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Feasibility of On-Farm Reduction of Nitrate Pollution in Subsurface Drainage Water

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This thesis is submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

I dedicate this thesis to my mother,

Rosemary Lorna Hughes

1934 - 1996

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Abstract

Nitrate polluted water from agricultural drainage systems is currently treated by the water industry for mains supply. Moves towards a polluter pays policy resulting from European Union legislation and consumer pressure will, however, have major implications for the agricultural industry. The aim of this study was to identify, investigate and evaluate on-farm strategies for reducing nitrate pollution in subsurface drainage water.

The high peaked nitrate rich discharges of drainflows from agricultural catchments in late autumn present significant management problems. One possibility which was examined is for polluting drainflows to be identified, intercepted and diverted into a holding pond or reservoir. Once stored, possible effective handling strategies include: long term storage, dilution, recirculation of the polluted water back onto the land, and anaerobic treatment. Anaerobic treatment is a method of reducing nitrate to harmless nitrogen gas, however, lower temperatures in late autumn would suppress microbial activity, and possibly treatment performance. Further detailed laboratory study was carried out to assess the potential of anaerobic treatment during winter.

Initially, the hypothesis that the nitrate concentration of drainage water could be reduced when applied to soil was tested, leading to the conclusion that it was only possible when a readily utilisable carbon source was continuously present. Glucose was added to water with a nitrate concentration of 100mg/l and applied to soil columns. Complete reduction of nitrate concentration was achieved at 10°C, demonstrating the feasibility of anaerobic treatment during winter. The study also confirmed the optimum application ratio of glucose-carbon to nitrate-nitrogen as 1.65 to 1, and the environmental threshold

as a redox potential of 200mV. Attached growth water treatment systems which utilise soluble carbon sources are, however, unsustainable because clogging of the porous media by microbial biomass results in hydraulic failure.

The hypothesis that organic materials be used both as carbon source and the microbial growth site was tested. Provisional examination of the biodegradability of several organic materials demonstrated that sugar beet could upon degradation be a source of readily utilisable carbon. Sugar beet was subsequently used in small-scale laboratory based nitrate reducing water treatment systems. An average treatment performance of 23 grams of nitrate-nitrogen reduced per cubic metre of bio-reactor per day was achieved by maintaining a near neutral pH environment with the addition of crushed limestone. Clogging was not experienced and therefore flow rate was both sustainable and controllable. An empirical based model was developed to predict the required flow rate of drainage water through the bio-reactor for a specified nitrate concentration reduction, ammonