. Reduction in the magnitude of the negative zeta potential signifies a reduction in the repulsive electrostatic forces and a critical zeta potential can be reached where the attractive van der Waals forces overcome these electrostatic forces and thus particles agglomerate (Gregory, 2006).

When investigating coagulation in combination with dissolved air flotation (DAF) for clarification, the significance of particle charge control is even more pronounced. The DAF process utilizes many microscopic, negatively charged bubbles, generated using pressurized, air saturated, recycled water to float flocs produced by the preceding coagulation process. Successful flotation relies on successful particle-bubble

attachment which is subject to the same forces previously described for particle-particle interactions, specifically electrostatic repulsion and attractive van der Waals among others. One study claimed that the electrostatic character of the bubbles and particles was the most important parameter for governing the removal efficiency of a batch DAF reactor (Han, 2002).

The use of zeta potential for monitoring and controlling the coagulation of natural organic matter (NOM) has been well researched and found to be of great benefit (Sharp *et al.*, 2005; Sharp *et al.*, 2006). However, there have been fewer studies investigating the use of zeta potential for controlling algae treatment. There is evidence to suggest that it may not be as successful when compared to NOM and kaolin as a result of variable morphology which interferes with the mechanisms involved in coagulation (Bernhardt and Clasen, 1991). However, if the zeta potential is demonstrated to be a useful control parameter for algae removal, many difficulties associated with algae coagulation control that arise as a result of highly variable population loadings and species diversity could be overcome. Hence, the current paper assesses the applicability of zeta potential for controlling the coagulation of the dynamic and diverse algae cell communities.

4.2.2 MATERIALS AND METHOD

4.2.2.1 Algae cultivation procedure

The following algae species were obtained from the Culture Collection for Algae and Protozoa (CCAP), Oban, Scotland: 1) *Microcystis aeruginosa* (1450/3), a cyanobacteria; 2) *Chlorella vulgaris* (211/11B), a green algae; and 3) *Asterionella formosa* (1005/9), a diatom. *Melosira* sp. (JA386) obtained from Sciento, Manchester, UK. *M. aeruginosa* and *C. vulgaris* were grown under the same cultivating conditions, using sterile Jaworski Media, a growth temperature of 20 °C and constant 24 hour lighting using two Sun-glo 30 W fluorescent tubes. The suspensions were grown in 200 ml volumes and shaken at 75 rpm using a Patterson Scientific Bibby Stuart SO1. The diatoms, *A. formosa* and *Melosira* sp., favoured

slightly different conditions for optimum growth as follows: sterile Diatom Media; a growth temperature of 15 °C; a lighting cycle of 14 hours light/8 hours dark; and, agitation only once daily by hand. An Environmental Test Chamber (Sanyo Versatile Environmental Test Chamber, MLK 350H), programmed to give a brightness of 1000 lx, was used for diatom growth.

4.2.2.2 Algae characterisation procedure

Algae systems comprise two major components: cells and algogenic organic matter (AOM). Algae cells were characterised in terms of concentration, size and surface area. Cell concentration was observed to increase according to a standard growth curve. Cell counting was completed using either a haemocytometer, for very concentrated samples, or a Sedgewick Rafter cell, for counting smaller populations, as appropriate. Owing to previous observations suggesting that growth cycle can affect system zeta potential, attributed to varying concentration and character of AOM (Edzwald and Wingler, 1990; Konno, 1993), it was ensured that algae were always harvested at the same stage of growth, selected as the onset of the stationary phase. At this stage, population density was at its highest and thus dilution to a concentration observed in the natural environment was undertaken prior to flotation experiments (Table 4.2.1). Dilution was achieved using deionised water that had been buffered to 0.5 mM using 1.0 M $NaHC0_3$ and made to a final ionic strength of 2.3 mM using 1.0 M NaCl.

The surface area and charge density of *M. aeruginosa*, *C. vulgaris* and *A. formosa*, and the surface area of *Melosira* sp. are summarised in Table 4.2.2. Note that charge density was determined according to an adapted back titration method (Kam and Gregory, 2001). Algae were specifically chosen to provide variations in terms of morphology and AOM as follows: 1) *M. aeruginosa* and *C. vulgaris* represent similarly shaped cells of simple spherical configuration from different phyla which have very different AOM character; 2) *A. formosa* has a more complex cell structure in comparison to *M. aeruginosa* and *C. vulgaris*; 3) *Melosira* sp. is a large, rigid,

filamentous diatom and has been specifically identified by water companies as a "problem" species that has proven difficult to treat.

Table 4.2.1 *Algae concentration reached in cultivating flasks and concentration used for flotation experiments.*

Algae Species	Stationary phase concentration (cells mL^{-1})	Experimental Concentration (cells mL^{-1})
Chlorella vulgaris	$1.1 \times 10^7 \pm 1 \times 10^6$	$5.0 \times 10^5 \pm 5.0 \times 10^4$
Microcystis aeruginosa	$1.3 \times 10^7 \pm 3 \times 10^5$	$6.0 \times 10^5 \pm 1.5 \times 10^4$
Asterionella formosa	$4.5 \times 10^5 \pm 7.6 \times 10^4$	$5.0 \times 10^4 \pm 1.2 \times 10^4$
Melosira sp.	$2.0 \times 10^4 \pm 4.8 \times 10^2$	$2.0 \times 10^3 \pm 2.0 \times 10^2$

Table 4.2.2 *Summary of algae cell characteristics in terms of cell surface area and charge density.*

Algae Species	Surface Area Equation	Average Surface Area (μm^2 $cell^{-1}$)	Charge Density (neq $cell^{-1}$)	Charge Density per Surface Area (μeq m^{-2})
Chlorella vulgaris	Sphere: $4 \pi r^2$ where r = 2	55	1.1×10^{-5}	300
Microcystis aeruginosa	Sphere: $4 \pi r^2$ where r = 2.75	95	1.9×10^{-6}	40
Asterionella formosa	Cylinder : $2 \pi r^2$ + $2 \pi r$ h where r = 1.4 and h=40	370	6.8×10^{-5}	180
Melosira sp.	Cuboid = 2ab + 2bc + 2ac where a = 55 ; b = 22 ; and c = 22	6000	1.88×10^{-3}	310

4.2.2.3 Flotation procedure

Batch Dissolved Air Flotation (DAF) experiments were undertaken using an EC Engineering Dissolved Air Flotation Batch Tester, Model DBT6 (Alberta, Canada). The coagulation/flocculation/flotation program comprised a 2 minute rapid mix (200 rpm), 15 minute slow stir (30 rpm) and 10 minute flotation time. Aluminium sulphate coagulant was added to 1 litre of algae suspension during the rapid mix stage at which time correction to pH 7 using 0.1 M HCl or 0.1 M NaOH as appropriate was undertaken. A coagulation pH of 5 was used in addition for *C. vulgaris* experiments to determine the impact of pH on zeta potential control. A recycle ratio of 12 % and a saturation pressure of 450 kPa were used for flotation. Ionic strength was kept constant throughout the experiments at 2.3 mM using NaCl. It was ensured that the saturated water used in flotation matched buffering, ionic strength and pH conditions set for algae suspensions. At the end of the flotation period, samples were extracted to measure for residual cell count, by microscopic analysis using the haemocytometer or Sedgewick Rafter cell as appropriate. Finally, all samples were tested for zeta potential as described in a later section.

Flotation experiments at pH 7 were repeated for AOM samples that were prepared by centrifuging the algae for 15 minutes at 10,000 G and filtering through a 0.7 μm filter (Whatman GF/F). Dissolved Organic Carbon (DOC) of the AOM was analysed using a Shimadzu TOC-5000A analyser. Initial DOC was adjusted to 5 mg L^{-1} and the residual DOC and zeta potential of each system was analysed.

4.2.2.4 Zeta potential

Zeta potential measurements were obtained using a Malvern ZetaSizer 2000 (Malvern, UK). The ZetaSizer 2000 measures the electrophoretic mobility (EM) and then converts this to zeta potential based on the Smoluchowski Equation. This is appropriate when $\kappa a >> 1$. Given the relatively high ionic strength (2.3 mM, equating to a κ value of 0.16 nm^{-1}) and algae cell size (3.2 μm or more) use of the Smoluchowski equation was appropriate for all cell systems. Consideration was also

given to zeta potential measurements involving solely AOM, given that the colloid size was much smaller. However, based on molecular weight analysis of AOM (Henderson *et al.*, 2007), it can be assumed that the majority of the AOM had a radius greater than 7 nm, which is that required for $\kappa a >> 1$.

All zeta potential results were obtained in triplicate. Furthermore, it was ensured that all samples extracted for zeta potential analysis had been exposed to the aluminium coagulant for at least 7 minutes. Previous research has demonstrated that zeta potential can take up to 7 minutes to stabilise after coagulant addition in algae systems depending on the dose administered (Clasen *et al.*, 2000). As anticipated, no difference was observed between zeta potential measurements obtained during the last five minutes of the slow stir period and at the end of the flotation period.

4.2.3 RESULTS AND DISCUSSION

4.2.3.1 Dose response curves

The algae cells under investigation in the current paper had cell surface areas that vary by orders of magnitude and cell charge densities that were similarly variable. For example, the surface areas of *M. aeruginosa* and *C. vulgaris* were 50 and 34 μm^2 $cell^{-1}$ respectively whilst their charge densities were 1.9×10^{-6} and 1.1×10^{-5} neq $cell^{-1}$ (Table 4.2.2). In contrast, *Melosira* sp. and *A. formosa* had much larger surface areas of 6000 and 370 μm^2 $cell^{-1}$ and charge densities of 1.88×10^{-3} and 6.8×10^{-5} neq $cell^{-1}$. Coagulant demands for the different algal systems were observed to vary by similar degrees (Figure 4.2.1). For example, *M. aeruginosa* and *C. vulgaris* both had relatively small coagulant demands at optimum removal of 0.0014 and 0.0057 ng Al $cell^{-1}$ respectively. It is interesting that despite the similarity of surface areas of *M. aeruginosa* and *C. vulgaris* cells, the much higher charge density of *C. vulgaris* resulted in approximately 3 times the coagulant demand. The much larger cells of *A. formosa* and *Melosira* sp. required 0.0314 ng Al $cell^{-1}$ for 98.9 % removal and 0.29 ng Al $cell^{-1}$ for 99.7 % removal respectively. This represents a respective increase of 22

and 207 times the coagulant required for *M. aeruginosa*. The increases in charge density for *A. formosa* and *Melosira* sp. were of the same order of magnitude at 35 and 990 times greater respectively compared to *M. aeruginosa*, while the surface areas were only 3.9 and 63 times greater. This indicates that increases in coagulant demand per cell are more strongly related to cell charge density rather than surface area. In the UK, the most common way of monitoring algae is by concentration, either by counting total algae cells microscopically or, even more generically, by analysing the chlorophyll *a* content in the influent water to give an indication of the overall algal activity. The implication of these results is that monitoring cell concentration will give limited information with respect to controlling coagulant dose at a water treatment plant as it is the overall charge density that determines coagulant demand.

Charge neutralisation was examined in the current study by zeta potential analysis. It was observed that for each species examined there was a decrease in the magnitude of the zeta potential which coincided with coagulant dose. For example, dosages of 0.0028, 0.0057, 0.05 and 0.29 ng Al $cell^{-1}$ were required to neutralise *M. aeruginosa*, *C. vulgaris*, *A. formosa* and *Melosira* sp. systems respectively (Figure 4.2.1). The onset of optimum removal was observed as the zeta potential approached more neutral values. Hence, charge neutralisation is an important mechanism for flocculation and subsequent removal of algae cells at pH 7, and additionally infers that there is a potential for utilising zeta potential for process control. This has frequently been reported in previous studies. For example, optimum removal of *Cyclotella* and *Chlorella* using aluminium at pH 6.5 by DAF coincided with the reduction of the electrophoretic mobility (Edzwald and Wingler, 1990).

4.2.3.2 The zeta potential "operational window"

The zeta potential operational windows for the species tested are varied (Figure 4.2.2). To illustrate, optimum removal for *C. vulgaris* was observed at zeta potential values of or less negative than -17 mV, whilst that of *M. aeruginosa* and *A. formosa* required zeta potential values of or less negative than -16 mV and -13 mV

respectively. Good removal of *Melosira* sp. was observed at all dosages for which zeta potential values ranged from -13 mV to 11.6 mV, although optimum removal was observed at a zeta potential of 1.4 mV. The good removal that was observed can be attributed both to the relatively low zeta potential observed for the system and to the relatively large size of the *Melosira* sp. cells, which were approximately 20 μm width by 55 μm length and linked together to form long filaments. This would mean that even at low coagulant doses good particle-bubble collisions and attachment could occur.

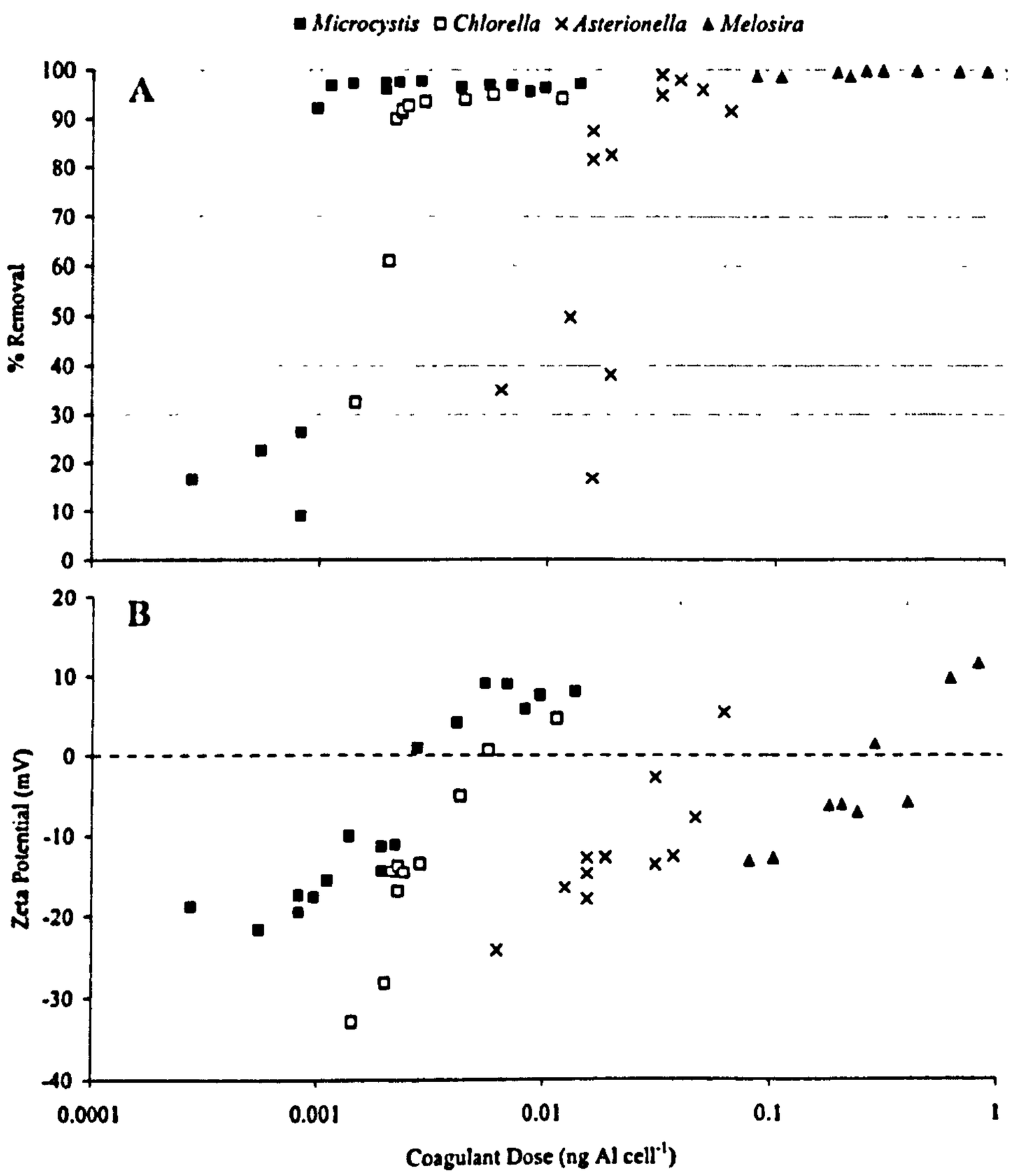

Figure 4.2.1 *A. Dose response curves for M. aeruginosa, C. vulgaris, A. formosa and Melosira sp. and B. corresponding zeta potential values (Henderson et al., 2007).*

Overall, reduction of the magnitude of the zeta potential to approximately, or less negative than, -10 mV would ensure removal for all algae species. It was noted that no decrease in removal efficiency was observed at positive zeta potentials. This is typical of coagulation experiments of organic components conducted at pH 7. At the relatively high doses required to instigate charge reversal, removal efficiency is not observed to decrease, generally attributed to a change in dominating coagulation mechanism from charge neutralisation to sweep flocculation (Duan and Gregory, 2003). This explains the observations that the system does not restabilise.

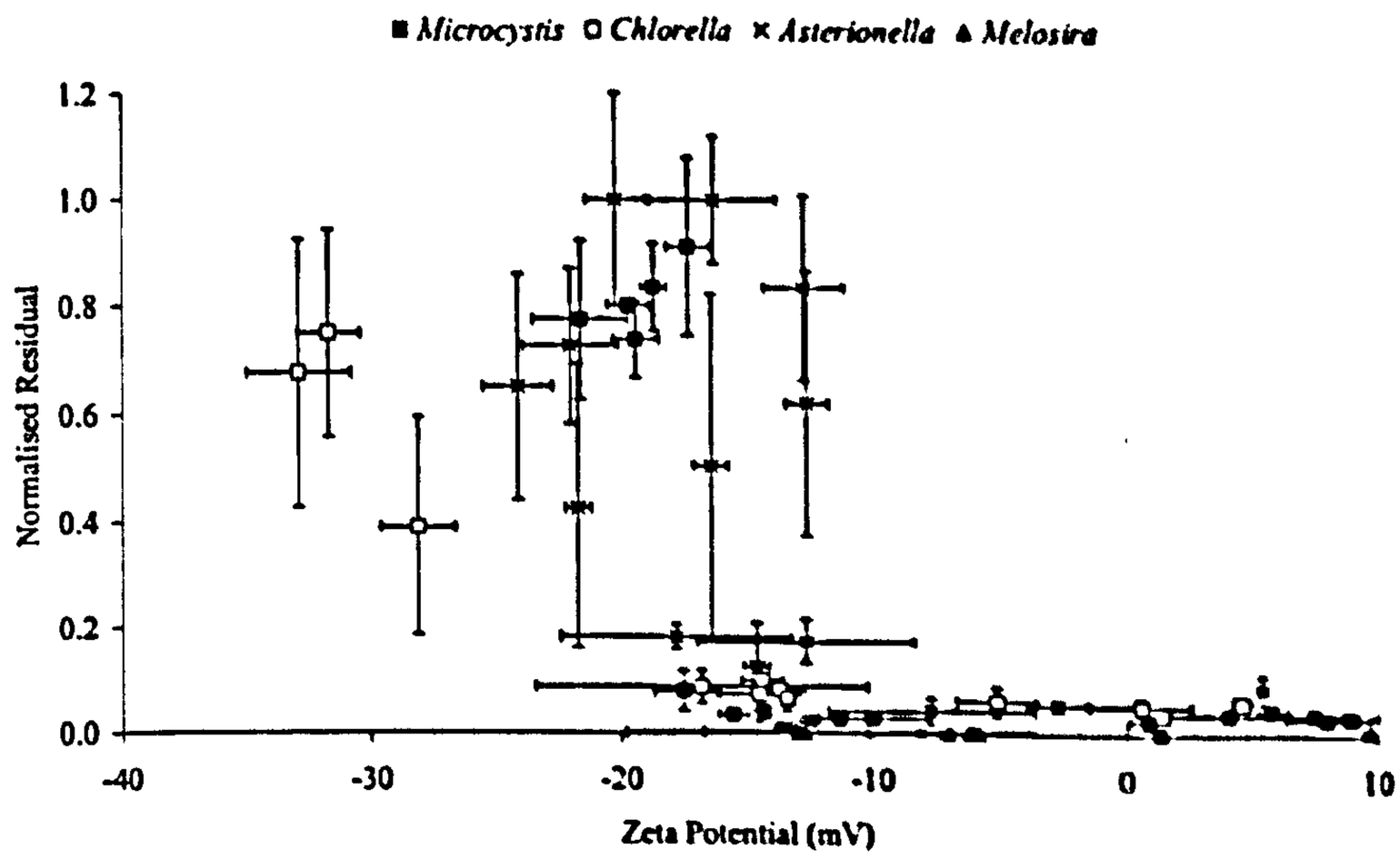

Figure 4.2.2 *The zeta potential operational window for M. aeruginosa, C. vulgaris, A. formosa and Melosira sp.*

Similar operational windows have previously been observed when treating algae using aluminium based coagulants and DAF. For example, treatment of *Cyclotella* was optimised at zeta potential values less negative than -15 mV compared to -13 mV for *Chlorella* (Edzwald and Wingler, 1990). Similarly, it was demonstrated that a critical minimum reduction in zeta potential to -10 mV was required to ensure optimum removal for NOM when using a ferric based coagulant, irrespective of whether the zeta potential is altered using coagulant dose or pH adjustment (Sharp *et al.*, 2006). Furthermore, the same study showed that if zeta potential values become too positive, +3 mV, then poor removal was observed. It has also been observed that

zeta potential operational ranges may become smaller when sedimentation as opposed to DAF is employed for clarification. For example, operational windows of -8 mV to 0 mV and -5 mV to 0 mV were observed for a mixed algae sample and for a sample dominated by *Melosira* and *Pediastrum* respectively during sedimentation processes Mouchet and Bonnélye, 1998). However, Bernhardt and Clasen (1991) showed that while zeta potential could be related to coagulant demand for many species, there was no relationship for cells with more complex morphologies, such as the diatoms *Stephanodiscus hantzchii*, which had long spines, and *Fragillaria crotonensis*, a large colony forming algae. This is in contrast to the current study where good correlation was obtained for complex species *A. formosa* and *Melosira* sp. The previous study utilised direct filtration and optimum coagulant demand was determined based on the run length. Direct filtration is susceptible to filter clogging when large algae of complex morphologies are introduced, which severely limits run times, irrespective of system zeta potential. DAF is not subject to these limitations and hence is a far more robust process for algae removal and therefore the removal of the large complex algae tested in the current paper (*A. formosa* and *Melosira* sp.) could still be controlled using zeta potential.

The importance of pH was investigated for *C. vulgaris*, where the zeta potential operational window obtained for pH 7 was compared with that at pH 5 (Figure 4.2.3). The primary observation was that optimum removal was obtained irrespective of pH if the zeta potential was maintained between -10 mV and +2 mV. Secondly, coagulation experiments conducted at pH 5 achieved more positive zeta potential values than those obtained at pH 7 and this coincided with a decrease in cell removal. This was attributed to restabilisation of the system as a result of the extremes of positive charge which cause electrostatic repulsion (Duan and Gregory, 2003). The data points at extreme positive charge (+14 to +18 mV) that demonstrated good removal (Figure 4.2.3) were obtained during a secondary zone of removal that was observed at high coagulant doses (0.02 ng Al $cell^{-1}$) which can be attributed to sweep flocculation mechanisms. This follows a pattern commonly observed for NOM and kaolin systems (Duan and Gregory, 2003). An additional observation was that the zeta potential operational window was narrower for pH 5 experiments. For example,

at pH 7, optimised removal was obtained by -16.8 mV whereas at pH 5 by -16.1 mV only 60 % of the algae had been removed. This can be explained by the presence of high concentrations of cationic amorphous aluminium hydroxide precipitates at pH 7 and low concentrations of dissolved cationic hydrolysis species which is in contrast to that which occurs at pH 5 (Duan and Gregory, 2003). The precipitates can take part in sweep flocculation whilst the dissolved species are more important in charge neutralisation. This suggests that at pH 7, the high rates of removal observed at relatively high negative zeta potential values (-16.8 to -13.4 mV) are a consequence of not only charge neutralisation but also sweep flocculation mechanisms. It is interesting to note that there was no apparent difference in removal efficiency between pH 5 and pH 7. This supports conclusions made previously for NOM where it was stated that provided zeta potentials within the operational range were obtained, the pH or coagulant dose used to achieve that zeta potential was unimportant (Sharp *et al.*, 2006).

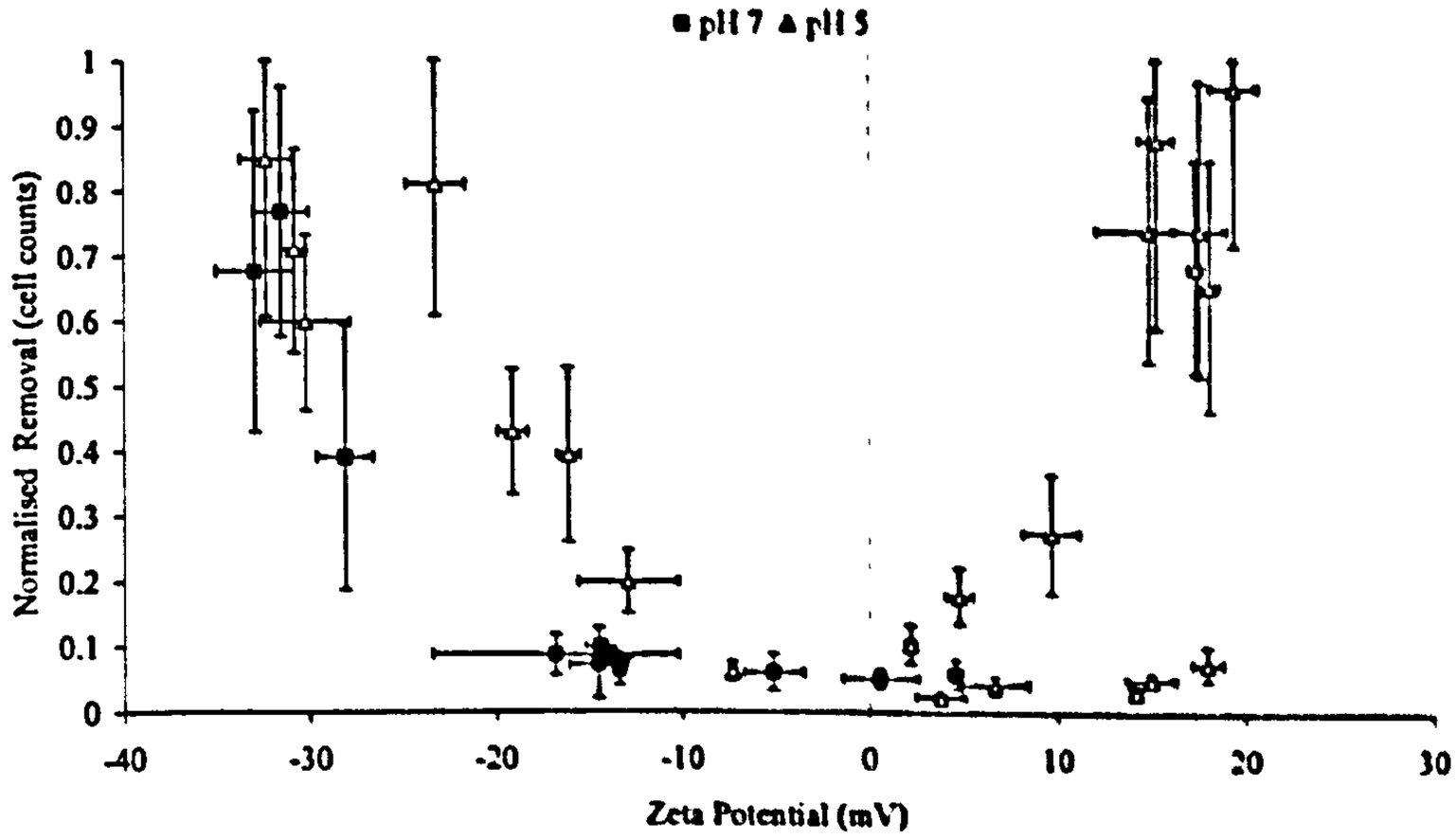

Figure 4.2.3 *A comparison of the zeta potential operational window for C. vulgaris at pH 7 and pH 5.*

A further benefit of using zeta potential as a control method for removal is that it takes into account removal of both components of the algae system: algae cells and AOM. Overall, optimum AOM removal (measured as DOC) was observed at -10 mV or less (Figure 4.2.4). Coagulant demands for optimum removal were 0.89, 1.25 and

1.56 mg Al mg^{-1} C (not illustrated here) for AOM of *C. vulgaris*, *M. aeruginosa* and *A. formosa* respectively. In the case of *A. formosa*, the optimum AOM removal required the same reduction in zeta potential as was observed for the cells of approximately -12 mV. However, AOM originating from *C. vulgaris* and *M. aeruginosa* required a reduction in the zeta potential to at least -10 mV for optimum removal, whilst optimum cell removal was observed at -16.8 mV and -15.5 mV respectively (Figure 4.2.2). Again, the zeta potential operational range observed for AOM was very similar to that observed for NOM (-10 mV to +5 mV) (Sharp *et al.*, 2006).

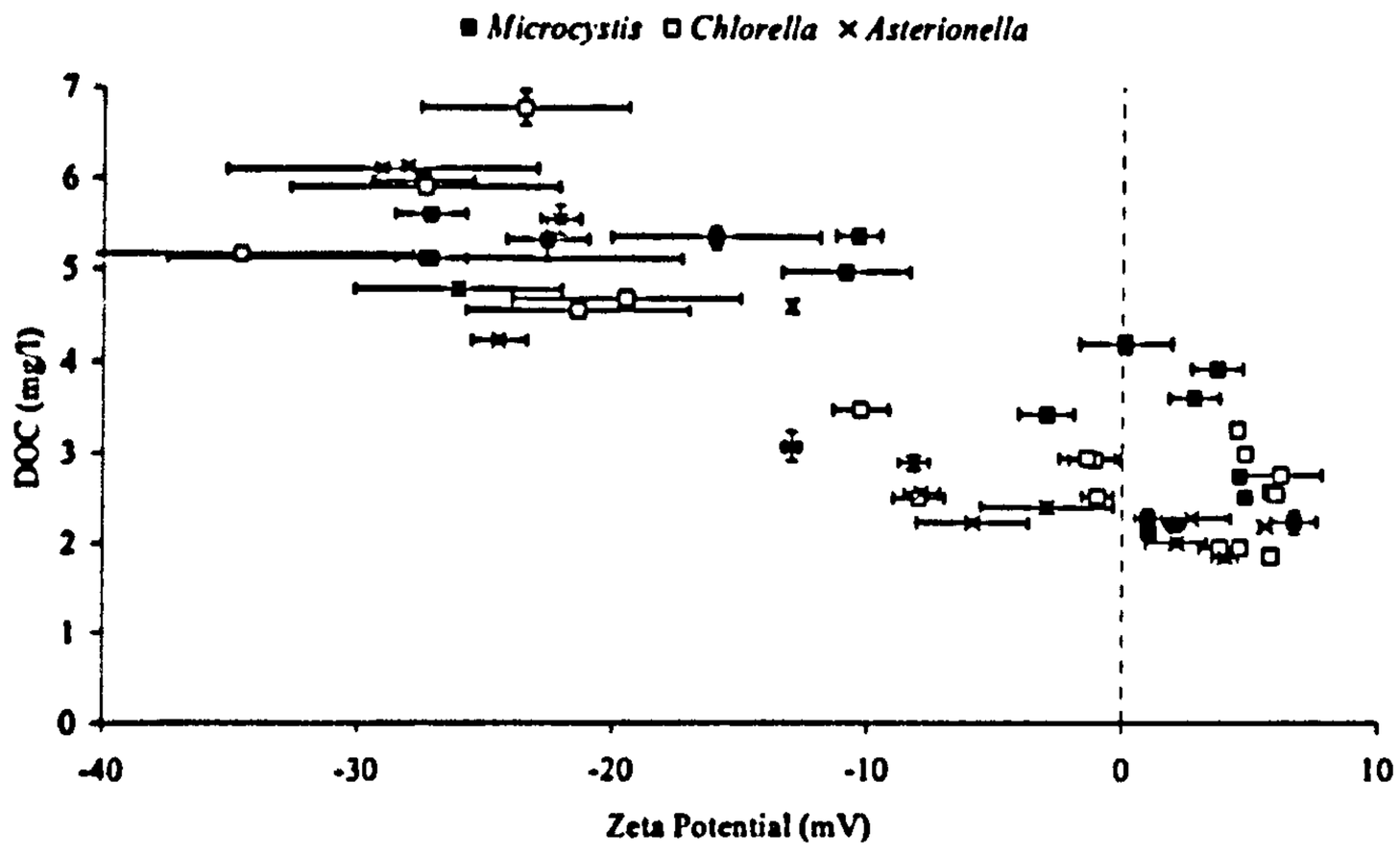

Figure 4.2.4 *The zeta potential operational window for the EOM of M. aeruginosa, C. vulgaris, and A. formosa.*

Overall, reducing the magnitude of the zeta potential to -10 mV for a pH between 5 and 7 ensured optimum cell removal was achieved. This was accomplished by either adjusting the pH or the coagulant dose, or a combination of these two actions. The removal efficiency did not depend on how the zeta potential was controlled for the two pH conditions examined. Optimum removal of the dissolved organic component of the organic system was also achieved at -10 mV or less, indicating that both components of the algal system are satisfactorily removed within the same operational window.

It is advised that a zeta potential operational window of -5 to 0 mV is targeted if using this technique for process control purposes. This range is within the operational window and additionally has outer margins to aid with process robustness. For example, if the algae population was to increase or decrease rapidly, as is frequently observed, there is leeway in the system for the zeta potential to be raised or decreased allowing time for pH or coagulant adjustment. At present one issue with using zeta potential for process control is the lack of an on-line instrument. Previously, studies have attempted to utilise a streaming current detector to determine coagulant dose using charge neutralisation principles (Bernhardt and Schell, 1993); however, practically, these instruments have been unpopular due to difficulty in data interpretation and instrument calibration. The development of an on-line zeta potential meter would allow a relatively straightforward method for controlling coagulant demand.

4.2.4 CONCLUSIONS

It can be concluded that monitoring cell concentration will give limited information with respect to controlling coagulant dose at a water treatment plant. A more informative approach is desired for robust process control. It was determined that provided the zeta potential range was kept between -10 mV and +2 mV, through a combination of coagulant dose and/or pH adjustment as preferred, optimum removal efficiency of both cells and AOM occurred. This operational range is very similar to that required for optimal removal of NOM, suggesting that no matter the influent organic character, optimal particle/colloid removal should be achieved. Overall, the use of zeta potential for process control is a viable tool for algae removal.

4.2.5 ACKNOWLEDGEMENTS

The authors would like to thank the Engineering and Physical Sciences Research Council (EPSRC), Yorkshire Water, Thames Water, Northumbrian Water and Anglian Water for their financial support.

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CHAPTER 5: POSIDAF

Paper 7 The potential for using bubble modification chemicals in dissolved air flotation – PosiDAF

Ready to submit: *Water Research*

Paper 8 Surfactants as bubble surface modifiers in the flotation of algae - PosiDAF

Ready to submit: *Environmental Science and Technology*

Paper 9 Polymers as bubble surface modifiers in the flotation of algae - PosiDAF

Ready to submit: *Water Research*

5. POSIDAF

5.1 THE POTENTIAL FOR USING BUBBLE MODIFICATION CHEMICALS IN DISSOLVED AIR FLOTATION - POSIDAF

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ABSTRACT

This paper investigates the potential for using surface modified bubbles in the treatment of algae using dissolved air flotation (DAF) instead of upstream coagulation and flocculation. Bubble modification is attempted by adding either metal coagulant, surfactant or polymers direct to the saturator. In this way, the chemical characteristics most suitable for removing small algae cells using this technique are examined. Optimum removal using metal coagulant, aluminium sulphate, was 60 %; however, both a decrease in the magnitude of the zeta potential and microfloc generation occurred concurrently, thus accounting for the improved removal. In contrast, there was no change in system zeta potential and no microfloc generation when using cationic surfactant cetyltrimethyl-ammonium bromide (CTAB), for which 63 % removal was achieved. An average of 95 % removal was achieved using the cationic polymer, polyDADMAC, with no change to system zeta potential. The results therefore confirm that there is a potential for adapting the conventional DAF process to operate without upstream coagulation and flocculation. A chemical with both a hydrophobic component in addition to a high molecular weight, hydrophilic, highly charge component is advised for the process.

Keywords: bubble, coagulant, dissolved air flotation, polymer, surfactant.

5.1.1 INTRODUCTION

Dissolved air flotation (DAF) is frequently used to treat algae laden water as it takes advantage of both their natural tendency to float and the very low density flocs that form on coagulation (Haarhoff and Edzwald, 2004). The DAF process floats particles using microscopic bubbles that are produced by saturating recycled water with air at high pressure and subsequently releasing it at atmospheric pressure. The generated bubbles attach to influent particles, raising them to the surface. Efficient flotation relies on effective collision and attachment of bubbles and particles, achieved by coagulating influent particles to increase their size and decrease their negative charge respectively (Han, 2002; Haarhoff and Edzwald, 2004). The latter minimises repulsive effects between the particles and the strongly negatively charged bubbles. However, coagulation of algae is frequently reported to fail due to variable morphology (Bernhardt and Clasen, 1991), metabolically excreted organic matter (Bernhardt *et al.*, 1985), and their ability to react to changes in their immediate environment (Pieterse and Cloot, 1997; Clasen *et al.*, 2000), leading to poor flotation. A flotation process that did not rely on coagulation would therefore be advantageous.

It is proposed that surface modification of the bubble, as opposed to the particle, could remove the requirement for upstream coagulation. This can be achieved by adding chemicals to the saturator of DAF process, which then coats the bubbles as they form, generating functionalised surfaces. This concept of particle collector modification has been investigated for depth filtration where media has been functionalised with cationic collectors such as metal hydroxides (Truesdail *et al.*, 1998). However, contamination of such collectors can occur in water with high influent loadings, reducing the lifetime of such media (Chen *et al.*, 1998). This issue is averted in modification of bubble surfaces as they are continually replenished. There is also potential to utilise surface modified bubbles in addition to upstream coagulation in order to further improve the particle removal obtained conventionally or reduce dose requirements. The specific surface function of the bubble depends on the chemical coating. For example, anionic surfactants have been demonstrated to make bubbles surface more negative (Skrylev *et al.*, 1984; Laskowski *et al.*, 1989) while the use of

cationic surfactants have been shown to produce positively charged bubbles (Skrylev *et al.*, 1984; Laskowski *et al.*, 1989; Kubota and Jameson, 1993; Cho *et al.*, 2005). Similarly, the application of di- and tri-valent metal coagulants have been shown to alter bubble charge, attributed to adsorption of the positively charged aluminium and magnesium hydroxide precipitates with hydroxylated dissolved species making a more minor contribution. For example, bubble charge reversal occurred at pH 10 using 10^{-3} M $MgCl_2$ (Li and Somasundaran, 1991) and 10^{-2} M $MgCl_2$ (Han *et al.*, 2006) while aluminium was observed to reverse charge at pH 7 for concentrations of 5×10^{-6} M (Li and Somasundaras, 1992), 10^{-5} M (Han *et al.*, 2006) and 10^{-4} M (Yang *et al.*, 2001). The use of a poly(diallyldimethyl ammonium chloride) (polyDADMAC) type polymer, Catfloc, has also been shown to create positively charged bubbles (Malley, 1995). Algae are negatively charged (Clasen *et al.*, 2000), thus in order to ensure good bubble-cell attachment, generating bubbles with positively charged functional sites is appropriate. However, charge may not be key to guaranteeing bubble-cell attachment. For example, polymer bridging between bubble and cell may also be important.

To date, only one published study has applied the approach outlined in this paper for particle removal. Malley (1995) revealed that, when using positively charged bubbles created by dosing Catfloc to the saturator, comparable removal to that obtained by conventional coagulation-DAF was obtained when treating low colour, low turbidity water. Overall, while it is acknowledged that surface modified bubbles can be produced, little research has been conducted in applying these bubbles in DAF for cell removal. Consequently, there is no understanding of which would be the most appropriate chemical to use. Hence, the current study investigates the potential for treating algae by flotation using surface modified bubbles without pre-coagulation. Bubble modification is attempted by dosing positively charged surfactant, metal coagulant and polymers into the saturator. Given the use of positively charged chemicals, this novel process will be referred to as PosiDAF. Specifically, this research aims to assess the maximum removal efficiency achievable for the microalgae, *Microcystis aeruginosa*, without coagulating conventionally. A further

aim is to determine the most appropriate chemical character for use in the production of surface modified bubbles for algae treatment.

5.1.2 MATERIALS AND METHODS

Cultivation of *Microcystis aeruginosa*. Cultures of *M. aeruginosa* (1450/3 – freshwater, Esthwaite Water, Cumbria, England) were obtained from the Culture Collection for Algae and Protozoa (CCAP), (Oban, Scotland). Cells were cultivated in sterilised Jaworski Media using conical flasks that were shaken at 75 rpm (Patterson Scientific Bibby Stuart SO1 shaker, Luton, UK) and incubated at 20 °C under 24 hour radiation using Sun-glo 30 W aquatic lighting. Algae were harvested at the onset of the stationary phase when they had reached maximum cell concentration of 2×10^7 cells mL^{-1}. *M. aeruginosa* cells had an average diameter of 5.5 µm respectively as measured microscopically for 100 cells.

PostDAF Chemicals. The chemicals trialled in this research are outlined in Table 5.1.1 and include the metal coagulant, aluminium sulphate, the surfactant cetyltrimethyl-ammonium bromide (CTAB), and polymers poly(ethyleneimine) (PEI), chitosan, polyamines – Magnafloc LT31 and Agefloc A50, and polyDADMAC. With the exception of aluminium sulphate, the charge density of each chemical was analysed using a back titration method (Kam and Gregory, 1991). Initially, the charge density of cationic surfactant, CTAB, was calculated from the chemical formula and subsequently used to standardise the charge of anionic poly (vinylsulphonic acid) sodium salt (PVSA). A 1 meq g^{-1} solution of PVSA was then used to measure the charge density of the remaining chemicals by back titration. Each solution was buffered to pH 7 or pH 5 as appropriate using 1 mM NaH_2PO_4/Na_2HPO_4 or $CH_3COOH/NaCH_3COO$ respectively) and the indicator ortho-Toluidene blue was added. Decrease in the UV_{635} absorbance (Jenway 6505 UV/Vis) signified neutralisation, coinciding with a colour change from blue to pink-purple. Three different volumes of cationic chemical were analysed in this way.

Table 5.1.1 *Description of chemicals utilised in the chemical trial.*

Chemicals	Supplier	Structure	Molecular Weight (Da) or Intrinsic Viscosity (cp)	Preparation of Stock Solution	Charge Density of Stock (meq g^{-1})
Metal coagulant					
1. Aluminium sulphate	Fisher Scientific (UK)	$Al_2(SO_4)_3.14H_2O$	594	10 mg mL^{-1} as Al prepared using DI water	Not measured
Surfactant					
2. Cetyltrimethyl-ammonium bromide (CTAB)	Sigma (UK)	$CH_3(CH_2)_{15}N(CH_3)_3Br$	364.5	1 mM prepared using DI water	2.8
Polymers					
3. Poly(ethyleneimine) (PEI)	Fisher Scientific (UK)	$(-CH_2CH_2NH-)_n$	50,000-60,000	1 mg mL^{-1} prepared using DI water	12.1
4. Chitosan (deacetylating grade)	Fisher Scientific (UK)	$(C_6H_{11}NO_4)_n$	100,000-300,000	1 mg mL^{-1} prepared using 1 mM acetic acid	4 at pH 5 / 2.9 at pH 7
5. Magnafloc LT31 (Polyamine)	CIBA (UK)	Not disclosed (N.D.)	N.D., 350-650 cp	1 mg mL^{-1} prepared using DI water	7.2
6. Agefloc A50 (Polyamine)	CIBA (UK)	Not disclosed (N.D.)	N.D., 600-900 cp	1 mg mL^{-1} prepared using DI water	7.2
7. PolyDADMAC	Sigma Chemicals (UK)	$((CH_2)_2C_4H_6(CH_3)_2N^+)_n$	100,000-200,000	1 mg mL^{-1} prepared using DI water	6.2

PosiDAF Experiments. A bench scale flotation jar tester was used for all PosiDAF experiments (EC Engineering Dissolved Air Flotation Batch Tester, Model DBT6, Alberta, Canada). One litre samples were prepared by diluting the concentrated algae solution to $7.5 \times 10^5 \pm 2.3 \times 10^4$ cells mL^{-1} using DI water buffered and ionised with 0.5 mM $NaHCO_3$ and 1.8 mM NaCl and adjusted to pH 7 using HCl. The same buffered DI water was added to the saturator along with aliquots of stock chemical, shaken vigorously, and adjusted to pH 7. The solution was then saturated with air at a pressure of 450 kPa, shaken for 30 seconds (until gauge pressure stabilisation) and then added to the algae solution using an equivalent recycle ratio of 20 %. The algae were allowed to float for 10 minutes. When using chitosan, experiments were undertaken additionally at pH 5. For comparison, conventional coagulation and flotation was undertaken whereby chemical was added to 1 L of algae sample (prepared as described previously) at the beginning of a 2 minute rapid mix (200 rpm) during which pH was adjusted. The suspension was then mixed at 30 rpm for 15 minutes after which the paddles were gently removed and pressurised (450 kPa), air saturated, buffered deionised water was supplied at an equivalent recycle ratio of 12 %. Algae flocs were floated for 10 minutes. Residual samples were analysed as follows: cell counting using a haemocytometer and light microscope (100 cells were counted per analyses); zeta potential (ZP) using a Malvern Zetasizer 2000HSA (Malvern, UK); and DOC using a Shimadzu TOC-5000A analyser. All analyses were performed in triplicate and with respect to DOC errors were less than 2 %. ZP analyses was undertaken at least 7 minutes after chemical addition to ensure that no further change in ZP would occur (Clasen *et al.*, 2000). At no time was bubble charge measured. Chemical addition was reported in terms of the effective concentration added to the 1 L cell suspension.

5.1.3 RESULTS

5.1.3.1 Coagulant

The maximum removal obtained when using aluminium sulphate in the saturator was 60 % which occurred over the dose range of 1.46 to 2.9 mg L^{-1} over which the removal was at a plateau (Figure 5.1.1). This plateau coincided with a decrease in ZP

from -18.5 to -1.3 mV, significantly less than the initial ZP of -22.6 ± 1.2 mV. Microflocs were observed when analysing residual cells microscopically and by the naked eye after the algae system was exposed to the turbulent flotation conditions. The DOC of the system remained constant at 0.68 ± 0.1 mg L^{-1}. In comparison, when the coagulant was added traditionally to the jar, removal of 98% was consistently observed once the dose exceeded 0.72 mg L^{-1} corresponding to ZP values between -10 ± 2.6 mV to 4.1 ± 2.4 mV (Figure 5.1.1). This demonstrates that upon coagulating conventionally there was more interaction of the coagulant with the algae cells in comparison to when using PosiDAF, suggesting that a proportion of the coagulant remained associated with the bubble. However, both the slight decrease in ZP and microfloc generation indicates that a significant proportion of the coagulant was associated with the cells in the bulk aqueous phase. Optimum dose ranges for PosiDAF convert to 5×10^{-5} to 10^{-3} M and are therefore within the concentration that has been determined to generate positive bubbles (Yang *et al.*, 2001; Han *et al.*, 2006).

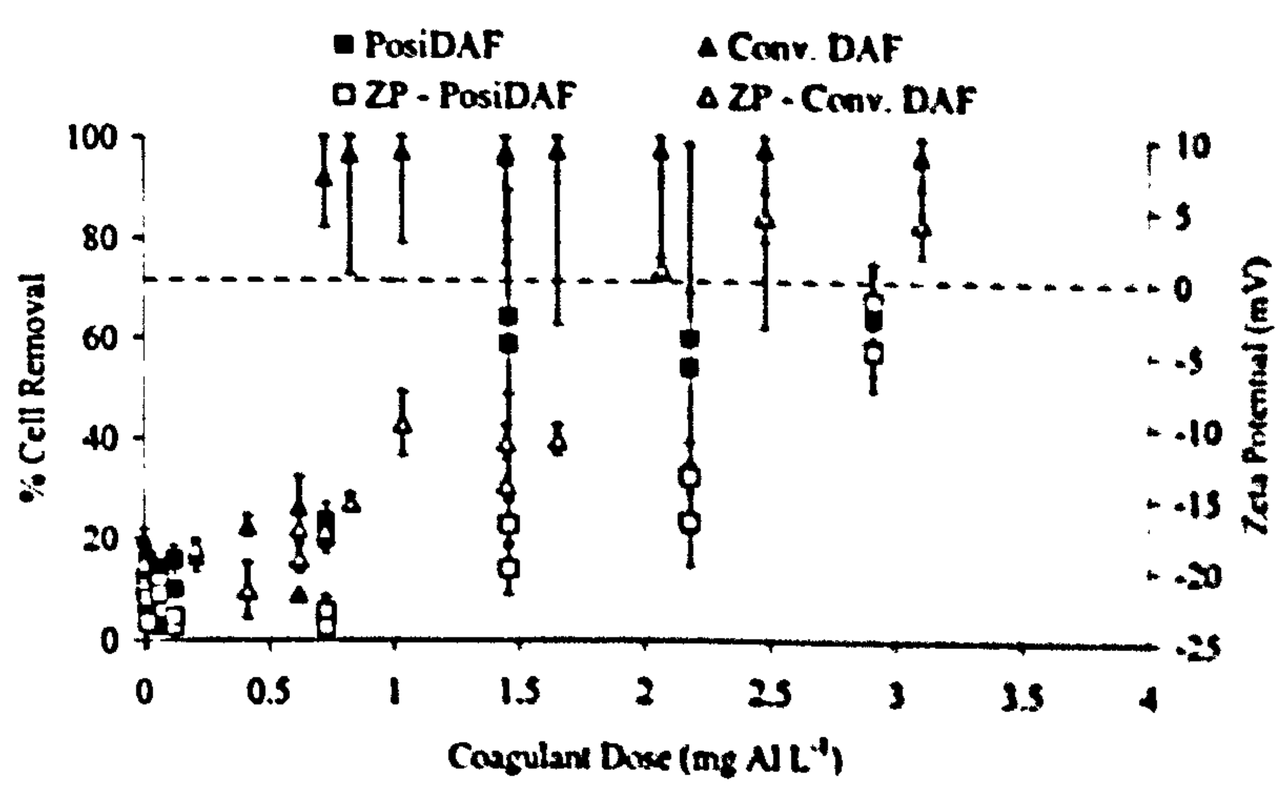

Figure 5.1.1 *Comparison of PosiDAF and conventional coagulation and flotation using aluminium sulphate at pH 7.*

5.1.3.2 Surfactant

The optimum removal obtained when adding the cationic surfactant, CTAB, to the saturator was 63 % (Figure 5.1.2) which occurred over the dose range of 0.0022-0.0040 mM (meq L^{-1}). Removal then decreased, stabilising at a removal of 42 %.

The ZP remained constant at -19.3 ±1.3 mV and microflocs were not observed at any time. The DOC remained consistent at 0.72 ± 0.29 mg L^{-1}. In comparison to when the surfactant was utilised as a conventional coagulant it was observed that aggregation of the algae cells did not occur until a dose of 0.14 mM had been added and optimum removal of 97 % required addition of 0.39 mM, approximately 100 times more chemical than that required for PosiDAF, indicating that a different removal mechanism was predominant. Optimum removal by conventional methods coincided with charge reversal of the ZP to 6.2 ± 0.0 mV. Hence, the surfactant did not interact with the algae until relatively large doses had been added. This suggests that bubble modification occurred and was responsible for the improved removal observed at low surfactant doses. The CTAB dose required for optimum removal by PosiDAF is comparable with that required to create a positively charged bubble as measured by Kubota and Jameson (1992) who showed a concentration of greater than 0.001 mM was required for charge reversal.

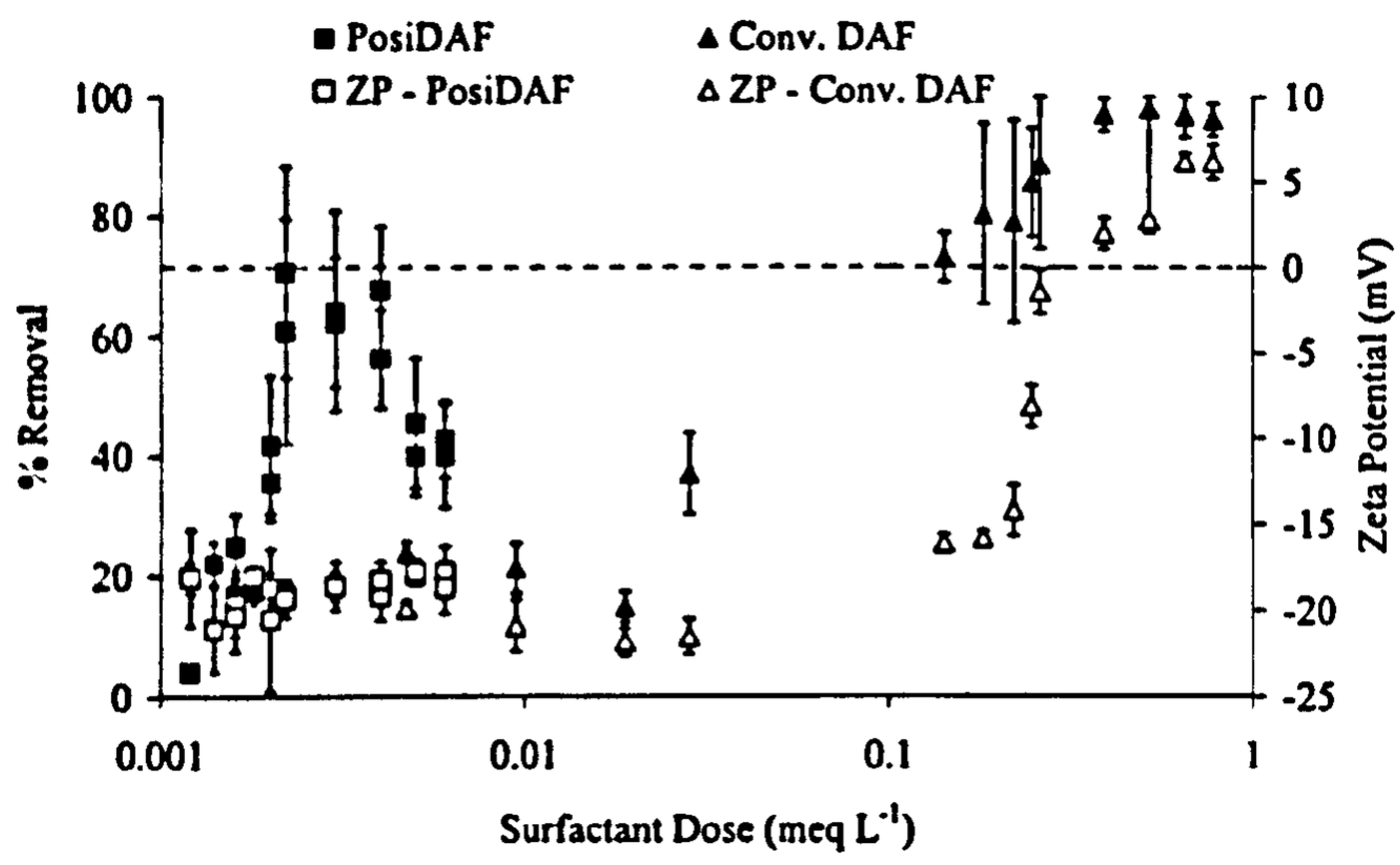

Figure 5.1.2 *Comparison of PosiDAF and conventional coagulation and flotation using cationic surfactant (CTAB) at pH 7.*

5.1.3.3 Polymers

Optimum removal obtained for PosiDAF using polymers was variable and in the order 68, 76, 78, 87 and 95 % for chitosan (pH 7), Magnafloc LT31, PEI, Agefloc

A50 and PolyDADMAC respectively. With the exception of PEI, all exhibited a peak in removal followed by a subsequent decrease, stabilising at approximately 45 %, as illustrated in the example of polyDADMAC (Figure 5.1.3). The optimum dose range was narrowest in the case of chitosan (pH 7), where optimum removal was obtained at 0.0024 meq l^{-1} (0.83 mg L^{-1}), and widest for polyDADMAC at 0.0020-0.0027 meq L^{-1} (0.32-0.44 mg L^{-1}) (Figure 5.1.3). The dose range obtained for the latter was much lower than the 8 mg L^{-1} of cationic Catfloc required to produce a positively charged bubble (Malley, 1995). Similarly, in the same study the dose range used for treatment of humic acid and clay by chemical dosing to the saturator was higher than that required in the current study, at 0.8-6 mg L^{-1} depending on the relative concentrations of humic acid and clay present. A direct comparison in terms of charge added was not possible as Catfloc charge density had not been measured. The residual DOC for PosiDAF experiments with polymer addition did not vary significantly in the case of PEI, chitosan, or polyamines or polyDADMAC, such that levels of 0.66 ± 0.17, 0.9 ± 0.3, 1.12 ± 0.24, 1.13 ± 0.14 and 1.34 ± 0.45 mg L^{-1} were obtained respectively.

Magnafloc LT31 and Agefloc A50 had similar patterns of removal and optimum dose ranges of 0.0019-0.0023 meq L^{-1}, despite the differing removal efficiencies. In the case of Magnafloc LT31, the ZP changed relative to the initial value for the optimum dose range, decreasing to -16.4 ± 0.2 mV, while that of Agefloc A50 remained stable. Structurally, these polyamines only differ in terms of their intrinsic viscosities (Table 5.1.1), which can be related to molecular weight and thus indicates that the MW of Agefloc A50 is greater than that of Magnafloc LT31. Hence, differences arising in PosiDAF performance for these polyamines can be related to MW. PEI required a much greater dose of 0.004 meq L^{-1} for optimum removal which remained constant for the dose range tested – up to 0.01 meq L^{-1}. The ZP changed with increasing removal, decreasing to -16.8 ± 1.0 mV at the onset of optimum removal and reversing the charge to $+2.7 \pm 1.2$ mV at the maximum dose and microflocs were visible to the naked eye during optimum removal. Chitosan experiments that were conducted at both pH 5 demonstrated improved removal at 82 % in comparison to pH 7 results, at a dosage of 0.0012 meq L^{-1}, 2.5 times lower than at pH 7. However, at pH 5, the ZP changed from -16.5 ± 0.9 mV to -13.3 ± 2.4 mV at the onset of optimum removal and

microflocs were observed. In comparison, the ZP values of chitosan experiments undertaken at pH 7 remained stable at -21.8 ± 0.2 mV for the optimum dose range.

A comparison of cell removal achieved by PosiDAF and conventional coagulation and flotation was conducted for polyDADMAC (Figure 5.1.3) and Agefloc A50 (not illustrated here). It was observed that although similar removal efficiencies were obtained using both procedures, the onset of removal by conventional methods was achieved at slightly higher doses of 0.0028 meq L^{-1} and 0.0027 meq L^{-1} for polyDADMAC and Agefloc A50 respectively compared to 0.0020 and 0.0019 meq L^{-1} as observed using PosiDAF. When using PosiDAF, optimum removal was obtained for a relatively constant ZP of -20.1 ± 3 mV and -20.2 ± 2.8 mV for the same chemicals and decreases in removal efficiency were concurrent with decreases in the magnitude of the zeta potential of the bulk solution when using PosiDAF. Similarly, at the onset of removal by conventional methods the ZP in the case of polyDADMAC remained constant, although it increased slightly in the case of Agefloc A50 to -16.0 ± 0.7 mV. In contrast to PosiDAF, good removal continued to be achieved for conventional treatment when the ZP decreased to -3.5 mV and 6.5 mV for polyDADMAC and Agefloc respectively. Notably, for polyDADMAC, the removal efficiency achieved by conventional means then decreased when charge reversal occurred. Importantly, when using PosiDAF, no microflocs were observed at optimum removal for either system. However, flocs were formed when treating by conventional methods.

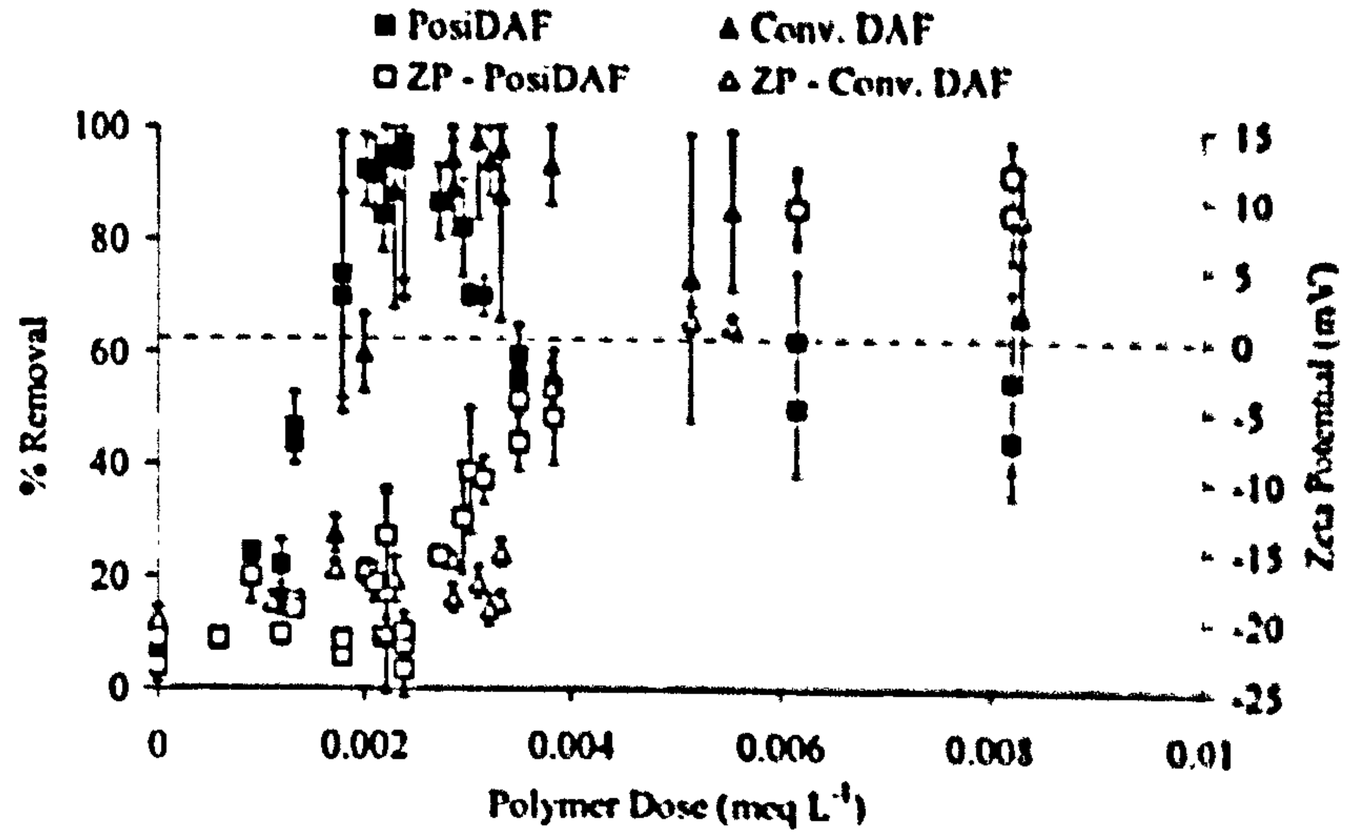

Figure 5.1.3 *Comparison of PosiDAF and conventional coagulation and flotation using cationic polymer (polyDADMAC) at pH 7.*

5.1.3.4 Overall comparison

The average removal at optimum dosage was continually greater than that obtained when no chemical was added to the saturator (Figure 5.1.4). Specifically, an average of 9 % cell removal was obtained without PosiDAF, which increased in the range of 60-95 % for the chemicals trialled thus demonstrating that a certain degree of removal was possible without upstream coagulation. Removal efficiency was lowest for aluminium and highest for polyDADMAC. The best removal was achieved for polymers as opposed to surfactant or metal coagulant. Overall, the dose range of the chemicals was most comparable in terms of charge added. For example, a dose of 0.0023-0.0024 meq L^{-1} ensured optimum removal for most chemicals, excepting aluminium, PEI and chitosan (pH 5). This indicates that a charge dependent mechanism is important in achieving removal. The charge concentration of the system was -0.0014 meq L^{-1} (Henderson *et al.*, 2007) and is therefore approximately 1.6 times smaller than the charge required for optimum removal.

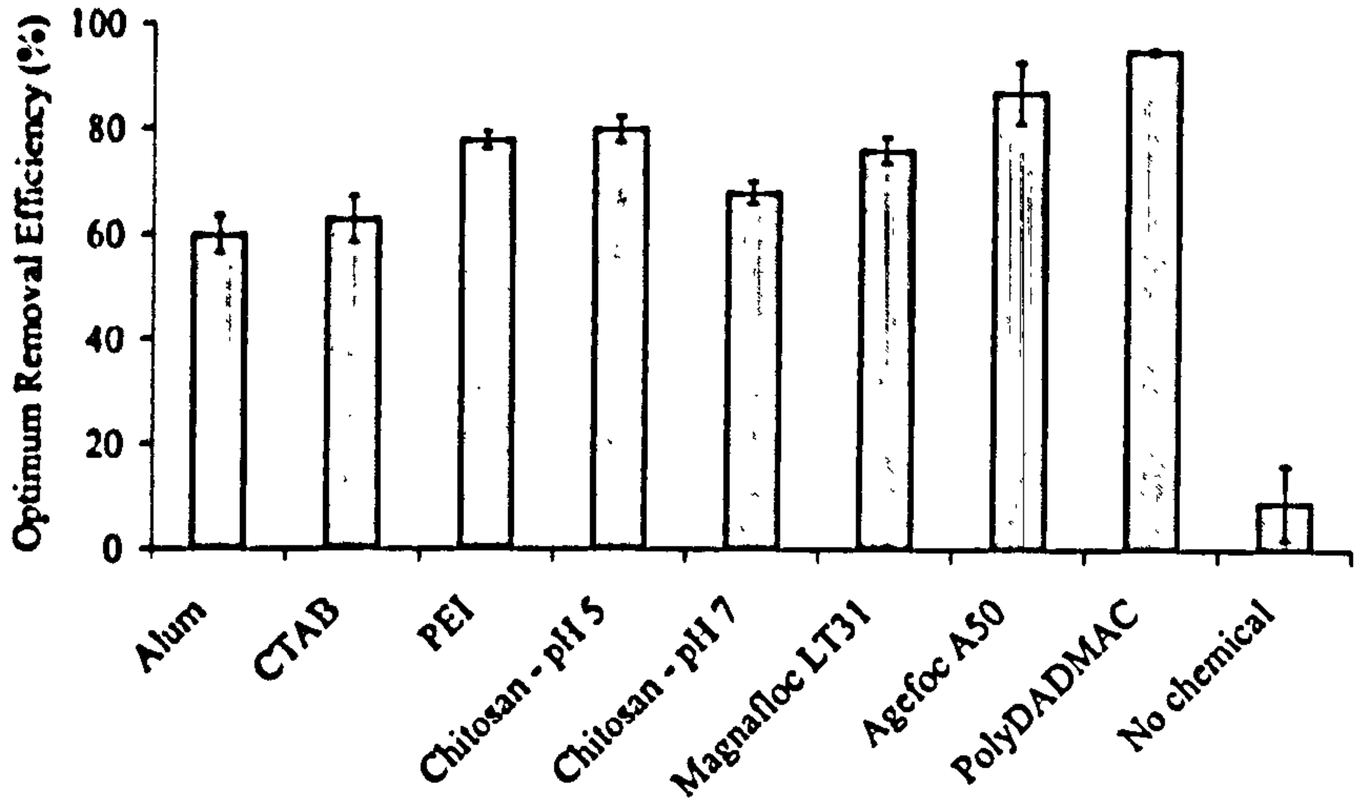

Figure 5.1.4 *Optimum removal efficiencies obtained using PosiDAF for all chemicals trialled.*

5.1.4 DISCUSSION

Each chemical trialled demonstrated that improved removal could be achieved by adding a positively charged chemical to the saturator compared to no chemical addition. However, different degrees of removal occurred depending on the characteristics of the chemical utilised. Furthermore, corresponding residual zeta potential values also differed, thus indicating that different removal mechanisms were responsible for the overall cell removal achieved.

Whilst no direct measurements of bubble charge were possible in the investigation, the associated data strongly suggests that the modifying chemicals, particularly CTAB, chitosan (pH 7), polyDADMAC and Agefloc A50, are predominantly associated with the bubble surface and hence with the generation of a positive bubble. In each case, the removal increases to an optimum without the zeta potential of the algae increasing or the observation of microflocculation. In parallel studies, where the chemical was added directly to the jar, removal proceeded by flocculation and, with the exception of polyDADMAC, an associated decrease in zeta potential was observed. Moreover, when compared with conventional methods, a lower dose overall was required for removal using PosiDAF, particularly in the case of CTAB

where the dose was 100 times less. In contrast, the observation of microflocculation in the instances of aluminium sulphate, PEI and chitosan (pH 5), in addition to the concurrent decreases in ZP relative to initial values for the same chemicals, suggest that chemical was more closely associated with the cell. While no microflocculation was observed when using Magnafloc LT31, the decrease in ZP observed relative to the initial value again suggests that in contrast to the similar chemical Agefloc, a certain amount of chemical is associated with the cells as opposed to the bubbles. Hence, the character of CTAB, chitosan (pH 7), Agefloc A50 and polyDADMAC allows them to associate more closely with the bubble, whilst the character of aluminium sulphate, PEI, and chitosan (pH 5), and to an extent Magnafloc LT31, means these chemicals will preferentially bind to the cells.

Decrease in removal that was observed beyond the point of optimum removal for all polymers, with the exception of PEI, is likely to be due to a combination of positive patches on the algae surface forming through adsorption of polymer and also steric repulsion between polymer chains at these higher concentrations, as commonly described when using cationic polymers for coagulation by polymer bridging (Hunter, 2001). In the case of surfactants, the chains are expected to be orientated such that the hydrophilic head faces the bulk solution, and hence the decrease is principally attributed to electrostatic patch repulsion (Eastoe, 2005). For example, when a previous study investigated the interactions between particles and bubbles in surfactant solutions, it was observed that the energy change due to steric repulsion was not significant compared to hydrophobic and electrostatic forces (Somasundaran *et al.*, 1983). The removal efficiency plateau that was achieved when using aluminium sulphate and PEI, in addition to the observed microflocculation, suggests that repulsive effects of the type previously described are not occurring. Firstly, there will be no steric hindrance from long chain polymers, particularly for PEI, which is 50-60 kDa in comparison to 100-300 kDa for polyDADMAC. Secondly, if there is no or very little chemical associated with the bubble, as implicated in earlier discussion then electrostic patch repulsion between bubble and particle is unlikely to occur.

The variation in removal efficiencies from 63 < 68 < 87 < 95 % for CTAB, chitosan (pH 7), Agefloc A50, and polyDADMAC respectively (Figure 5.1.4), indicate that although the underlying mechanism in terms of removal by positive bubble remain the same, differences in the specific chemical characteristics affect overall removal efficiencies. This can be attributed to the way in which the chemical interacts at the interface. Figure 5.1.5 demonstrates how modification of the bubble surface may be envisaged in the case of surfactant and polymers (Eastoe, 2005; Cosgrove, 2005). The surfactant is likely to lie relatively close to the bubble surface as a result of both the low MW of the chemical, 365 g mol^{-1}, and the hydrophobic tail adsorbing tightly at the air-liquid interface. The hydrophilic head will lie on the outer edge of the bubble, thus generating positively charged regions in the current example of CTAB. In contrast, the polymers will be more loosely associated as they are more hydrophilic than the surfactants. Consequently, it is likely that the polymer will project from the bubble into the bulk solution, thus intercepting the commonly described trajectory of a particle around its collector (Leppinen, 1999). The extent to which the polymer reaches into solution will depend on the relative hydrophilic/hydrophobic portions and also on the molecular weight. For example, shorter chain polymers have lower affinity isotherms (Eastoe, 2005). Overall, there are two implications. Firstly, that the "swept volume" will increase for polymers with larger molecular weights increasing cell-bubble collision and attachment efficiency (Figure 5.1.5) and secondly, that longer chain polymers will more strongly adsorb to the interface. Long chain polymers could therefore act as bridges between cells and bubbles. Comparison of the removal exhibited by Magnafloc LT31 to that of Agefloc A50 supports this suggestion as the only difference between them is associated with their MW. The lack of association of PEI with the bubble surface can be attributed to both its relatively low molecular weight of 50-60 kDa and also to its highly hydrophilic nature. Hence, although CTAB will be most tightly adsorbed to the bubble surface, the swept volume will be smallest (Figure 5.1.5) and thus the removal efficiency will be low in comparison to the long chain polymers.

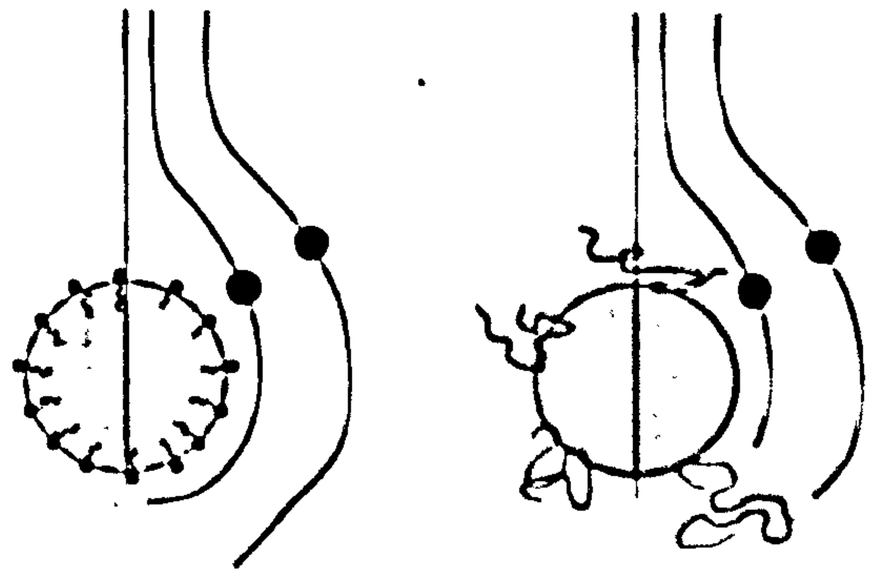

Figure 5.1.5 *Modification of bubble surface using surfactants and polymers leading to differences in removal efficiencies.*

A theoretical evaluation determined using the white water model described in detail by Haarhoff and Edzwald (2004) and assuming an average bubble size of 40 μm and saturator efficiency of 0.7, suggests that optimum removal for a cell of this diameter should be 40-70 % for attachment efficiencies of 0.5-1.0 respectively. This compares well with removal efficiencies obtained for CTAB and chitosan (pH 7); however it is considerably less than those achieved for Agefloc A50 and polyDADMAC. The increased swept volume of the polymer explains this discrepancy. It is suggested that these polymers bridge between cells and bubbles, forming an effective mesh, entrapping cells that fall into it as the bubble and associated polymer rise. Hence, 95 % removals can be obtained.

Overall, it was revealed that metal coagulants were not suitable for modifying bubble surface as they preferred to associate with the cell surfaces. Similarly, small, hydrophilic chemicals were also shown not to be suitable as again they associated preferentially with the cell surface. Chemicals with a defined hydrophobic component, such as that exhibited by the surfactant, ensured no interaction with the cell surface; however, removal was reduced as a result of its tight adsorption and small molecular weight. When the chemical was more loosely associated with the bubble and furthermore was of a significant MW, projection into the bulk phase could occur, increasing the swept volume of the bubble. Ideal chemical character for PosiDAF would therefore be a chemical that had a strong hydrophobic component to

allow tight adsorption to the bubble surface as it rises vertically, but additionally a hydrophilic charged component with long chain length that would project far into solution to collect cells on its upward path. For example, a co-polymer containing strongly hydrophobic and hydrophilic blocks, such as that synthesised and characterised by Lieske and Jaeger (1998) which incorporates both hydrophobic poly(ethylene glycol) and more hydrophilic PolyDADMAC, contains these attributes.

5.1.5 CONCLUSIONS

1. Greater than 60 % removal of un-coagulated cells can be obtained by adding a cationic chemical to the saturator of a DAF unit, although removal occurs by different mechanisms dependent on the chemical characteristics.
2. Removal obtained by small, hydrophilic polymers and metal coagulants was the result of microflocculation occurring during the turbulent flotation regime rather than by the production of positive bubbles.
3. It was possible to obtain 95 % removal efficiencies for algae cells using polyDADMAC, a cationic polymer, despite theoretical removal efficiencies of only 48-70 % being calculated for the cell diameter. This was attributed to the polymer projecting into the bulk phase from the bubble surface, capturing increased cell numbers as a result of increased swept volume.
4. Overall, three criteria are required for the chemical utilised to generate a positively charged bubble for successful cell removal: 1) positively charged region; 2) a hydrophobic component to encourage adsorbance at the bubble surface; 3) high molecular weight both to increase the swept volume or enmeshment area.

5.1.6 ACKNOWLEDGEMENTS

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5.2 SURFACTANTS AS BUBBLE MODIFIERS IN THE FLOTATION OF ALGAE – POSIDAF

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ABSTRACT

The current paper presents an investigation into the potential for using bubbles modified with surfactants in DAF for algae treatment. Surfactants of varying character were trialled and it was determined that removal of the algae system was only improved when using cationic surfactant. For each cationic surfactant chemical, optimum removal of a *Microcystis aeruginosa* system was obtained for the same equivalent charge dosed; however the magnitude of the removal differed according to the hydrophobicity of the surfactant. Specifically, the more efficiently the surfactant adsorbed at the bubble interface the better removal until a plateau was achieved at pC_{20} values of 3.6 or greater. When keeping the dose to the saturator constant and varying recycle ratio, the removal increased with increasing recycle ratio and thus bubble volume, largely according to values predicted by a theoretical model. Bubble efficiency was constant irrespective of influent cell number or recycle ratio where a maximum of two cells were collected per bubble. Treatment of additional species in this way revealed a relationship between increasing size and both increasing removal efficiency and decreasing surfactant dose, which is supported by theoretical relationships.

5.2.1 INTRODUCTION

Dissolved air flotation (DAF) is frequently used at water treatment works where algae blooms in supply water sources are common place. However, although improved removal of algae is achieved with DAF when compared to sedimentation (Edzwald, 1993), operational difficulties are still reported. DAF removal efficiency is governed

primarily by electrostatic interactions between the bubble and particle (Han and Dockko, 1999). The air bubbles formed in DAF are negatively charged (Dockko and Han, 2004), as are influent particles, thus the magnitude of this electrostatic barrier needs to be reduced before bubble-particle attachment will take place. Upstream coagulation fulfils this requirement by the addition of cationic chemicals, typically trivalent metal coagulants, which reduce the negative charge of particles, destabilising the system and allowing bubble-particle contact. However, algae coagulation is notoriously difficult as a result of differing influent algae system character (Pieterse and Cloot, 1997) and inefficient coagulation can result, which consequently leads to poor flotation. An alternative to cell surface modification is bubble surface modification, which could remove the requirement for upstream coagulation (Malley, 1995; Dockko and Han, 2004). This technique could also be used in addition to conventional coagulation/flotation to provide an overall improvement in removal.

Bubble surface modification can be achieved by dosing chemical direct to the saturator that is used to pressurise recycled water for microbubble generation. Specifically, the production of a positive bubble has been demonstrated for metal coagulants (Han *et al.*, 2006), cationic surfactants (Kubota and Jameson, 1993; Cho *et al.*, 2005) and cationic polymers (Malley, 1995). However, to date only two studies have demonstrated flotation of particles using bubbles modified by chemical dosing to the saturator. For example, Malley (1995) dosed the cationic polymer, Catfloc, to the saturator and achieved comparable removal to conventional coagulation/flotation for low colour and low turbidity water. More recently, a chemical trial conducted by the authors, in which metal coagulant, surfactant or polymer was added to the saturator, determined that use of cationic surfactant and polymer achieved removal of 63 % and 95 % of algae cells respectively. No change to bulk system character in terms of zeta potential or microfloc production was observed leading to the conclusion that removal was attributed to bubble surface modification, specifically a reduction in the magnitude of the bubble zeta potential. In contrast, metal coagulant was not a good bubble surface modifier. The technique was referred to as PosiDAF and this name will be used from hereforth to describe flotation that utilises a chemically modified bubble. The current paper further explores the use of surfactants as bubble modifiers

for the flotation of algae cells. Specifically, the aims are to determine a) the most appropriate surfactant character for PosiDAF; b) the most appropriate PosiDAF operational parameters; and c) PosiDAF performance for a variety of algae species.

5.2.2 MATERIALS AND METHOD

Algae. Cultures were obtained from the Culture Collection for Algae and Protozoa (CCAP), Oban, Scotland as follows: cyanobacteria *Microcystis aeruginosa* (1450/3), green *Chlorella vulgaris* (211/11B) and the diatom *Asterionella formosa* (1005/9). The diatom *Melosira* sp. was obtained from a natural bloom in a reservoir situated in the London area and was used in the experiments as received. The cell culturing procedure has previously been described (Henderson *et al.*, 2007). Prior to use cultures were diluted, using DI water that was buffered using 0.5 mM $NaHC0_3$ and brought to the ionic strength of 2.3 mM using NaCl, to achieve concentrations as follows: *C. vulgaris* – $5.0 \times 10^5 \pm 5 \times 10^4$ cells mL^{-1}; *M. aeruginosa* – $7.5 \times 10^5 \pm 1.5 \times 10^4$ cells mL^{-1}; *A. formosa* – $1.7 \times 10^5 \pm 2.5 \times 10^4$ cells mL^{-1}. *Melosira* sp. had a concentration of 1900 ± 550 cells mL^{-1}. Average cell sizes for the spherical *C. vulgaris* and *M. aeruginosa* were 4.0 ± 1.1 μm and 5.4 ± 0.8 μm respectively, while *A. formosa* and *Melosira* sp. had diameters of 4.2 ± 0.9 μm and 24.0 ± 0.8 μm and lengths of 26.0 ± 3.1 μm and 60.7 ± 11 μm respectively.

Surfactants. A range of surfactants including non-ionic – Triton X-100 (TX100), anionic – dodecylsodium sulphate (DSS) and octadecylsodium sulphate (OSS), and cationic – dodecyl-, myristyl-, cetyl- and octadecyl-trimethylammonium bromide (DTAB, MTAB, CTAB and OTAB respectively) and benzalkonium chloride (BKC), were tested for use in PosiDAF. The structure, supplier and chain length are highlighted in Supplementary Information (SI, Section 5.2.5). Surfactant solutions were prepared using deionised (DI) water containing 0.5 mM $NaHC0_3$ and 1.8 mM NaCl and adjusted to pH 7. Characterisation of surfactants was achieved by measuring the decrease in surface tension using a surface tensiometer, ST500man (Nima Technology Ltd., UK), with the increasing concentration of surfactant. This procedure allows calculation of the following parameters (Rosen, 2004): a) the critical

micelle concentration (CMC), which is the concentration at which surface tension plateaus indicating micellisation; b) the surface excess, Γ, which is determined using the gradient on a surface tension, γ, vs ln[concentration] plot in combination with the Gibbs Adsorption Equation (Equation 5.2.1), and enables the calculation of the degree of packing of surfactant at the interface surface (mol m^{-2}); and c) the pC_{20}, which is the –log C_{20}, where C_{20} is the concentration of surfactant required to reduce the surface tension by 20 mN m^{-1} and is thus an indicator of efficiency of surfactant adsorbance. Using Equation 5.2.2, the pC_{20} and surface excess can be related to the standard free energy change, ΔG, involved in the transfer of the surfactant from bulk phase to interface. ΔG can be used to determine whether adsorption is spontaneous and the magnitude of the driving force.

$$\Gamma = \left(\frac{-1}{RT}\right)\left(\frac{\delta\gamma}{\delta \ln C}\right) - \text{non-ionic surfactants} \qquad \text{(Eq. 5.2.1a)}$$

$$\Gamma = \left(\frac{-1}{2.303RT}\right)\left(\frac{\delta\gamma}{\delta \ln C}\right) - \text{ionic surfactants} \qquad \text{(Eq. 5.2.1b)}$$

$$pC_{20} = -\left(\frac{\Delta G}{2.303RT}\right) + 1.74 + \left(\frac{20}{2.303RT\Gamma_m}\right) \qquad \text{(Eq. 5.2.2)}$$

Flotation Method. An EC Engineering Dissolved Air Flotation Batch Tester, Model DBT6 (Alberta, Canada) was used for the flotation jar testing. Initially, increasing concentrations of each surfactant was added direct to the saturator with DI water which contained 0.5 mM $NaHC0_3$ and 1.8 mM NaCl, and was adjusted to pH 7. This mixture was pressurised and shaken until achieving stabilisation of gauge pressure at 450 kPa. The pressurised solution was released at an equivalent recycle ratio of 20 % to 1 L of *M. aeruginosa* sample adjusted to pH 7 and left to float for 10 minutes. The impact of bubble:particle ratio was investigated by varying recycle ratio and initial cell number. Using a recycle ratio of 20 %, the removal efficiency for algae of varying character was examined by obtaining surfactant dose response curves for the other algae species. Pre-coagulation was also investigated (see SI, section 5.2.5, for further information and results). Residual analyses after each of the aforementioned

experiments included cell counting of 100 cells using a haemocytometer or Sedgewick Rafter counting cell and zeta potential (ZP) analysis using a Zetasizer 2000HSA (Malvern, UK). Each analyses was preformed in triplicate. Comparisons were made to a theoretical model (Haarhoff and Edzwald, 2004). See SI for further details (Section 5.2.5).

5.2.3 RESULTS AND DISCUSSION

5.2.3.1 Surfactant characterisation

The CMCs of anionic surfactants ranged from 6.32 mM to 0.02 mM for DSS and ODS respectively; those of cationic surfactants varied from 13 mM to 0.12 mM as chain length increased from C=12 to 18 for DTAB to OTAB respectively, while BKC had a CMC of 3.36; and the non-ionic surfactant, TX-100, had a CMC of 0.17 (Table 5.2.1). With the exception of TX-100, CMC values measured in the current study were consistently less than those of the literature (Mukerjee and Mysels, 1971). This is a result of the ionic strength, which was set at the level used in the further experiments, of the solutions which reduces the CMC, particularly that of ionic surfactants (Hunter, 2001). The surface excess concentration at maximum surfactant coverage, Γ_m, was in the range of 1-4 × 10^{-6} mol m^{-2} (Table 5.2.1). There was only a small decrease in Γ_m when carbon chain length was increased from C=12-16 as demonstrated by the values of Γ_m for DTAB to CTAB of 2.6 mol m^{-2} to 2.2 mol m^{-2} respectively. However, on transition from C=16-18, there was a significant decrease to 1.4 mol m^{-2}. This is attributed to coiling of the long chain, thus increasing the area of adsorption required by the molecule (Rosen, 2004). TX-100 had the greatest Γ_m which is a result of the non-ionic head not being subject to repulsive forces at the interface (Rosen, 2004). The pC_{20} was in the range 2.3 to 5.2 and increased with increasing carbon chain length for both anionic and cationic surfactants. For example, DTAB, MTAB, CTAB and OTAB had pC_{20} values of 2.3, 3.0, 3.6 and 4.6 respectively (Table 5.2.1). This indicates an increase in the efficiency of surfactant adsorption and is a result of the greater hydrophobicity of the surfactants. The ΔG was negative in all cases, indicating that the adsorption at the air-water interface is spontaneous. Again, the ΔG increased with increasing carbon chain length from -10 to -29 kJ mol^{-1} for DTAB through to OTAB and from -13 to -29 kJ mol^{-1} for DSS to

OSS respectively, demonstrating that the driving force of this adsorbance is greater for more hydrophobic surfactants. Finally, the CMC/C_{20} ratio ranged from 2.4 to 5.3 for cationic surfactants, from 2.8 to 3.4 for anionic surfactants and was greatest for the non-ionic surfactant at 13.6 (Table 5.2.1). There was only a small increase of 0.4 for increases in carbon chain length from 12 to16 for cationic surfactants. However, when increasing carbon chain length to 18, the ratio increases significantly from 2.8 to 4.8 (Table 5.2.1). The CMC/C_{20} ratio for TX-100 was significantly greater than that of all other surfactants. This indicates that micellisation is inhibited more than adsorption for TX-100 and similarly for OTAB in comparison to DTAB (Rosen, 2004).

Table 5.2.1. *Summary of surfactant characterisation data, where CMC a) is current data and b) is literature data (Mukerjee and Mysels, 1971).*

Surfactant	CMC (mM)		Surface excess, Γ_m $\times 10^6$ (mol m^{-2})	pC_{20}	ΔG (kJ mol^{-1})	CMC/ C_{20}
	a	b[1]				
DTAB	13.0	14.2	2.6	2.3	-10	2.4
MTAB	2.60	3.5[2]	2.4	3.0	-15	2.6
CTAB	0.66	1.0	2.2	3.6	-19	2.8
OTAB	0.12	0.3[3]	1.4	4.6	-29	4.8
BKC	3.36	5.0[4]	2.5	3.2	-16	5.3
DSS	6.32	8.1	2.3	2.6	-13	2.8
OSS	0.02[5]	0.3[5]	2.5	5.2	-27	3.4
TX100	0.17	0.17	3.3	4.9	-23	13.6

(1) Measured at 25 °C, in DI water, using equivalence conductance graphs unless otherwise stated
(2) Surface tension log plot
(3) Streaming current at 23 °C
(4) Iqbal *et al.* (2007)
(5) Measured at 50 °C

5.2.3.2 Impact of surfactant character on algae removal

Maximum removal efficiencies of 45 ± 4, 64 ± 5, 10 ± 4, 30 ± 7 and 65 ± 3 % were achieved on applying concentrations of 0.002-0.0021, 0.0022-0.004, 0.0020, 0.002-0.004 and 0.0022-0.0042 mM (meq L^{-1}) for cationic surfactants MTAB, CTAB, DTAB, BKC and OTAB respectively (MTAB and OTAB examples are presented in Figure 5.2.1). The impact of dose was the same in all cases with an increasing removal up to an optimum dose whereafter removal remained stable or decreased. To illustrate, removal obtained using OTAB increased from 12 % to 65 % for a dose of 0.002 meq L^{-1} where after removal remained stable up to the maximum dose tested of 0.0045 meq L^{-1} (Figure 5.2.1). For all cationic surfactants except OTAB, further increases in concentration resulted in a decrease in removal efficiency. At no time did the zeta potential of the residual bulk cell suspension alter as a result of the addition of the surfactant. In fact, when using CTAB as a conventional coagulant, 50 times more surfactant was required to alter zeta potential and destabilise the algae cell system, coinciding with the CMC. This suggests that the removal efficiency obtained by cationic surfactants in the current study is the result of creating bubble surfaces with positively charged sites.

Use of non-ionic surfactant, TX-100, was investigated to ensure that other beneficial factors introduced by surfactant usage in addition to the alteration of bubble charge, such as a decrease in bubble size, were not responsible for the observed increases in removal efficiency. However, the removal efficiency obtained using TX-100 remained on average 12 % (Figure 5.2.1) and thus did not deviate from removal efficiencies obtained with no chemical. This provides a strong indication that improved removal was solely a consequence of the generation of positively charged sites on the bubble surface. The results obtained when investigating anionic surfactants, with similar chain length and surfactant character to the cationic surfactants (Table 5.2.1), further confirmed this supposition. Use of OSS did not alter removal efficiency from 12 % (Figure 5.2.1), as was observed for DTAB (not illustrated), while that of DSS decreased to 4 %. Similar observations were obtained when floating *Scenedesmus quadricauda* using Dispersed Air Flotation (DiAF) (Chen

et al., 1998) where CTAB, TX-100 and DSS were used as frother and collector. Cationic CTAB was found to be most effective in comparison to the non-ionic and anionic surfactants. Thus a similar observation to those made in the current study was reported in that electrostatic interactions between collector and cell surfaces played a key role in flotation efficiency.

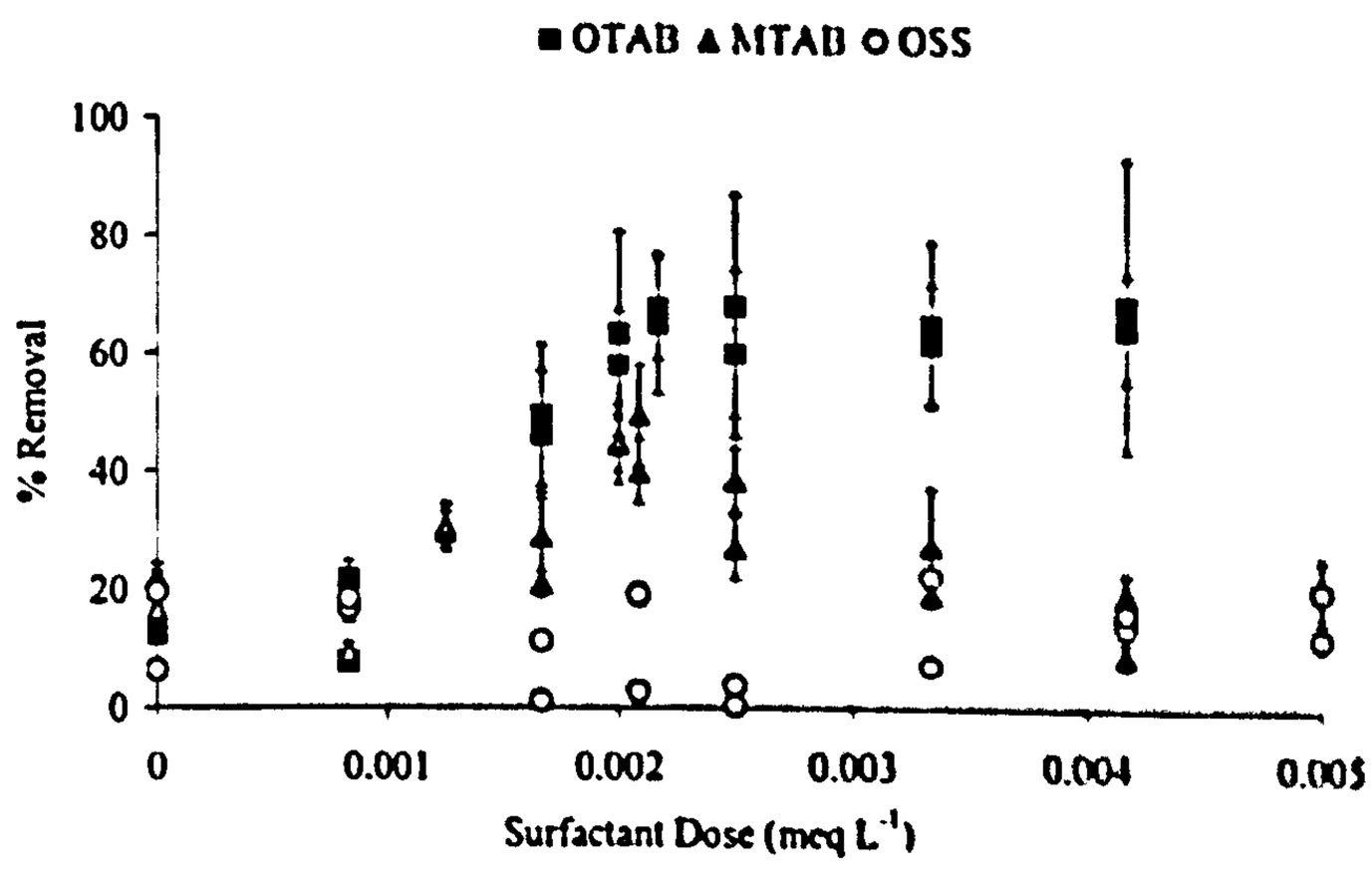

Figure 5.2.1. *The removal efficiency achieved for OTAB and MTAB – cationic surfactants, and OSS – anionic surfactant.*

Further assessment of the impact of surfactant character within the cationic grouping was achieved by making comparisons between removal efficiency and surfactant characterisation data (Table 5.2.1). There was no relationship between the removal efficiency and CMC/C_{20} thus indicating that inhibition of micellisation relative to adsorption did not impact on removal. However, good relationships were observed between % removal and Γ_m and pC_{20} (Figure 5.2.2), and also CMC and ΔG. For example, as Γ_m decreased the removal efficiency increased linearly until reaching a plateau at values less than 2.2 mol m^{-2} and as the surfactant adsorption efficiency, pC_{20}, increases so did removal efficiency, again reaching a plateau at values greater than 3.6 (Figure 5.2.2). The latter correlation reveals that the more efficiently the surfactant adsorbs at the air-liquid interface the better the removal. Equilibria exist between surfactant adsorbed at the gas-liquid interface and free in bulk solution. A higher pC_{20} value indicates the equilibrium favours adsorbance to the bubble and is

observed for surfactants with longer chain length and thus increased hydrophobic character. This is supported by the increases in the force driving adsorbance (ΔG) with increasing removal. The correlation with Γ_m demonstrates that removal does not increase with increasing packing of charge at the bubble surface. Rather, a decrease in packing indicates an increase in chain length and hydrophobicity and thus relates well to pC_{20} observations. Overall, as carbon chain length increases, hydrophobicity increases and thus adsorption efficiency increases. Importantly, while the generation of cationic sites is key, hydrophobicity of the cationic surfactants is also important. Similarly, both electrostatic and hydrophobic interactions were shown to be important in previous studies investigating bubble-particle attachment with respect to interaction forces including electrostatic, hydrophobic, steric and van der Waals (Somasundaran *et al.*, 1983; Perea-Carpio *et al.*, 1988). It was demonstrated that optimum removal efficiency correlated well with the maximum interaction energy between particles and bubbles. A decrease in removal efficiency at higher surfactant concentrations, such as that observed at increased surfactant concentrations in the current study, was attributed to decreases in both the hydrophobic and electrostatic interactions between bubble and particle (Somasundaran *et al.*, 1983). Hydrophobic interactions were the result of transfer of adsorbed surfactant molecules from the solid-liquid to gas-liquid interface. However, in the current study, it is assumed that the reverse would occur, further strengthening the attachment efficiency.

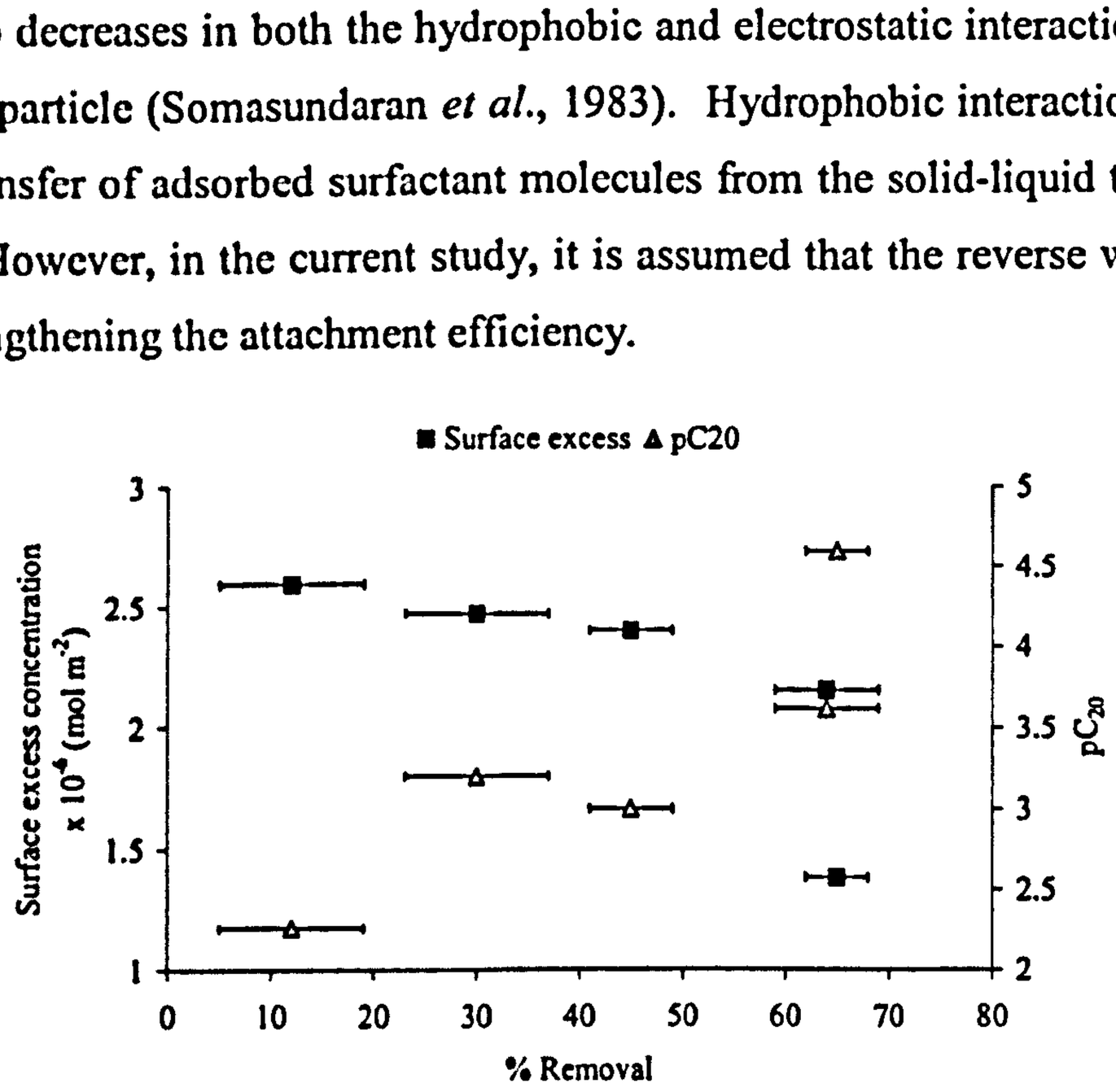

Figure 5.2.2. *The relationship between surfactant character, specifically surface excess concentration and pC20, and % removal.*

The majority of studies examining bubble zeta potential in surfactant solutions have investigated a single bubble in solution of certain surfactant concentration and so cannot be compared directly with the current study. However, in each study the bubble isoelectric point (i.e.p.) was achieved at concentrations that increased with chain length and thus increased with CMC (Kubota and Jameson, 1993; Cho *et al.*, 2005). This indicates that in the current study, bubbles generated using MTAB and DTAB would have been more negative (or less positive) than those of CTAB and OTAB. According to Cho *et al.* (2005), the degree of dissociation (α_s) at the air-water interface can be determined by $\alpha_s = [CTAB^+]/\Gamma$ and for DTAB, MTAB and CTAB this is 0.024, 0.015 and 0.013 respectively. The Γ_s measured at the concentration used for optimised removal were 0.00213, 2.8×10^{-4}, 3.5×10^{-5} and 2.3×10^{-5} mmol m^{-2} for OTAB, CTAB, MTAB and DTAB respectively. Hence, on combination with α_s, it was revealed that the concentration of positively charged sites on bubble surfaces for the same surfactants were approximately 30, 4, and 1.2 and 0.6 neq m^{-2}. These values will be underestimated since α_s was previously calculated for an ionic strength of 0.01 M, in comparison to 2.3 mM used in the current study. Further investigations were carried out for MTAB and DTAB at higher concentrations to give comparable charge densities at the bubble surface of 10 and 16 neq m^{-2} respectively; however removal was still low at 24 % and 5 % for the same surfactants. Furthermore, the magnitude of the zeta potential was now decreased to -17 and -18 mV for MTAB and DTAB respectively from an initial value of -21 mV indicating that interactions between cell surface and surfactant were occurring. This further supports the overall conclusion that removal efficiency is governed by both hydrophobic and electrostatic interactions.

5.2.3.3 Optimisation of PosiDAF operating conditions

Optimum dose ranges were consistently in the range of approximately 0.0022 to 0.004 meq L^{-1}, reported as effective dose to the 1 L sample. Hence, when examining the impact of varying recycle ratio (R_r) on removal using CTAB, three scenarios were investigated.

1. The dose applied to the saturator was kept constant at 0.017 meq L^{-1} as R_r was varied, thus keeping the surfactant to bubble ratio constant. This was set to investigate whether dose was related to bubble surface area.
2. Secondly, the dose to the saturator was adjusted depending on the R_r to ensure that the effective dose to the jar was kept constant, at 0.0034 meq L^{-1}, thus investigating whether the dose is a reflection of the algae charge.
3. Increasing Rr without dosing any chemical to the saturator to ensure that improvements in removal are not only related to increases in bubble concentration.

In Scenario 1, little removal was observed at low R_r of 2.5-7.5 %; however from 10-20 % a linear relationship was observed between increasing removal efficiency from 15-51 % (Figure 5.2.3). The removal efficiency obtained was slightly lower for R_r=20 % than reported previously and was a result of the batch of *M. aeruginosa* cells used for this particular set of experiments being slightly smaller in size at 4.5 μm compared to 5.4 μm. At R_r = 25-60 %, removal efficiencies continued to increase to 87 %, although at a lower rate, and stabilised at this level for R_r>60 %. Different results were obtained for Scenario 2 (Figure 5.2.3). Improved removal in comparison to Scenario 1 was obtained at low R_r values of 5-10 %. For example, at R_r = 10 %, removal efficiency of 40 % was achieved in comparison to only 15 % for Scenarios 1 and 2 respectively. However, at Rr values of 20-50 % there was a plateau at an average of 58 %. This then decreased to 48 % for higher R_r values. Hence, for R_r values of 30 % and greater, removal efficiency for Scenario 2 was lower in comparison to Scenario 1. At no stage was improved removal observed for Scenario 3, indicating that increased R_r for Scenarios 1 and 2 are directly related to surfactant addition. This indicates in the case of the latter that at higher R_r there was not sufficient surfactant in the saturator to coat the bubbles effectively. This further supports indications that removal is a result of surfactant interacting at the bubble surface, thus producing a certain degree of positively charged sites at the bubble. The attachment efficiency remains consistent in Scenario 1, whereas in Scenario 2 it is initially increased but then decreases such that, even at high bubble concentrations, collisions do not result in such successful attachment.

Further insight was gained from comparisons with the theoretical model (Haarhoff and Edzwald, 2004) (Figure 5.2.3), where input parameters were particle diameter of 4.5 μm and an attachment efficiency of 1.0. Hence it was assumed that due to surfactant adsorption at the bubble surface each collision resulted in successful attachment. From R_r = 2.5-15 % the theoretical model data was more similar to that of Scenario 2, while for Rr of 20 % and upwards, theoretical results matched those obtained for Scenario 1. This suggests that at low recycle ratios, attachment efficiencies were not optimised in Scenario 1. This may be the result of the very low effective concentration of surfactant being supplied to the jar, thus decreasing the surface excess concentration and therefore the charge density at the bubble surface.

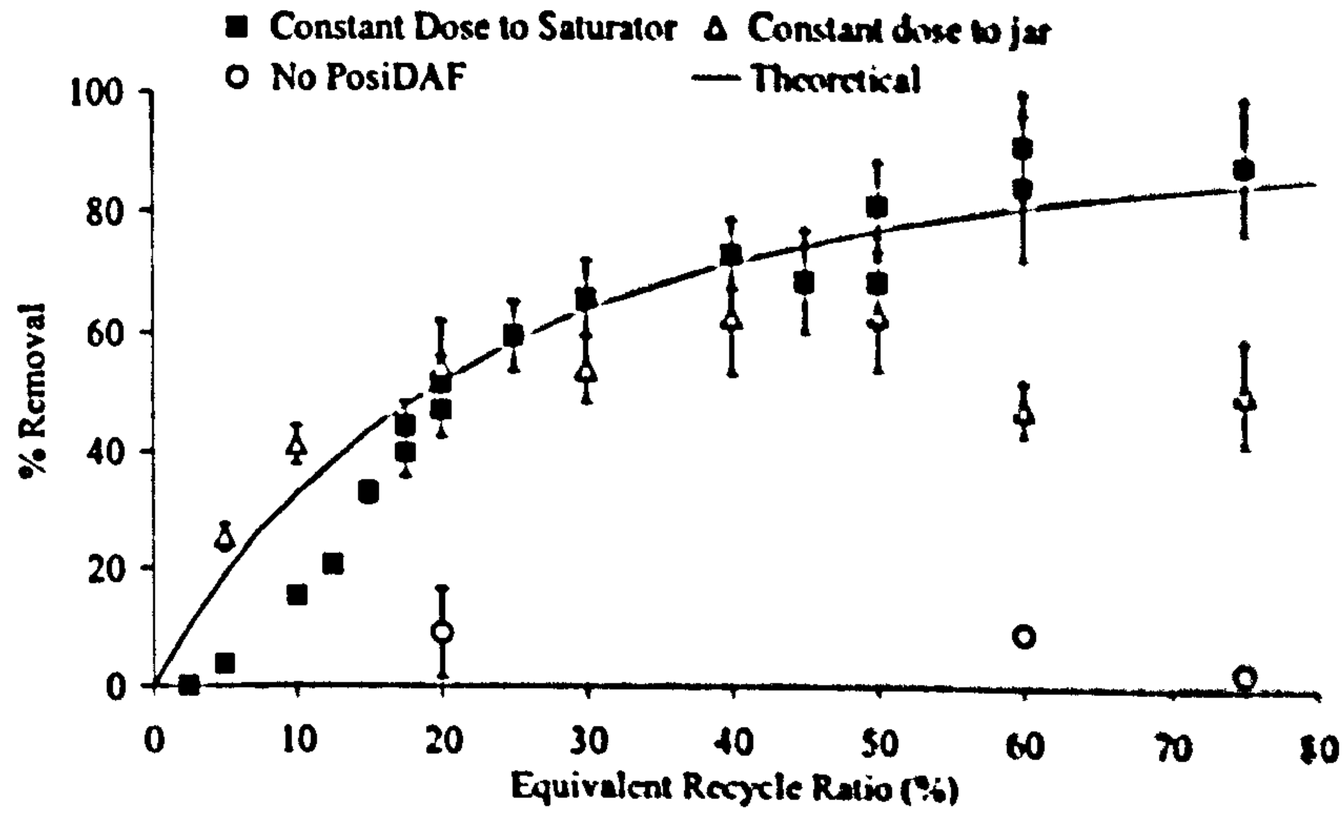

Figure 5.2.3. *% Removal vs increasing recycle ratio for 1. Constant dose to the saturator; 2. Constant dose to the jar; 3. No chemical; and 4. Theoretical data.*

Air:solids mass ratios and bubble:particle number ratios are typical DAF operating parameters. The latter is applicable in the current study as there was no flocculation. Overall, improved removal correlated with increased bubble number:particle number ratio, reaching maximum removal of 80 % at a bubble:particle ratio of 0.64. This was far less than the typical ratio for conventional treatment, which was calculated to be approximately 2-5 for a typical algae system with mass of 10 mg L^{-1} and floc size of 100 μm (Haarhoff and Edzwald, 2004), and reflects the fact that cells remained

unicellular. At Rr = 20 % the maximum bubble collection efficiency of 2 cells bubble^{-1} was achieved.

Bubble capacity was investigated further by varying cell concentration and measuring cell removal at R_r = 20 and 60 % using a constant dose to the saturator of 0.017 meq L^{-1}. Both achieved maximum removal for an initial cell count of 5.7×10^5 and 6×10^5 cells mL^{-1} for R_r = 20 and 60 % respectively (Figure 5.2.4a). At increased cell concentrations removal steadily decreased, although R_r = 60 % consistently had 1.6 times greater removal efficiency. Converting initial cell concentrations to the equivalent bubble:particle ratio revealed that maximum removal occurred at 0.2 and 0.67 for R_r = 20 and 60 % respectively (Figure 5.2.4b). At lower ratios there was a sharp decrease in removal efficiency suggesting that there were insufficient bubbles for optimised removal. The decrease observed at bubble:particle ratios greater than optimum indicates that when cell concentrations are more dilute relative to the bubble concentration, removal is reduced as the probability of bubble-particle collision is lowered. At R_r = 20 %, the maximum cell removal occurred at a lower bubble:particle ratio than for R_r = 60 % suggesting that in more dilute systems there was less opportunity for bubble-cell collision. The same log linear relationship between the number of cells removed per bubble collector and the bubble:particle ratio (Figure 5.2.4c) was obtained both 20 % and 60 recycle ratios, indicating that bubble efficiency is consistent irrespective of the recycle ratio or particle number. At low bubble:particle ratios, more cells have to be removed per bubble, leading to the reduction in removal efficiency. Optimum removal efficiencies were consistently achieved when bubble collector efficiency was in the range 1-2 cells bubble^{-1}.

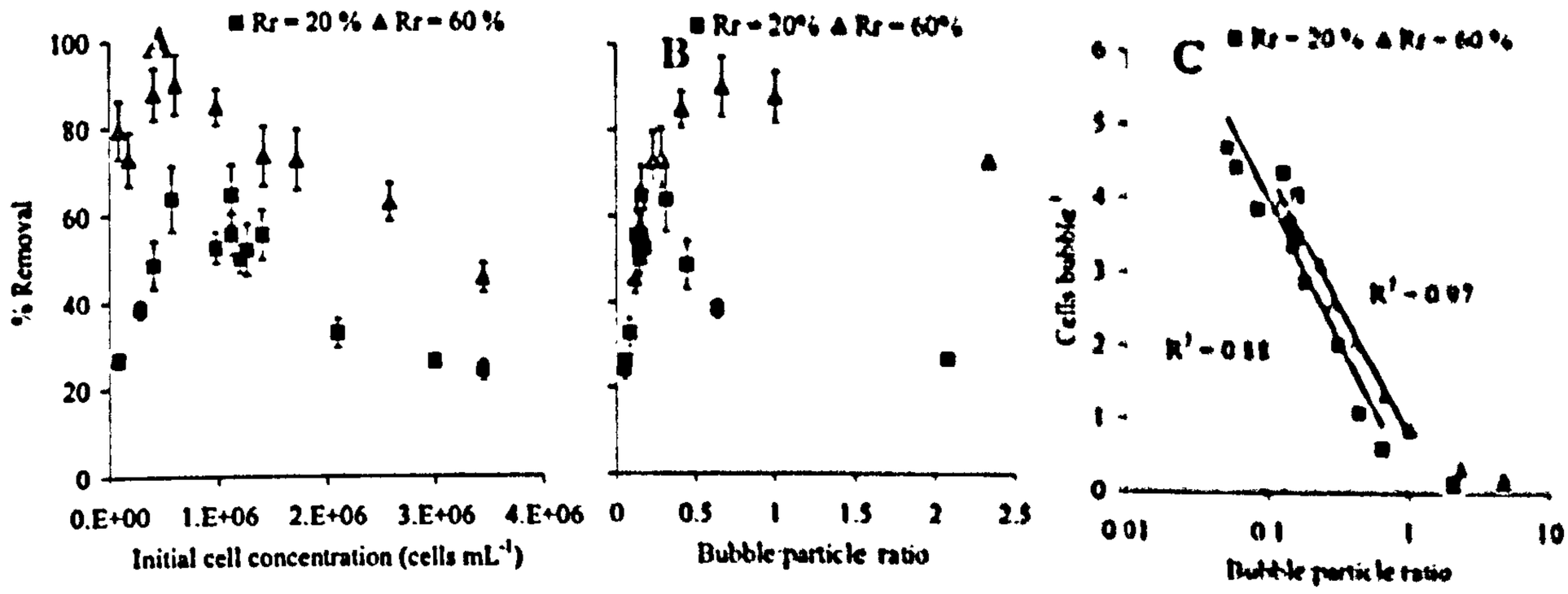

Figure 5.2.4. *% Removal vs A. Variable initial cell concentration and B. Bubble:particle ratio and C. Bubble collector efficiency, for R_r = 20 and 60 %.*

5.2.3.4 The impact of varying algae species on removal by PosiDAF

Dose response curves were obtained using CTAB for *C. vulgaris*, *A. formosa* and *Melosira* sp. with maximum removal efficiencies of 54 ± 0.4, 89 ± 4.1 and 97 ± 2.7 % respectively for effective doses to jar of 0.005, 0.0008 and 0.0005 meq L^{-1} for the same species. In the instance of *A. formosa* and *Melosira* sp. removal efficiency reached a plateau while that of *C. vulgaris* decreased slightly after achieving a maximum. Overall, removal efficiencies increased with increasing particle size and the dose required for optimum removal decreased with increasing particle size (Figure 5.2.5). The particle size for the *A. formosa* and *Melosira* sp. was calculated using the diameter of a sphere that had the equivalent surface area. Stabilisation of both surfactant dose and % removal occurred for particle sizes greater than 10 µm. Theoretical removal efficiencies, calculated assuming an attachment efficiency of 1.0, corresponded well with experimental removal efficiencies (Figure 5.2.5). This suggests that attachment efficiency was optimised as a result of bubble surface modification. Previous investigations of dispersed air flotation of *Scenedesmus quadricauda* using CTAB demonstrated 90 % cell removal (Chen *et al.*, 1998). *S. quadricauda* has an effective diameter of 24 µm and thus comparisons with theoretical data reveal that these removal efficiencies are achievable.

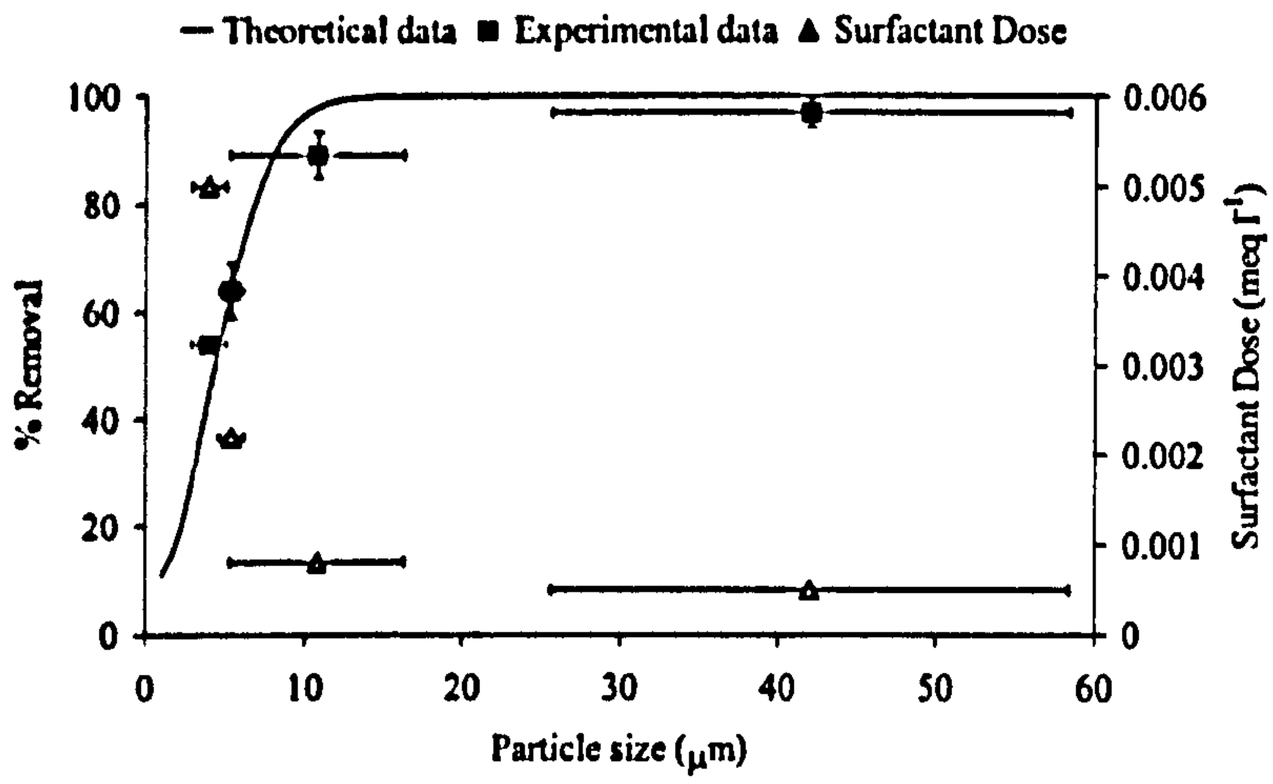

Figure 5.2.5. *The impact of cell size on: a) the optimum removal efficiency and b) the required chemical dose for this removal, for PosiDAF experiment and comparisons with theoretical data.*

Based on initial cell counts and corresponding charge densities (Henderson *et al.*, 2007), the charge concentrations of *M. aeruginosa*, *C. vulgaris*, *A. formosa* and *Melosira* sp. were 0.0014, 0.0056, 0.01 and 0.0036 meq L^{-1} respectively. Hence, there was no relationship between dose and system charge concentration. This indicates that the surfactant dose was related to the size of the algae particles. The relationship between increasing size and decreasing dose suggests a balance between collision and attachment efficiencies where the latter does not have to be optimised to the same extent if the size of the cell is sufficiently large. This is supported by the *white water* model which relates removal efficiency to the product of collision and attachment efficiencies (Haarhoff and Edzwald, 2004).

5.2.4 ACKNOWLEDGEMENTS

The authors would like to thank the Engineering and Physical Sciences Research Council (EPSRC), Anglian Water, Northumbrian Water, Thames Water and Yorkshire Water for their financial support.

5.2.5 SUPPLEMENTARY INFORMATION

5.2.5.1 Surfactant Information

Table 5.2.2. *Summary of surfactants utilised in the study*

Surfactants	Supplier	Molecular Composition	Molecular weight ($g\ mol^{-1}$)	No. of CH_2
Cationic surfactants				
Dodecyltrimethyl-ammonium bromide (DTAB)	Sigma, UK	$CH_3(CH_2)_{11}N^+(CH_3)_3Br^-$	308	11
Myristyltrimethyl-ammonium bromide (MTAB)	Acros Organics, UK	$CH_3(CH_2)_{13}N^+(CH_3)_3Br^-$	336	13
Cetyltrimethyl-ammonium bromide (CTAB)	Aldrich, UK	$CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$	365	15
Octadecyltrimethyl-ammonium bromide (OTAB)	Aldrich, UK	$CH_3(CH_2)_{17}N^+(CH_3)_3Br^-$	392	17
Benzalkonium chloride (BKC)	Fisher Chemicals, UK	$CH_3(CH_2)_nN^+(CH_3)_2$ $CH_2(C_6H_5)Cl^-$	321-409	8-16
Non-ionic surfactants				
Triton X-100 (TX100)	Acros Organics, UK	$(CH_3)_3CCH_2C(CH_3)_2(C_6H_4)$ $O(CH_2CH_2O)_{10}H$	647 (x=10)	10
Anionic surfactants				
Dodecylsodium sulphate (DSS)	Fisher Chemicals, UK	$CH_3(CH_2)_{11}SO_4^-Na^+$	288	11
Octadecylsodium sulphate (OSS)	Aldrich, UK	$CH_3(CH_2)_{17}SO_4^-Na^+$	372	17

5.2.5.2 The *White Water* model

The *white water* model uses performance Equation 5.2.3 to describe removal in the contact zone of a DAF unit.

$$\left(1 - \frac{n_{p,e}}{n_{p,i}}\right) = \left\{1 - \exp\left(\frac{-\frac{3}{2}\ \alpha_{pb}\eta_T\phi_b v_b t_{cz}}{d_b}\right)\right\} \qquad \text{(Eq. 5.2.3)}$$

$n_{p,e}$ and $n_{p,i}$ = number of particles in the effluent and influent water respectively;

α_{pb} = the attachment efficiency;

η_T = the dimensionless particle transport coefficient;

ϕb = the bubble volume concentration;

v_b = the bubble rise velocity;

t_{cz} = the time the bubble spends in the contact zone;

d_b = the bubble diameter.

It is of note that α_{pb} which includes the effects of repulsive electrostatic forces and attractive van der Waals forces, is evaluated empirically. Parameters were applied as follows:

Bubble diameter: 45 μm;

Saturator efficiency: 0.7;

Residence time: 180 s (as measured);

Density of algae: *M. aeruginosa* - 1020 kg m^{-3}, *C. vulgaris* – 1070 kg m^{-3} and diatoms – 1140 kg m^{-3} (Edzwald and Wingler, 1990).

5.2.5.3 Pre-coagulation

To investigate if there was improved removal when using PosiDAF for coagulated micro cells as opposed to uni-cells, *M. aeruginosa* cells were pre-coagulated by adding aluminium sulphate at varying concentrations and adjusting to pH 7 during a 200 rpm rapid mix phase, slow mixed for 15 minutes and then floated using PosiDAF. The resultant sizes of cell agglomerates were measured using a Mastersizer 2000 (Malvern, UK).

Overall, when using this CTAB near 100 % removal can be achieved for cells that are greater than approximately 15 μm (Figure 5.2.6). For cells smaller than this, such as *M. aeruginosa* and *C. vulgaris*, pre-coagulation to increase cell size prior to treating by PosiDAF could be implemented. When investigating this hypothesis it was observed that pre-coagulation at low aluminium doses improved removal slightly from 54 % to 67 %, while removal by conventional methods remained at 20-26 % for the same coagulant dose ranges. The zeta potential of the cell system decrease from -21 to -17.6 mV for this dose range. Removal at levels greater than 92 % for both conventional methods and PosiDAF occurred concurrently at zeta potentials of less than -17.5 mV. This was in spite of an average floc diameter of greater than 30 µm being achieved at the dose of 0.000814 ng cell^{-1} required to obtain 67 % removal. Greater removal was anticipated at this dosage, given that the average floc size was greater than the 15 µm required to achieve optimum collision efficiency. The fact that only 67 % occurred suggests that there was an increase in the barrier to bubble-particle attachment that may have been caused by the decrease in the magnitude of the zeta potential of the residual cells. This suggests that including pre-coagulation when using PosiDAF would not be advantageous.

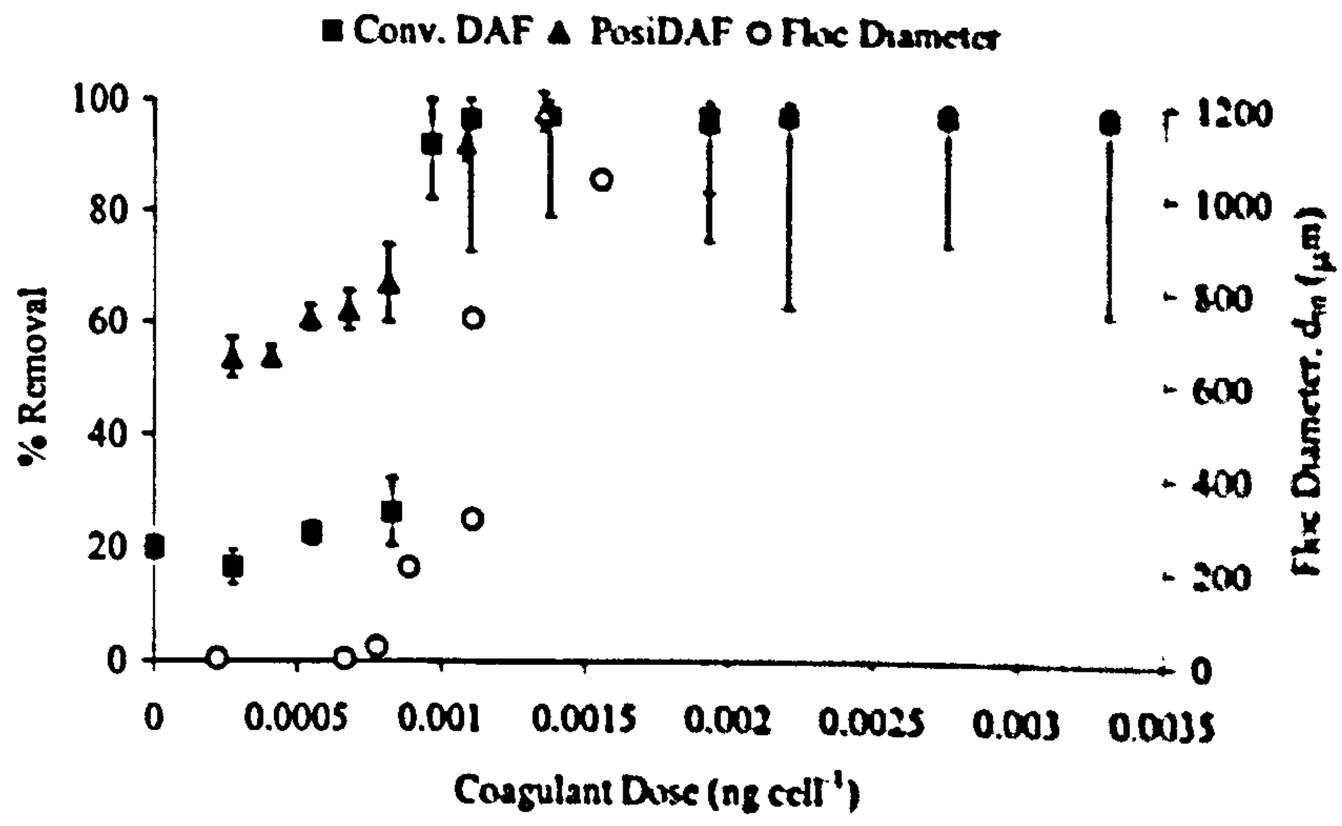

Figure 5.2.6. *% Removal obtained for PosiDAF with pre-coagulation and for conventional coagulation and flotation with respective floc sizes.*

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5.3 POLYMERS AS BUBBLE SURFACE MODIFIERS IN THE FLOTATION OF ALGAE - POSIDAF

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ABSTRACT

Research has demonstrated that dosing polymers direct to the saturator of a dissolved air flotation (DAF) process with no upstream coagulation can achieve comparable algae removal to conventional treatment due to bubble modification. This research further explores the application of polyDADMAC as a bubble modifier in this adapted DAF process – PosiDAF. It was determined that removal improved with increasing polyDADMAC molecular weight, attributed to enhanced adsorption at the bubble surface and increased extension distances of the polymer from the bubble. Projection of the polymer into the aqueous phase increased the swept volume of the bubble, such that obtained removal efficiencies were much greater than those predicted theoretically. PolyDADMAC dose and the resultant removal efficiency were dependent on the character of the associated algogenic organic matter (AOM). Specifically, AOM with high MW, low charge and significant hydrophobicity and protein content enabled co-operative binding. In contrast, for systems with AOM of high charge and lower molecular weight and hydrophobicity, removal efficiency was reduced due to interactions of polyDADMAC with AOM and lack of co-operative binding.

Keywords: algae; bubble surface modification; dissolved air flotation; polyDADMAC.

5.3.1 INTRODUCTION

Traditionally, treatment of high population densities of algae was accomplished using coagulation and flocculation followed by sedimentation. However, algae are naturally buoyant and form very low density flocs; therefore in more recent years this clarification process has been replaced by dissolved air flotation (DAF) (Edzwald, 1993). Microscopic bubbles, generated by the release of pressurised, air saturated recycled water to the flotation tank, collect influent coagulated particles and float them to the surface. Coagulation is important as it lowers the magnitude of the negative charge of algae cells through the addition of cationic chemicals which modify the cell surface (Gregory, 2006). If this is not accomplished, then attachment of the particle to the bubble, which is also negatively charged, will not occur as the energy barrier to contact will be too high (Somasundaran *et al.*, 1983). Hence, unsuccessful coagulation results in unsuccessful flotation. Complications in optimising coagulation of algae cells are frequently reported, resulting in poor flotation (Pieterse and Cloot, 1997; Jun *et al.*, 2001; Kempeneers *et al.*, 2001) and thus an adaptation of the original DAF process has been conceived to circumvent the problem.

This adaptation removes, or at least reduces the requirement for, the coagulation stage by instead dosing cationic chemicals to the saturator of the DAF unit. The aim is to create a functionalised bubble surface as opposed to attempting to manipulate the cell surface. Specifically, positively charged bubble surfaces can be generated that will attract negatively charged algae cells. A similar concept has been investigated for depth filtration where the filter media was modified using metal hydroxides to create positively charged sites (Truesdail *et al.*, 1998). However, in contrast to DAF, the collector media is not continually replenished, and thus contamination can greatly reduce the lifetime of modified surfaces (Chen *et al.*, 1998). While many studies have investigated the charge of bubbles treated with a variety of chemicals, including cationic surfactants (Skrylev *et al.*, 1984; Laskowski *et al.*, 1989; Kubota and Jameson, 1993; Cho *et al.*, 2005), metal coagulants (Li and Somasundaran, 1992; Yang *et al.*, 2001; Han *et al.*, 2006) and cationic polymers (Malley, 1995), very few

have actually applied this research for particle removal in DAF. Only the latter study utilised the positively charged bubbles for particle removal. Malley (1995) demonstrated that by adding Catfloc, a polydiallyldimethylammonium chloride (PolyDADMAC) type chemical, to the saturator of a DAF unit, the treatability of water containing low concentrations of turbidity and humic acid was similar to that obtained by conventional coagulation. Subsequently, a recent study by the current authors investigated dosing of surfactants, metal coagulant and polymers to the saturator to investigate the potential for algae removal (Henderson *et al.*, 2007a). The process was termed PosiDAF due to the addition of cationic chemicals. It was determined that polyDADMAC could achieve comparable removal to that obtained using conventional coagulation and flotation. Notably, the removal achieved when using polyDADMAC was far greater than that obtained when using metal coagulants, surfactants or other polymers.

The aim of this study is to improve the understanding of the mechanisms by which polyDADMAC addition to the saturator achieves algae removal and thereby determine the potential for using polyDADMAC type polymers in the PosiDAF process. Specific objectives were as follows: a) to determine the removal efficiencies achieved when using polyDADMAC chemicals of varying molecular weight (MW); b) to investigate the optimum operating conditions; and c) to determine the impact of varying algae character on removal efficiency.

5.3.2 MATERIALS AND METHOD

Algae. Cultures were obtained from the Culture Collection for Algae and Protozoa (CCAP), Oban, Scotland as follows: cyanobacteria *Microcystis aeruginosa* (1450/3), green *Chlorella vulgaris* (211/11B) and the diatom *Asterionella formosa* (1005/9). The diatom *Melosira* sp. was obtained from Sciento, Manchester, UK. *M. aeruginosa* and *C. vulgaris* cells were cultivated in sterilised Jaworski Media that was shaken at 75 rpm (Patterson Scientific Bibby Stuart SO1 shaker, Luton, UK) and incubated at 20 °C under 24 hour radiation using Sun-glo 30 W aquatic lighting. Diatoms were grown in sterile Diatom Media, using a growth temperature of 15 °C and a lighting

cycle of 14 hours light/8 hours dark that was provided using an Environmental Test Chamber (Sanyo Versatile Environmental Test Chamber, MLK 350H), programmed to give a brightness of 1000 lx. Agitation of diatoms was performed by shaking by hand once per day. Algae were harvested at the onset of the stationary phase. Prior to use cultures were diluted, using DI water that was buffered using 0.5 mM $NaHCO_3$ and brought to the ionic strength of 2.3 mM using NaCl, to achieve concentrations as follows: *C. vulgaris* – $9.2 \times 10^5 \pm 7 \times 10^3$ cells mL^{-1}; *M. aeruginosa* – $7.5 \times 10^5 \pm 1.5 \times 10^4$ cells mL^{-1}; *A. formosa* – $3.7 \times 10^4 \pm 500$ cells mL^{-1}, and *Melosira* sp. – 1100 ± 50 cells mL^{-1}. Average cell diameters for the spherical *C. vulgaris* and *M. aeruginosa* were 4.0 ± 1.1 µm and 5.4 ± 0.8 µm, while *A. formosa* and *Melosira* sp. had diameters of 4.2 ± 0.9 and 24.0 ± 0.8 µm and lengths of 26.0 ± 3.1 and 60.7 ± 11 µm respectively.

Chemicals. A variety of polyDADMAC chemicals were used as follow: Very low MW (<100 kDa), low MW (100-200 kDa), medium MW (200-350 kDa), and high MW (400-500 kDa) polyDADMAC were obtained from Aldrich, UK. Zetag 7125 and Magnafloc LT35, both polyDADMAC type chemicals of intrinsic viscosities of 1000-3000 cp and 700-1400 cp respectively (no molecular structure or MW information was available) were obtained from CIBA Chemicals, UK. However, intrinsic viscosity can be related to MW (Cosgrove, 2005); hence, while the MW was not disclosed, it is known that Zetag 7125 is a bigger polymer than Magnafloc LT35. The charge density of these chemicals was determined by back titration method (Kam and Gregory, 2001) using the anionic polyelectrolyte poly (vinylsulphonic acid) sodium salt (PVSA).

Flotation Method. An EC Engineering Dissolved Air Flotation Batch Tester, Model DBT6 (Alberta, Canada) was used for the flotation jar testing. A number of different experiments were undertaken as follows:

1. Increasing concentrations of each polymer was added direct to the saturator with deionised water containing 0.5 mM $NaHCO_3$ and 1.8 mM NaCl, adjusted to pH 7. This mixture was pressurised and shaken until achieving stabilisation of gauge pressure at 450 kPa. The pressurised solution was released to 1 litre of *M.*

aeruginosa sample adjusted to pH 7, at an equivalent recycle ratio of 20 %, and left to float for 10 minutes.

2. Low MW polyDADMAC was dosed directly to the jar, stirred vigorously for 1 minute and left for 10 minutes. The exact same system of *M. aeruginosa* cells was subjected to polymer dosing via the saturator as in Experiment 1. The system zeta potential after 10 minutes was compared that measured when using PosiDAF.
3. Using the optimum polymer concentration determined in Experiment 1, the recycle ratio was varied. The experiment was undertaken as a function of firstly constant dose to saturator and secondly constant dose to jar.
4. The impact of changing the influent particle loading was investigated at an equivalent recycle ratio of 20 % by varying cell concentration.
5. Finally, the removal efficiency for algae of varying character was examined at a recycle ratio of 20 % by obtaining polymer dose response curves for the other algae types.

Residual analyses after each of the aforementioned experiments included cell counting of 100 cells using a haemocytometer or Sedgewick Rafter counting cell and zeta potential (ZP) analysis using a Zetasizer 2000HSA (Malvern, UK). Each analysis was preformed in triplicate.

Theoretical Model. Experimental results were compared to those calculated using a theoretical model described in detail by Haarhoff and Edzwald (2004). This particular model uses the *white water* model performance Equation 5.3.1 to describe removal in the contact zone of a DAF unit.

$$\left(1-\frac{n_{p,e}}{n_{p,i}}\right) = \left\{1-\exp\left(\frac{-\frac{3}{2}\,\alpha_{pb}\eta_T\phi_b v_b t_{cz}}{d_b}\right)\right\} \qquad \text{(Eq. 5.3.1)}$$

Where $n_{p,e}$ and $n_{p,i}$ relate to the number of particles in the effluent and influent water respectively; α_{pb} is the attachment efficiency; η_T is the dimensionless particle transport coefficient; φb is the bubble volume concentration; vb is the bubble rise velocity; t_{cz} is the time the bubble spends in the contact zone; and d_b is the bubble diameter. α_{pb}

which includes the effects of repulsive electrostatic forces and attractive van der Waals forces, is evaluated empirically. Parameters were applied as follows: bubble diameter – 45 μm; saturator efficiency – 0.7; residence time – 180 s (as measured); density of algae: *M. aeruginosa* - 1020 kg m^{-3} (Haarhoff and Edzwald, 2004), *C. vulgaris* – 1070 kg m^{-3} and diatoms – 1140 kg m^{-3} (Edzwald, 1993).

5.3.3 RESULTS

5.3.3.1 Comparison of polyDADMAC chemicals

Removal efficiencies of 74 ± 2, 94 ± 2, 94 ± 1, 89 ± 2 %, 80 ± 1.5 % and 96 ± 1 % were obtained for very low, low, medium and high MW polyDADMAC (Figure 5.3.1) and Magnafloc LT35 and Zetag 7125 (Figure 5.3.2) respectively at dose ranges of 0.0018-0.0025, 0.002-0.0027, 0.0022-0.0027, 0.0022-0.0031, 0.0016-0.0017 and 0.0016-0.0020 meq L^{-1} for the same chemicals. Furthermore, PosiDAF with low MW polyDADMAC was trialled for pH 7-9 and the removal efficiency achieved was consistent at 98. 2 ± 0.7 % (not illustrated), indicating that the mechanism of removal is insensitive to changes of pH in this range. Comparisons with the literature reveal that these doses of 0.26-0.5 mg L^{-1} were lower than the 0.8-6 mg L^{-1} of Catfloc dosed to the saturator to treat NOM and kaolin (Malley, 1995). However, optimum dose ranges were similar to that obtained using cationic surfactant, cetyltrimethyl ammonium bromide (CTAB), for treating the same algae system, at 0.0022-0.0040 meq L^{-1} (Henderson *et al.*, 2007a).

The lowest removal efficiencies were obtained for very low MW polyDADMAC and Magnafloc LT35, the smallest of the CIBA chemicals. Furthermore, the rate of decrease in removal efficiency at relatively high doses increased for lower MW polymers. For example, the gradient for very low MW polyDADMAC was -55114 L meq^{-1} compared to -13476 L meq^{-1} for high MW polyDADMAC (Figure 5.3.1). The lower optimum removal efficiency obtained for high MW polyDADMAC in comparison to low and medium MW polyDADMAC can be attributed to float stability. The float of the former was much more likely to collapse due to heavy

flocs formed within the float (Figure 5.3.3b). In contrast, the cells remained separate, contained in a gelatinous film (Figure 5.3.3a) for low MW polyDADMAC.

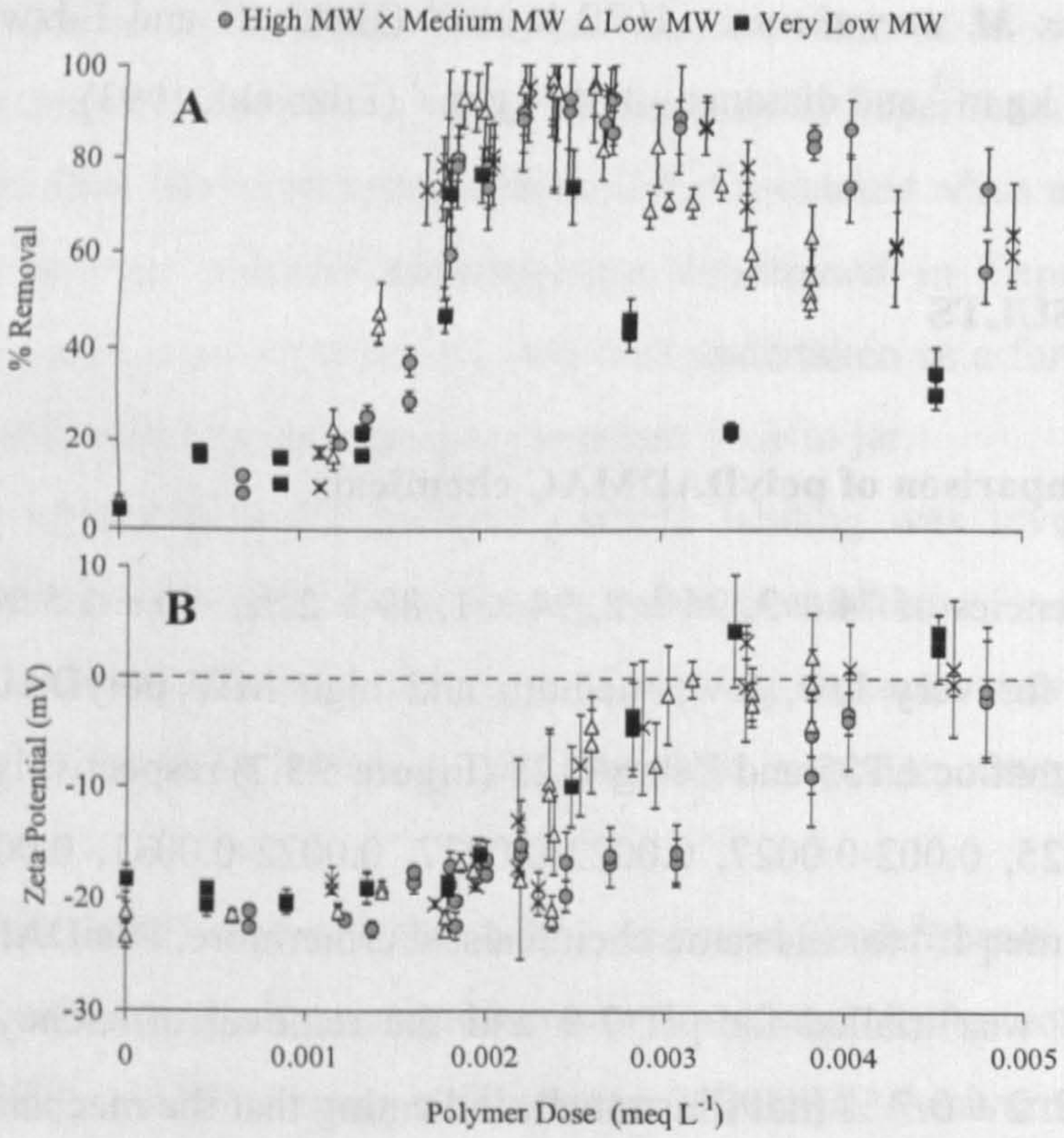

Figure 5.3.1 *Comparison of very low-high MW polyDADMAC for A. M. aeruginosa removal efficiency and B. Zeta potential.*

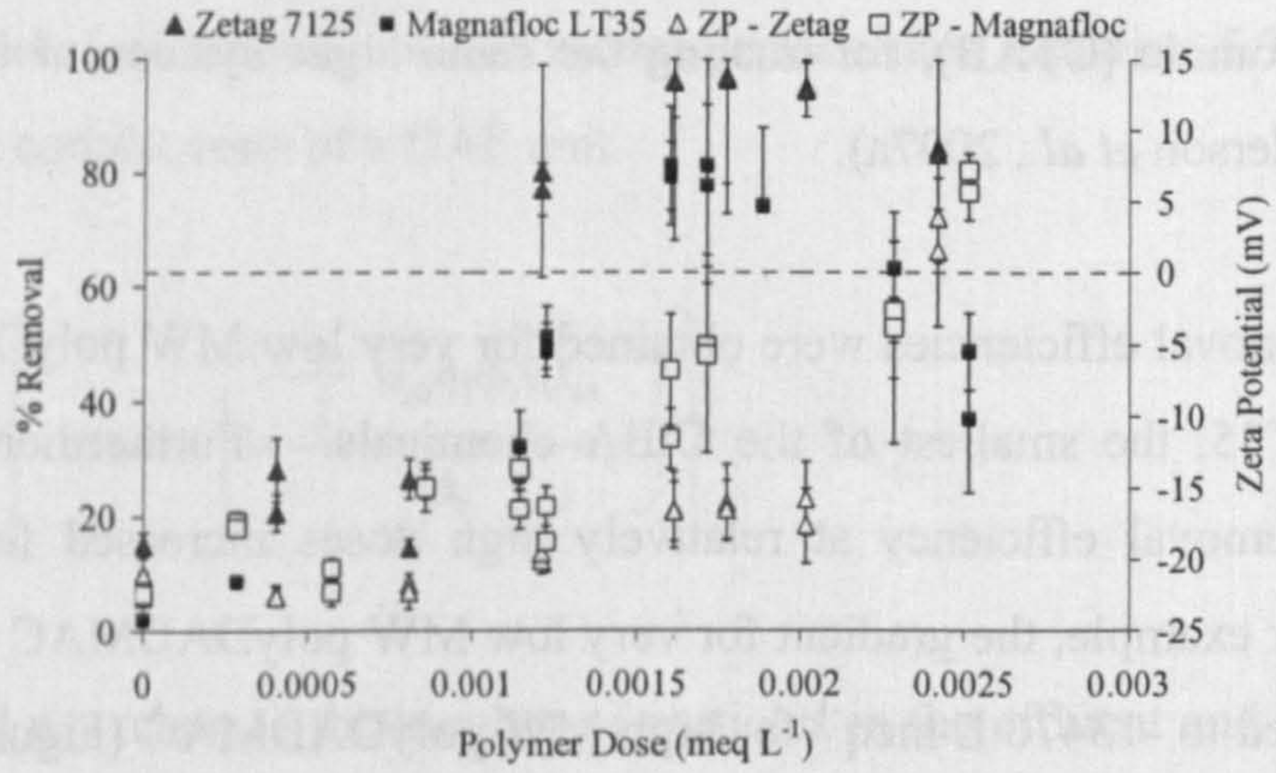

Figure 5.3.2 *Comparison of the removal efficiency and residual zeta potential for Zetag 7125 and Magnafloc LT35.*

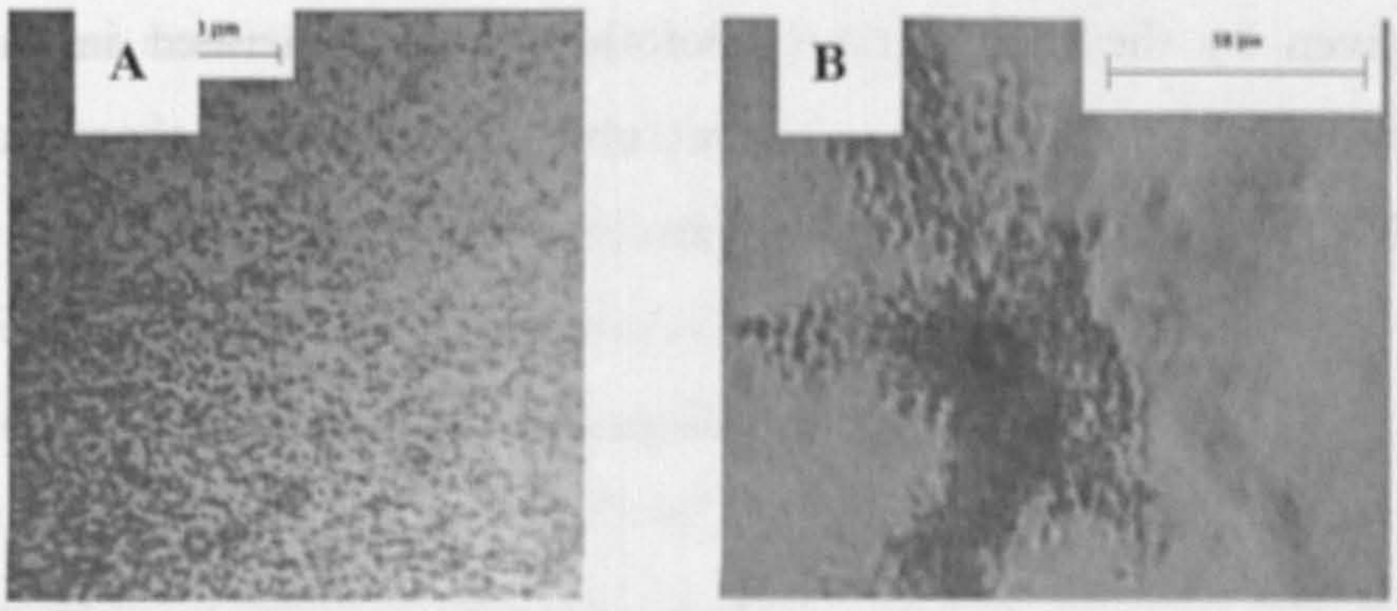

Figure 5.3.3 *Microscopic images of the float obtained from A. Low MW and B. High MW polyDADMAC experiments.*

The initial ZP of the system with no added polymer was -19.8 ± 1.5 mV and, in each polyDADMAC experiment, this did not change until a dose of 0.0023 meq L^{-1} had been added. In the instance of very low, low and medium MW polyDADMAC experiments, the magnitude of the ZP then began decreasing, achieving the isoelectric point (i.e.p.) at a dose of 0.003 meq L^{-1}, indicating that polymer residue was present in the bulk solution (Gehr and Henry, 1982). The decrease in ZP was concurrent with the decrease in removal efficiency. The ZP obtained when using high MW polyDADMAC remained stabilised at the initial value until 0.0031 meq L^{-1} had been added, reaching the i.e.p. at a dose of 0.0048 meq L^{-1} (Figure 5.3.1). This contrast between the ZP of low and high MW polymers was also observed for Magnafloc and Zetag 7125 where in the case of the former, the ZP began to decrease at the doses required for optimum removal. In contrast, the ZP of the Zetag 7125 remained stable until a dose of 0.0022 meq L^{-1} had been added (Figure 5.3.2). This has previously been observed for high and low MW polymers during conventional DAF treatment (Gehr and Henry, 1982). Comparison between the ZP of the system treated by 1. PosiDAF and 2. addition of polyDADMAC direct to the jar with no flotation, revealed that the ZP of the latter was decreased relative to the former at the doses required for optimum removal (Figure 5.3.4). For example, at a dose of 0.0022 meq L^{-1}, the ZP of the PosiDAF treated system was -21.3 ± 0.3 mV in comparison to -11.4 ± 4.1 mV for the untreated system. This suggests that polyDADMAC is primarily associated with the bubble as opposed to the cell system during PosiDAF treatment.

Further evidence supporting the theory that polyDADMAC is associated with the bubble is given by the fact that no microflocs were generated in the PosiDAF experiments. In contrast, flocs were observed in the experiments where polyDADMAC was added directly to the jar.

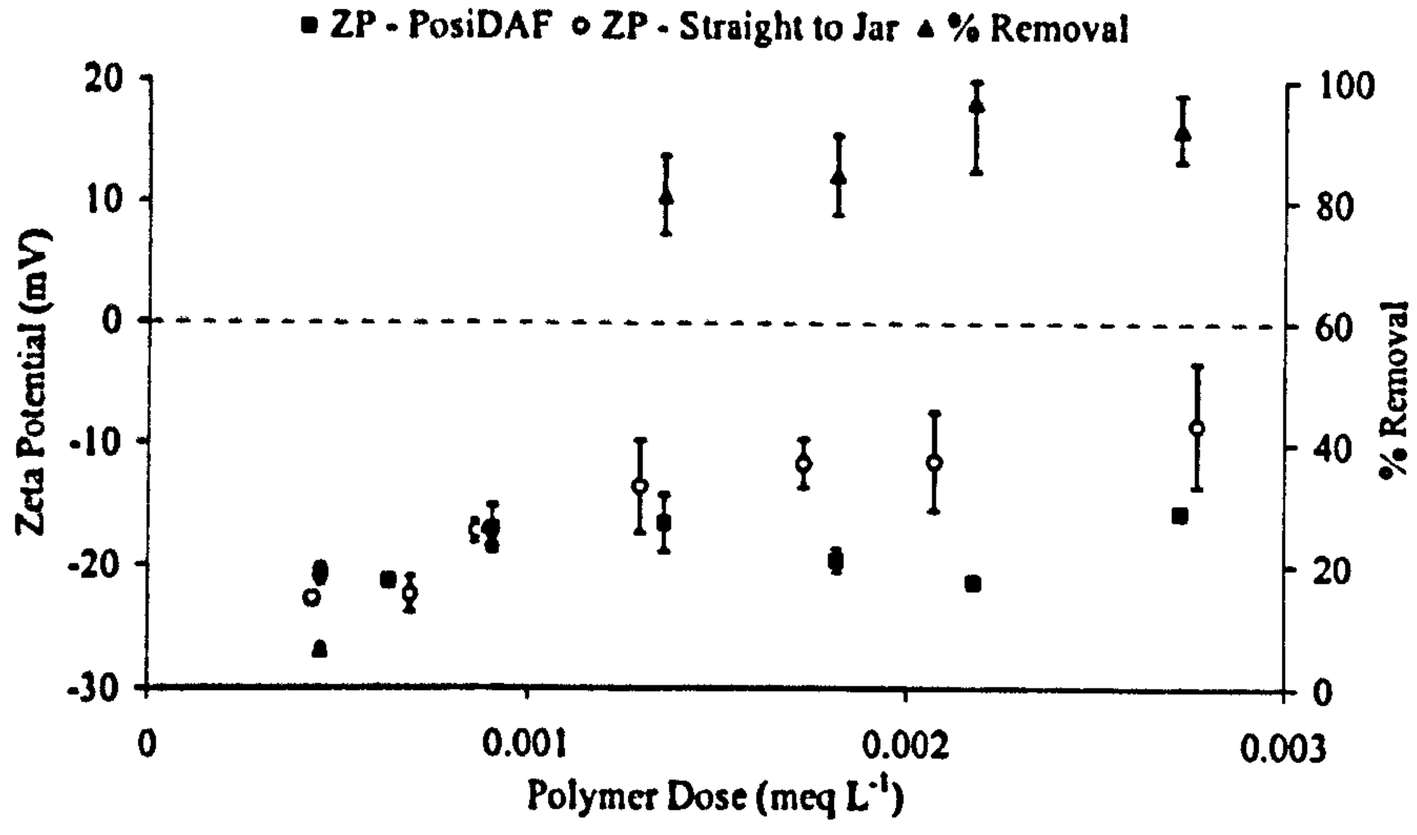

Figure 5.3.4 *Comparison of the residual zeta potential obtained when adding low MW PolyDADMAC direct to the jar or via the saturator.*

5.3.3.2 Optimisation of operational parameters

The impact of varying recycle ratio was examined by firstly applying a constant dose of 0.0124 meq L^{-1} to the saturator (Experiment 1) and secondly applying a constant dose of 0.0021 meq L^{-1} to the jar by adjusting the dose to the saturator (Experiment 2). The former allowed examination of the relationship between dose and bubble surface area while the latter investigated whether the dose was a reflection of the algae charge. Firstly, increasing bubble concentration without chemical addition did not improve removal (Figure 5.3.5). In contrast, during Experiment 1, the removal efficiency increased linearly from 7 ± 6 % to 83 ± 10 % with increasing recycle ratio from 5-15 %. It then reached a plateau at 94 ± 2 % for recycle ratios from 20-30 % before gradually decreasing at recycle ratios of 40 % and greater. During Experiment 2, even at a 5 % recycle ratio, greater than 90 % removal was achieved. From R_r = 5-

20 % removal was approximately equal at 96 ± 2 %. However, at R_r = 30 % or more, removal was less consistent and showed an overall decrease. Experiment 1 removal efficiencies exceeded theoretical values calculated using an attachment efficiency of 1.0 for R_r = 10-40 %, while for experiment 2, removal efficiencies were greater than calculated for R_r = 5-40 %. Experiments 1 and 2 achieved maximum removal at R_r = 20 %, achieving an additional 30 % cell removal when compared to theoretical calculations (Figure 5.3.5). The results of the same experiments when using CTAB have been previously reported (Henderson *et al.*, 2007a) where it was demonstrated that removal was similar to that calculated experimentally, indicating the removal mechanisms operating when using CTAB and polyDADMAC are very different.

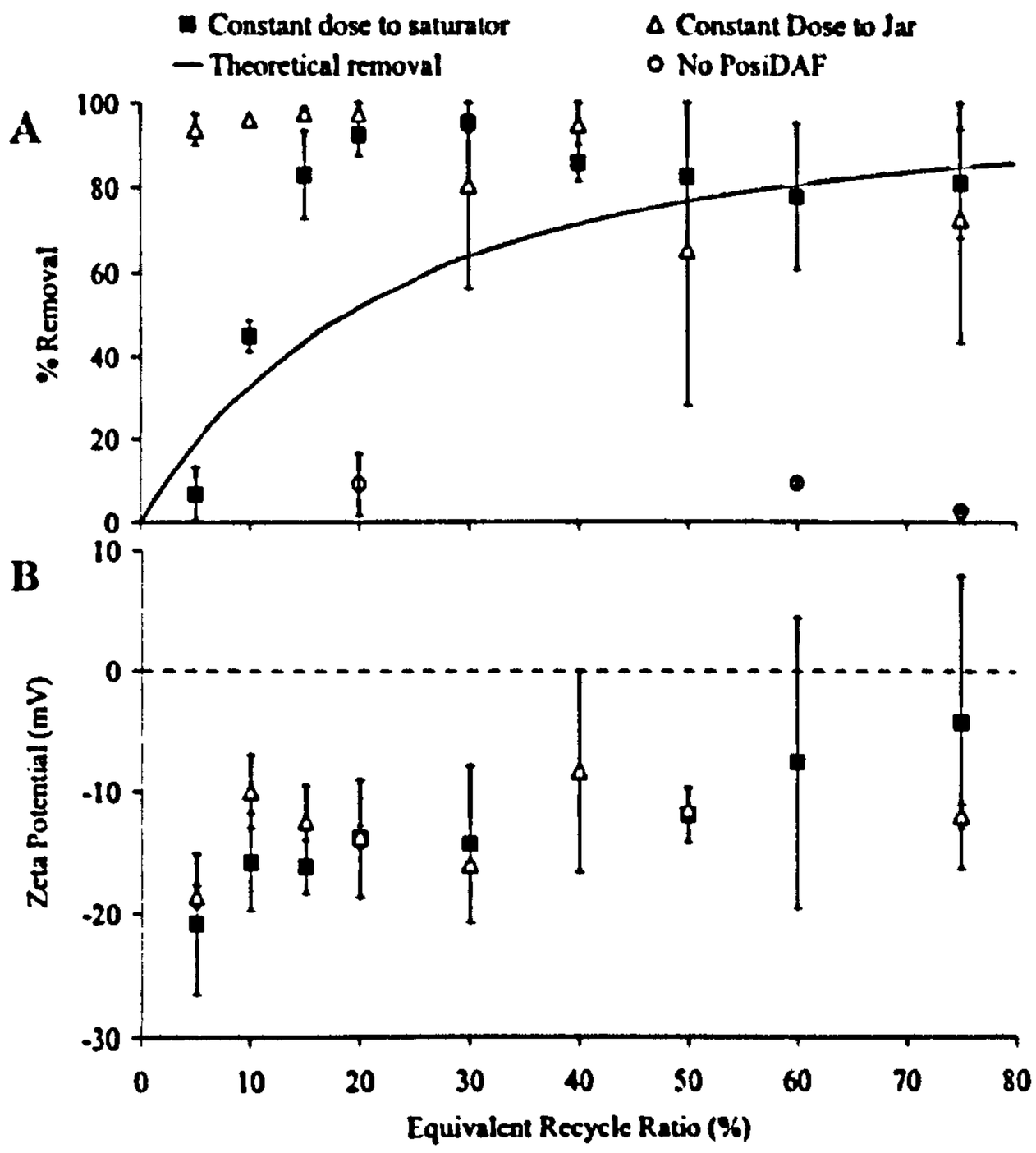

Figure 5.3.5 *A. % removal and B. zeta potential vs recycle ratio for a constant polymer dose to the saturator, to the jar and for no polymer dose.*

Overall, the results demonstrate that at R_r = 5-20 % the effective dose to the jar is most important with respect to obtaining good removal efficiencies, as opposed to the

dose to the saturator. For Experiment 1, the decrease in removal efficiency at $R_r > 30$ % can be attributed to the decreasing of the system ZP from -15 to -5 mV as was previously observed (Figure 5.3.1). For experiment 2, the dose to the jar was constant and thus there was no systematic decrease in ZP; however, removal became more scattered at R_r>20 %. This suggests that the poorer removal efficiency was the result of inconsistent surface coverage of bubble with polymer at these high bubble concentrations. Hence, the bubble-particle attachment efficiency would be decreased.

The maximum bubble:particle ratio, calculated using a 45 µm bubble diameter, was 0.64 at the recycle ratio of 80 % in the current study and optimum removal occurred at a bubble:particle ratio of approximately 0.3. This is 10 times less than that calculated based on conventional treatment – where a typical algae system with mass concentration of 10 mg L^{-1}, floc size of 100 µm and typical bubble number concentrations of 44-88 × 10^6 bubbles L^{-1}, yields a bubble:particle number of 2-5 (Haarhoff and Edzwald, 2004) – and is a reflection of cells remaining separately, rather than agglomerating. In the current research, bubble:particle numbers, as opposed to air:solids ratio, were used as the operational parameter as algae were not flocculated. The number of particles collected per bubble, the bubble collector efficiency, was calculated based on the removal efficiencies. At R_r=5 % in Experiment 2, 11.7 cells $bubble^{-1}$ were collected, decreasing to 3.4 cells $bubble^{-1}$ at Rr = 20 %. This was 1.4 times the maximum bubble collector efficiency of 2 cells $bubble^{-1}$ that was obtained for CTAB under the same conditions (Henderson *et al.*, 2007a); again indicating that a different mechanism predominates when removing cells with polymer.

When varying the initial cell concentration, the removal efficiency increased from 76 % to a maximum of 97 % for cell concentrations of 1.1 × 10^5 cell mL^{-1} to 5.6 × 10^5 cell mL^{-1} respectively (Figure 5.3.6). At higher cell concentrations, removal efficiencies remained relatively stable. Comparisons with results obtained when utilising CTAB as the PosiDAF chemical (Henderson *et al.*, 2007a) revealed that the optimum removal occurred at the same cell concentration, although the removal maximum was lower (Figure 5.3.6). Conversion to bubble:particle ratio demonstrated

that while relatively low removal was obtained at high bubble:particle ratios, as bubble:particle ratio decreased, a steep decline in removal efficiency, such as that demonstrated by CTAB, was not observed. Similar observations to those made when varying bubble:particle ratio through recycle ratio variations were noted when varying cell concentration. To illustrate, maximum removal efficiencies were obtained at a bubble:particle ratio of 0.3 for a bubble collector efficiency of 3.05 cells bubble^{-1} and a maximum of 12 cells bubble^{-1} was possible (Figure 5.3.6). The contrasting gradients of cell removal vs bubble:particle ratio for polyDADMAC and CTAB of -6.1 and -1.7 cells^{2} bubbles^{-2} respectively (Figure 5.3.6c) reveal that at high particle concentrations bubbles coated with PolyDADMAC are far more effective at capturing cells. The low removal efficiencies obtained at low cell concentrations, and therefore high bubble:particle ratios, indicate that a minimum number of cells is required for optimum collision, as was observed previously for CTAB (Henderson *et al.*, 2007a).

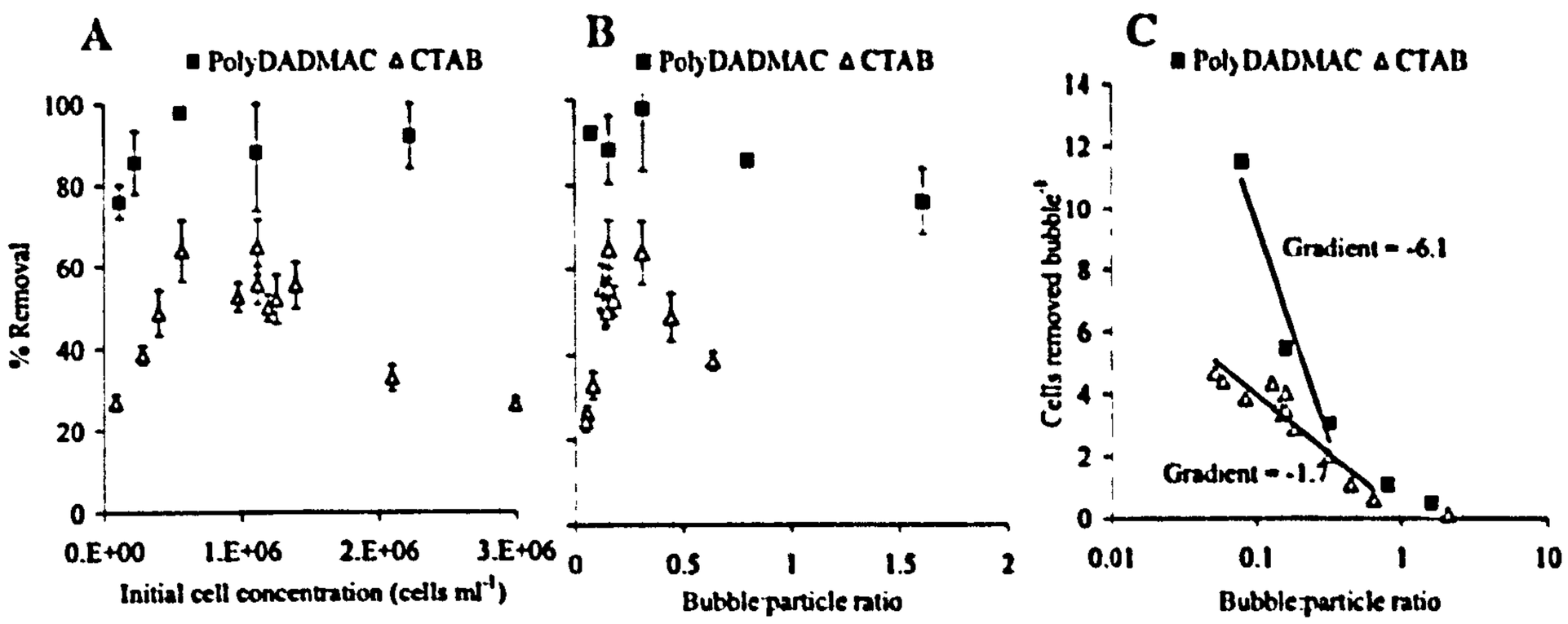

Figure 5.3.6 *% removal vs A. cell concentration and B. bubble:particle ratio and C. the bubble collector efficiency vs bubble:particle ratio for polyDADMAC and CTAB (Henderson et al., 2007a).*

5.3.3.3 Removal efficiency for various algae

Removal efficiencies of 76 ± 2, 49 ± 1 and 99.7 ± 0.5 % were obtained for *C. vulgaris*, *A. formosa* and *Melosira* sp. respectively for doses of 0.034-0.042 meq L^{-1}, greater than 0.00536 meq L^{-1} and 6.7 × 10^{-5} meq L^{-1} for the same species at a recycle ratio of 20%. In the instance of *C. vulgaris*, removal efficiency decreased at dosages

greater than 0.042 meq L^{-1} (Figure 5.3.7), while those of *A. formosa* (Figure 5.3.7) and *Melosira* sp. (not illustrated) stabilised. The ZP curves obtained were distinctly different to those obtained for *M. aeruginosa*. For example, in the case of *C. vulgaris*, the ZP decreased initially from -34 ± 0.9 mV to -21 ± 3 mV at a dose of 0.023 meq L^{-1} (Figure 5.3.7). It then stabilised until the removal maxima occurred upon which ZP continued a steady decrease achieving the i.e.p. at 0.046 meq L^{-1}. For *A. formosa*, the i.e.p. was achieved at 0.002 meq L^{-1}, and thus the ZP was positive, at +14 ± 0.8 mV, for the dose range of optimum removal (Figure 5.3.7). Good removal was achieved for *Melosira* sp. at very low dose ranges, corresponding to ZP values that were similar to the initial of -13.9 ± 1.3 mV. This is likely a result of the very low initial ZP which in combination with the much greater size of the cell leads to good removal efficiencies.

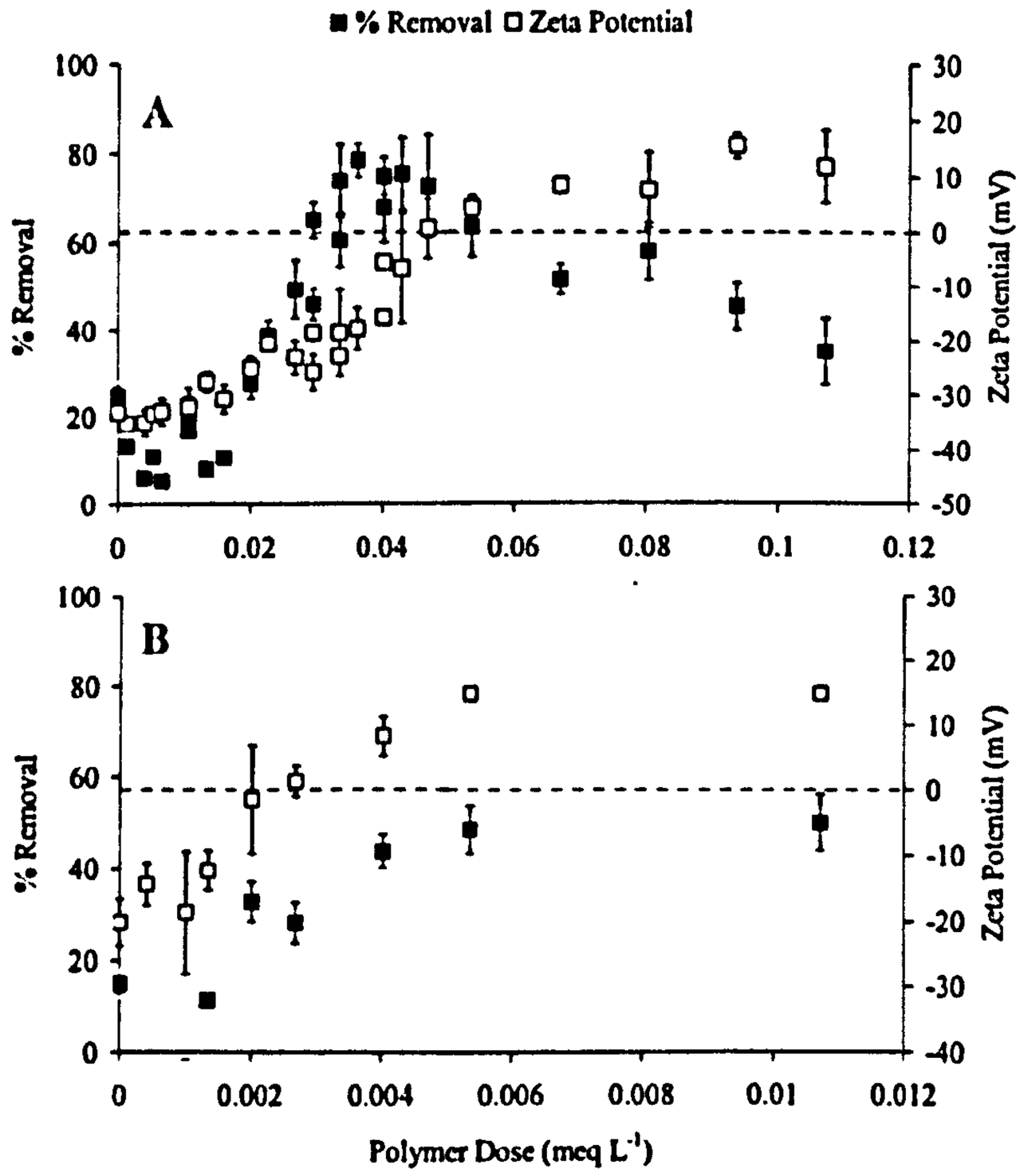

Figure 5.3.7 *% removal and zeta potential vs polymer dose for A. C. vulgaris and B. A. formosa.*

5.3.4 DISCUSSION

The two key observations with respect to utilising polymers as bubble modifers are that the removals are better than theoretically predicted and that there is variability in the results obtained for different algae types.

5.3.4.1 Mechanism of removal

It was hypothesised that when polyDADMAC is used in the PosiDAF process, removal is enhanced by the modification of the bubble surface such that it increases the attachment efficiency. A number of key observations have been made which enable this hypothesis to be verified: (1) comparison to the theoretical model shows an under prediction compared to experiments results, (2) the same model correctly predicts removal with CTAB as the bubble modifier (Henderson *et al.*, 2007a) and (3) the same dose with respect to charge equivalents added was required when using both CTAB and polyDADMAC.

The use of polyDADMAC as a bubble modifier has previously been shown to make the bubble charge positive (Malley, 1995) and as such, coupled to the third observation above, the original suggestion appears valid. The fact that removal is greater than predicted for polyDADMAC but not for CTAB, as reported previously (Henderson *et al.*, 2007a), reflects additional enhancements are occurring. In comparison to the surfactants used previously, polyDADMACs are much larger MW compounds and are more hydrophilic. These two factors indicate that the polymer will project away from the bubble surface generating a greater swept volume for the bubble, acting as a bridge between bubble and cell. This is supported by the fact that removal increases with increasing MW of the polymer. Whilst the exact enhancement of the swept volume has not been measured, typical spatial extension distances for polymers were calculated to be 15 nm and 40 nm for the very low and high MW polymers respectively, although this is a rough approximation to give an illustrative value (Napper, 1983). In the case of the surfactants, the molecules are small with MW of around 360 mol L^{-1} and so do not significantly enhance swept volumes. The impact of this is seen by the increase in the average bubble collector efficiency for

polyDADMAC. In the case of CTAB it was calculated that a maximum of 4 cells bubble^{-1} were removed but with polyDADMAC this increased to 12 cells bubble^{-1}. Corroborating observations have been made previously where the use of a polymer rich effluent for the recycle stream increased performance although this was reported to be due to increased flocculation (Gehr and Henry, 1982). The other observation that appears effected by MW is the robustness of the process. The impact of overdosing became less significant as the MW of the polymer increased as seen by the reduction in removal efficiency due to dosing beyond the optimum dose. This coincided with a smaller change in ZP indicating that less material was entering the bulk phase. Gehr and Henry (1982) also observed improved removal when dealing with higher MW polymers. Such observations fit in with established knowledge on polymers where more favourable adsorption is observed for long chain polymers as opposed to short chain polymers due to thermodynamics (Cosgrove, 2005). The reduced removal is observed due to the polyDADMAC in the liquid phase adsorbing onto the algae and generating steric repulsion with the polymer on the bubble surface.

5.3.4.2 Impact of varying algae character

Comparison of the results for different algae species demonstrated a significant difference due to species which was not observed during the surfactant trials (Henderson *et al.*, 2007a). This was manifested in different removal levels, dose requirements and zeta potential responses. For instance, the dose ratio in terms of charge of polymer to charge of algae (meq meq^{-1}) was 1.7, 3.0 and 2.2 for *M. aeruginosa, C. vulgaris and A. formsa* respectively, calculated using previously determined algal charge densities (Henderson *et al.*, 2007b). The major differences between the algae species are the balance of the charge associated to the cell and algal organic matter (AOM) (Henderson *et al.*, 2007b). In cases where the AOM contains a significant charge the polyDADMAC interacts with the AOM and reduces its effectiveness at binding with the cells directly. The reason for this is polyDADMAC is known to interact primarily with the AOM and secondly with the cells (Haarhoff and Cleasby, 1989), although the exact nature of this appears to depend on nature of the AOM. In the case of *M. aeruginosa*, the AOM contains little charge as only 6% of the total charge is associated with this dissolved organic component (Henderson *et al.*,

2007b). Hence, the removal levels are high as the polyDADMAC does not specifically interact with the AOM and so can bind to the algae. A second effect appears evident in that the AOM can also act a bridging chemical and, when the MW and character of the AOM is appropriate, this aids removal. In the case of *M. aeruginosa*, the AOM is of very high MW weight, as approximately 45% is greater than 500 kDa, and additionally is relatively hydrophobic and proteinaceous; characteristics which favour bridging flocculation (Tirado-Miranda *et al.*, 2003). The bubble-cell attachment is therefore enhanced by co-operative binding from the AOM. Observational evidence is provided as in these cases the float had a gelatinous consistency which is consistent with an enmeshment by both polyDADMAC and AOM. In contrast, removal was reduced for both *C. vulgaris and A. formosa,* attributable to the lower MW, hydrophobicity and protein content of the AOM and its increased charge density, which interfere with the enhanced bridging effect and the action of the polyDADMAC respectively. The same reduction was not observed when surfactants were used as the bubble modifiers where size governed the removal efficiency achievable (Henderson *et al.*, 2007a). However, CTAB does not interact with AOM like polyDADMAC, providing further evidence that the interaction of polyDADMAC with the AOM is attributable for the decreased removal.

This study therefore demonstrates that using polyDADMAC as a bubble modifier can achieve comparable removal to conventional treatment without the requirement for upstream coagulation and flocculation or pH control. Furthermore, this removal can be achieved using relatively low recycle ratios of 5 % (Figure 5.3.5). However, comparable removal to conventional treatment can only be achieved for algae of certain character. Specifically, certain AOM interferes with the polyDADMAC reducing the effectiveness of the polymer bridging and thus the bubble collector efficiency.

5.3.5 CONCLUSIONS

The use of polyDADMAC as a PosiDAF chemical achieved cell removal as a result of both modification of the bubble surface such that positive sites were created

increasing the attachment efficiency and by bridging between the bubble and cell surface. Hence, the polyDADMAC increased the swept volume of the bubble by projecting from the bubble into the media. This was concluded based on the following:

1. Cell removal increased with increasing MW of the polyDADMAC chemical.
2. Theoretically calculated removal efficiencies were lower than those achieved experimentally.
3. The bubble collector efficiency obtained when using polyDADMAC was three times greater than that obtained when using CTAB.

The interaction of polyDADMAC with AOM was critical in determining achievable cell removal efficiencies. Specifically, hydrophobicity, molecular weight and charge density had an impact on cell removal and dose required.

5.3.6 ACKNOWLEDGEMENTS

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CHAPTER 6: OVERALL IMPLICATIONS FOR ALGAE TREATMENT

6. OVERALL IMPLICATIONS FOR ALGAE TREATMENT

It was originally hypothesised that through a more thorough understanding of the characteristics of algae, the treatment of algae could be enhanced and further, that new technologies could be developed on the basis of this knowledge. The research presented in the previous four chapters supports this hypothesis.

6.1 CONVENTIONAL TREATMENT

The key observations within this thesis with respect to conventional flotation were:

1. Algae impact on a water treatment works in different ways depending on their characteristics – specifically those of size and charge density (Paper 1, 2, 5).
2. The character of the algal AOM was shown to impact directly on the coagulation process (Paper 3, 4, 5).
3. Successful coagulation and therefore flotation was achieved provided the correct coagulant demand was applied (Paper 5).
4. The appropriate coagulant demand was dependent to an extent on the cell surface area; although a stronger relationship between charge density of the algal system and coagulant demand was obtained (Paper 5).
5. Zeta potential (ZP) monitoring is a useful tool for determining the optimum coagulant dose for removal (Paper 6).

The above findings suggest that monitoring algae using indiscriminate parameters such as chlorophyll *a* concentration and total algae counts, can only give an indication of algae activity in source water and will not provide any information to aid process optimisation. In contrast, monitoring by identification of the specific species involved will give a clearer indication of the impact of algae on processes and more importantly provide an estimate of the coagulant dose. Once the species has been identified, the surface area is known which relates to coagulant demand. It was

determined that during algae blooms population diversity narrowed such that only a small number of species were present and that these species did not vary greatly with respect to both location and time for the WTW examined (Paper 2). This suggests that an algorithm linking surface area to coagulant demand could be devised for typical algae species, providing a straightforward assessment of the approximate coagulant demand once the influent algae had been identified. Equation 6.1 is derived based on Figure 4.1.5 (Paper 5):

$$\text{Dose} = \frac{\text{surface area}^{1.57}}{3000} \qquad \text{(Eq. 6.1)}$$

However, using an algorithm of this type would only ever be an approximation due to the accompanying dissolved algogenic organic matter (AOM). For example, using the algorithm for *M. aeruginosa* results in a theoretical coagulant demand of 0.0044 ng Al cell^{-1}; four times greater than that actually required. As removal does not decrease with overdosing at this level, good removal would still result in this instance. However, in the case of *C. vulgaris,* where a theoretical demand of 0.0019 ng Al cell^{-1} was 2.3 times less than that required experimentally, the application of the algorithm would not result in good removal. This implies a modified algorithm is required.

The best method for determining coagulant dose and thus ensuring successful removal is to measure the charge density of the system (Paper 5), which will change depending on the species, cell and AOM concentration, and age of the system. An algorithm could be devised to determine the coagulant demand based on charge density. For example, Equation 6.2 based on Figure 4.1.5 (Paper 5):

$$\text{Dose} = \left(\frac{\text{charge density}}{0.007}\right)^{0.81} \qquad \text{(Eq. 6.2)}$$

However, the charge density of a particular species may alter due to changes in character and concentration of AOM with age. For example, stationary phase *C. vulgaris* had a charge density of 1.12×10^{-5} neq cell^{-1}, of which 85 % of the charge

vulgaris had a charge density of 1.12×10^{-5} neq cell^{-1}, of which 85 % of the charge was associated with the AOM, resulting in a coagulant demand of 0.0054 ng Al cell^{-1}. In contrast, in the exponential growth phase, assuming approximately 85 % of the charge was still associated with the AOM, theoretical determinations are that the charge density would be 9.1×10^{-7} neq cell^{-1}, leading to a coagulant demand of 0.00071 ng Al cell^{-1}; 8 times lower than that required in the stationary phase. It was observed that for almost all species charge density of the AOM increased with age. For the only species where this was not the case, *M. aeruginosa*, the change in charge density was relatively small. Hence, if the algorithm is devised for stationary phase algae as opposed to exponential phase algae, the coagulant dose calculated should always be in excess of that required such that good removal should result. The physical form of this algorithm is perhaps best visualised as a look-up table that provides operators with a guide to coagulating algae based on species specific understanding and should result in more stable systems.

The implementation of an algorithm/look-up table may therefore result in overdosing which, while ensuring good removal and a robust process, would lead to increased chemical costs and sludge production. Hence, it is further suggested that the process can be further tailored by the use of charge measurement (Paper 6). For example, successful removal was observed whenever the zeta potential (ZP) post coagulation was within the range -10 mV to + 2 mV. Such a system can be delivered by either off-line ZP measurements or on-line using streaming current detectors (SCD). However, operational difficulties encountered during the use of such instrumentation have largely meant that the technique is not widely in use. As a result, research is currently on-going into devising an on-line zeta meter for this purpose (Sharp *et al.*, 2007). Inclusion of such monitoring should enhance the robustness of the removal process and enable instabilities to be appropriately managed.

From a practical point of view improvements can be made by tailoring the coagulation operation to the specific algae in the bloom. The findings above suggest better coagulation diagnostics are possible as a result of a better understanding of the coagulation process, which should lead to greater robustness in operation.

6.2 NOVEL TECHNOLOGY – POSIDAF

The key observations within this thesis with respect to PosiDAF were:

1. By treating algae using PosiDAF it was possible to achieve residual concentrations comparable with those achieved by conventional methods (Paper 7).
2. The use of a cationic chemical in the saturator was critical to provide a bubble surface with an affinity for algae cells (Paper 8).
3. An increase in chemical hydrophobicity improved the removal efficiency (Paper 7, 8).
4. Use of polymer improved the removal efficiency when compared to surfactant, suggesting that an increase in swept volume of the bubble is produced by projection of polymer chain from the bubble into solution (Paper (7, 9).
5. Algae character impacted on the removal efficiency of algae cells using PosiDAF
    a. When using surfactants, cell size as opposed to organic composition was the important characteristic (Paper 8).
    b. When using polyDADMAC, the organic composition as opposed to cell size was the important characteristic (Paper 9).

The above findings suggest that PosiDAF has potential as a treatment option. Considerations of the process suggest that there are several key advantages to implementation. However, there are challenges that need addressing to ensure that the process is a robust treatment alternative. The advantages of the process are focussed on removing the requirement for using coagulation upstream of the DAF unit. A detailed cost evaluation would include a great deal of uncertainty as the work has only been conducted at bench scale so far on algal suspensions of cultured mono species. However, based on the information provided in the thesis, a scoping assessment into the potential economic feasibility of PosiDAF is possible based on the information generated within this thesis. As such the following section examines the

application of PosiDAF using polyDADMAC, to illustrate the potential for saving with respect to changes in chemical consumption, sludge mass production and energy requirements. Costs were examined for *M. aeruginosa* and *C. vulgaris* at respective concentrations of 7.5×10^5 cells mL^{-1} and 9.2×10^5 cells mL^{-1}.

a) Chemical consumption. Using prices of £1.12 kg^{-1} of PolyDADAMAC and £0.08 kg^{-1} of alum (8 % as Al) (as supplied by water utilities), it was demonstrated when using PosiDAF for *M. aeruginosa*, that the chemical cost for a dose of 0.35 mg L^{-1} polyDADMAC (Paper 9) was £0.40 ML^{-1} while for conventional treatment, the cost using a dose of 10.3 mg L^{-1} as $Al_2(SO_4)_3.18H_2O$ (Paper 5) was £0.83 ML^{-1}. In contrast, the cost of conventional treatment chemicals was cheaper for *C. vulgaris* at £4.00 versus £6.16, due to the increased polymer dose that was required as a result of interactions with the AOM (Table 6.1). However, many WTW operate coagulation at a pH of approximately pH 6-7, using acid to lower the pH from the initial highly alkaline conditions generated during algae blooms. PolyDADMAC was shown to achieve good results at pH 7-9; hence, further savings could be incurred through a reduction in acid usage, although the specific savings are dependent on influent water chemistry, including alkalinity and hardness, and the pH at which a WTW chooses to operate coagulation.

b) Sludge mass production. The sludge resulting from the use of aluminium in conventional coagulation would be approximately 2.4 kg ML^{-1}, assuming that all aluminium is converted to aluminium hydroxide precipitate, $Al(OH_3)$. Whereas that of polyDADMAC for PosiDAF would be 0.35 kg ML^{-1}, nearly 7 times less than that of conventional treatment. The average cost of sludge treatment is £41 per tonne of dry solids (Parsons and Jefferson, 2006), leading to a saving of £0.09 ML^{-1} although treatment costs are known to vary considerably around the UK leading to a likely maximum saving of £0.70 ML^{-1}. There would be an average saving of £0.27 ML^{-1} incurred for sludge treatment of the *C. vulgaris* system; however this would not be sufficient to balance the additional cost required for polymer (Table 6.1). These calculations assume that the cost of treating polymer-algae sludge does not increase relative to that of the metal coagulant-algae sludge as a result of changes in

dewaterability. Insufficient information exists to quantify this at present but could prove important when conducting a more thorough whole life costing.

c) Energy requirements. For WTW that operate using flocculator tanks with energy powered impellors, as opposed to a baffled reactor, cost savings will be incurred through removing the need to flocculate. There may also be cost savings incurred through a reduction in the recycle ratio (R_r) of the dissolved air flotation (DAF) unit. Most WTW operate at R_r = 6-12 % (Markham *et al.*, 1997); however, when using polyDADMAC, good removal was achieved at R_r = 5 % (Paper 9), thus power savings could be incurred by lowering the amount of water recycled. Given that bubble generation currently accounts for approximately 50% of the total operating costs of a DAF plant (Parsons and Jefferson, 2006) the reduction in recycle ratio should generate a significant cost saving although such savings need to be confirmed at pilot as it is known that bench scale systems do not accurately predict recycle ratios in practice. It is suggested that polymer is dosed to the recycle line just prior to the inlet to the saturator with similar technology to that currently used to dose polyDADMAC at water works (Wetherill, 2007). Hence, there should be no significant additional cost incurred through polyDADMAC dosing compared to coagulant dosing.

Overall, the illustration indicates that in the case of *M. aeruginosa* a saving of £0.52 ML^{-1} (Table 6.1) could be achieved by using posiDAF in comparison to conventional DAF. The saving is generated by a 52 % saving in chemical cost and an 86 % saving in sludge cost. Such an analysis ignores potential additional savings due to reduction in energy as a result of lower recycle ratios and a lack of flocculation. Whilst, the analysis is an initial estimate it does indicate that, as well as being technologically feasible, PosiDAF is potentially cost effective making the technology a suitable candidate for further development. However, the comparison to *C. vulgaris*, where PosiDAF is more expensive than conventional DAF, indicates the importance of the interaction between polyDADMAC and AOM in determining the economic attractiveness of the process.

Table 6.1 *Example of approximate chemical consumption costs and sludge treatment costs for 7.5 × 10^5 cells mL^{-1} of M. aeruginosa and 9.2 × 10^5 cells mL^{-1} of C. vulgaris.*

	Conventional Treatment with Aluminium		PosiDAF Treatment with PolyDADMAC	
	M. aeruginosa	*C. vulgaris*	*M. aeruginosa*	*C. vulgaris*
Chemical Cost (£ ML^{-1})	0.83	4.00	0.40	6.16
Sludge Cost (£ ML^{-1})	0.1	0.5	0.014	0.23
Total Cost (£ ML^{-1})	0.93	4.5	0.41	6.39

In addition to cost savings, another driving force for implementing PosiDAF is to reduce the impact of changing algae character on treatment and thus improve process robustness. A barrier to implementation is therefore that polyDADMAC in particular was particularly sensitive to changing organic matter character. PosiDAF could therefore be subject to similar difficulties observed for conventional technologies. While the optimum dose could be controlled using similar charge based methods discussed for conventional treatment, the low removal efficiency observed for *A. formosa* (Paper 9) indicates that PosiDAF using polyDADMAC would only be suitable for algae with specific characteristics, such as high MW AOM. In contrast, PosiDAF that utilised highly hydrophobic surfactants such as CTAB was not sensitive to organic composition and all algae greater than ~10-15 µm were treatable using this chemical (Paper 8). However, micro-algae would not be sufficiently removed using surfactant based PosiDAF. Additionally, CTAB is more expensive at £9.95 kg^{-1} (as supplied by Macrofarm Chemicals Ltd., UK), leading to a chemical cost of £7.97 ML^{-1}, and furthermore, surfactants do not feature on the DWI list of approved chemicals (DWI, 2007).

Overall, the challenges to implementation are that surfactants are too expensive, not approved and inappropriate for removing micro-algae, while polyDADMAC is too sensitive to changing algae character to provide the process robustness that is desired. However, an understanding of the mechanisms by which chemically modified bubbles

can float algae cells has been acquired. Proper application of this knowledge could ensure that the aforementioned challenges to implementation are overcome. For example, a non-toxic chemical is required that is cationic in nature, highly adsorbant (similar to highly hydrophobic surfactants) and of a chemical structure that would increase the swept volume of the bubble (similar to the polyDADMAC). Novel chemicals that have all of these attributes, for example co-polymers, could be tested and DWI approval applied for. For instance, Lieske and Jaeger (1998) synthesised and characterised a co-polymer which incorporated both hydrophobic poly(ethylene glycol), as well as the more hydrophilic polyDADMAC, while still maintaining a surface activity similar to surfactants. It is therefore conceivable that a novel co-polymer, exhibiting surface tension vs polymer concentration plots comparable to OTAB and CTAB, as well as an increased swept volume by a polyDADMAC type chemical, could be purpose designed. It is suggested that by increasing the driving force for adsorption through the inclusion of a hydrophobic component, the impact of the polyDADMAC interaction AOM may be sufficiently reduced, as the neutralised polyDADAMAC containing co-polymer will still have an affinity for the bubble surface. In this way, removal of not only cells but also the AOM component of the system could be achieved. Commonly used polymers in water treatment are in the price range of £1.12 kg^{-1} (polyDADMAC) to £1.67 kg^{-1} (polymer LT25) indicating that the price of a co-polymer could be anticipated to be within this price range and thus would give comparable operational costs to those calculated for polyDADMAC. Indeed, it is likely that a newly developed polymer would need to be in this price range in order to provide an economic advantage to switching to PosiDAF

The PosiDAF process has the potential to be used not only in place of conventional DAF but also at the head of the works. Situated at the inlet pipe, a positively charged bubble curtain would float algae to the surface of the source water where it could be harvested. Application of modified bubbles in such a way would not have the aim of attaining 90-100 % removal efficiencies, but would simply act to reduce the overall concentration of influent algae to the works, allowing existing operations to operate as designed.

6.3 REFERENCES

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CHAPTER 7: CONCLUSIONS AND FURTHER WORK

7 CONCLUSIONS AND FUTURE WORK

7.1 CONCLUSIONS

The major conclusion is that the character of algae can be linked to treatment. A thorough understanding of the character can aid process optimisation by providing an indication of coagulant demand in addition to aiding novel process design.

Specific conclusions were as follows:

1. A review of the literature and historical data showed that algae character, specifically cell size, was shown to impact on treatment. Primarily, small, unicellular algae and motile algae were observed to pass through the unit operations, particularly filters, while large algae did not pass through filters but accumulated at the surface, altering their operation from one of depth filtration to surface filtration (Paper 1, 2). An increase in size was also approximately related to an increase in coagulant demand on a per cell basis (Paper 1). Overall, it was observed that algae should be monitored according to species and that more generic classifications would not provide informative data for process optimisation (Objective 1).

2. Key species demonstrated to be prevalent at WTW were diatoms, including *Asterionella*, *Melosira* and *Cyclotella/Stephanodiscus*, which dominated in spring and autumn; cyanobacteria, including *Microcystis*, *Aphanizomenon* and *Anabaena*, which dominated in late summer; green algae, including colonial green species such as *Scenedesmus* and *Sphaerocystis*, which dominated in summer; and flagellated species including *Rhodomonas* and *Chlamydomonas* which were present all year round (Paper 2) (Objective 1).

3. Comparisons of the coagulation and flocculation of algae with NOM and kaolin revealed that surface organic layers dominated the algae. Hence, AOM formed an important component of algae with respect to treatment, both increasing the coagulant demand and affecting the flocculation of the cell system (Paper 3) (Objective 2).

4. Characterisation of the AOM showed that the major differences of this dissolved component between species were related to molecular weight, hydrophobicity, protein:carbohydrate ratio and charge density (Paper 4). The charge of the system originated predominantly in the hydrophilic fraction as a result of acidic carbohydrates, while the hydrophobicity was not the result of humic/fulvic acids but of proteins. Both of the latter observations are in contrast to that reported for NOM systems indicating that relationships that hold between NOM character and treatability are not necessarily true for algae dominated systems (Objective 2).

5. When the correct coagulant dose had been applied, good removal at greater than 94 % was achieved by flotation for each cell system (Paper 5) (Objective 3).

6. Relationships were observed between algae character and coagulant demand. Increasing cell size was related to an increase in coagulant demand and corresponded well with literature review observations. A stronger relationship was observed between increasing charge density of algae systems and increasing coagulant dose, which takes into account the impact of the charge of the AOM. (Paper 5) (Objective 3).

7. Zeta potential monitoring could provide a valuable tool for ensuring the correct coagulant dose has been applied for algae systems (Paper 6) (Objective 3).

8. Chemical dosing to the saturator without pre-coagulation resulted in at least 60 % removal and as much 95 % removal of <10 μm, spherical cells depending on the chemical utilised providing the chemical was cationic in nature (Paper 7) (Objective 4).

9. Utilising cationic, highly hydrophobic surfactants in the PosiDAF process yielded removal efficiencies that could be theoretically predicted. This was attributed to the generation of positively charged sites on the bubble surface that improved the attachment efficiency such that when a bubble and cell collided, the collision led to successful attachment. The presence of AOM did not impact on removal efficiencies which were governed by the size of the cell (Paper 8) (Objective 5).

10. Utilising polyDADMAC, a cationic, hydrophilic polymer did not yield removal efficiencies that were theoretically predicted. In some instances, removal efficiencies were much higher than predicted as a result of the polymer extending from the bubble and interacting with cells at a greater distance. However, interference by AOM was observed when it had a relatively low molecular weight, high charge and hydrophilic character (Paper 9) (Objective 5).

11. The chemical utilised in the PosiDAF process should therefore be cationic – to ensure an attraction to the negative algae cell, have a hydrophobic component – to ensure the chemical tightly adsorbs to the bubble surface, as exhibited by the surfactants, and have a long chain hydrophilic component – to project into solution and increase the swept volume of the bubble, as exhibited by the polyDADMAC (Objective 5).

12. There is a potential for using the novel process PosiDAF for the treatment of algae.

7.2 FUTURE WORK

A number of areas for further research have been identified in the course of this thesis. These are detailed in turn below.

1. Relationships between both surface area and charge density of algae systems with the coagulant demand required for optimum removal need to be tested for mixed cultures and source water samples. Such testing would additionally need to be performed for linking coagulant dose to zeta potential. If the relationships still hold, a full-scale assessment would need to be performed prior to implementation of suggested algorithms which may alter from site to site depending on coagulation conditions and water conditions.

2. This research identified that algae floc growth, structure and strength was distinctly different from that of NOM and kaolin systems. Notably, indications were that they were more fragile on exposure to turbulent conditions and could take much longer to grow. Further research is required to assess the importance of these findings with respect to conventional treatment. Of key importance is determining firstly, whether current coagulation and flocculation regimes enable sufficient growth of algae flocs for clarification and secondly, whether algae flocs have sufficient strength to withstand turbulence imparted during dissolved air flotation.

3. Novel chemicals including co-polymers described in this thesis need to be trialled in the PosiDAF process to determine whether use of such chemical will overcome barriers to implementation incurred by changing algae character, specifically that of cell size and AOM.

4. The use of PosiDAF with mixed cultures, source water samples, and also with natural organic matter and clay based systems needs to be investigated.

5. Pilot and full-scale PosiDAF trials need to be completed in order to address practical and logistical aspects of implementation including process robustness, application of PosiDAF chemical to saturator, impact of chemical dosing on saturator performance, and manageability of sludge produced.

APPENDIX

Appendix 1 Growth phases for algae

APPENDIX 1: Growth Phases of Algae

Algal growth phases include in order the following growth phases: a lag phase, an exponential phase, a stationary phase and a decline phase. The exponential growth phase can be split further to show an unlimited growth phase followed by a linear growth phase. Growth phases are illustrated in Figure A.1.1 using the example of *Chlorella vulgaris*.

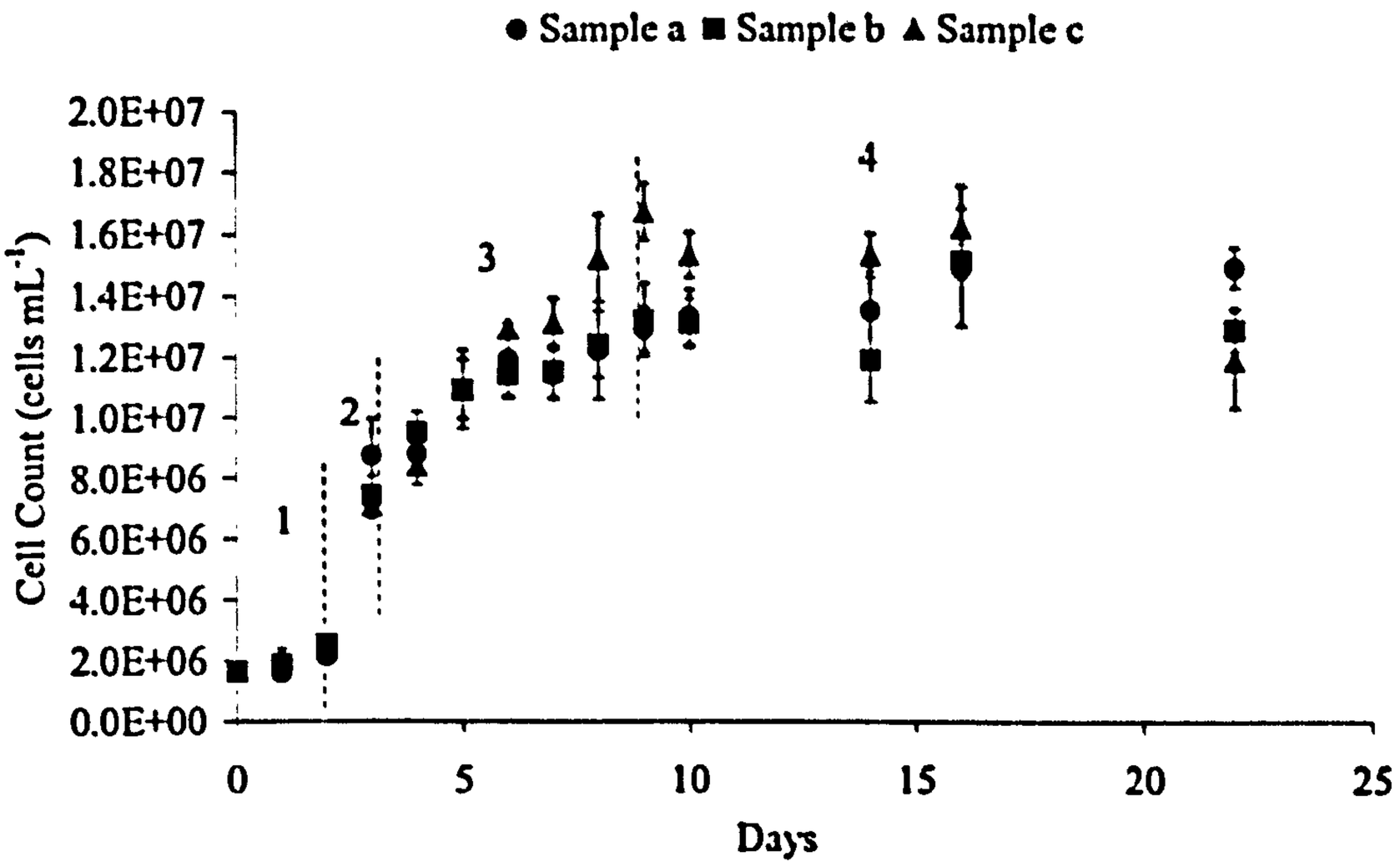

Figure A.1.1 *The growth phases of the algae, Chlorella vulgaris, where Zone: 1. Lag phase; 2. Unlimited growth; 3. Linear growth; 4. Stationary phase.*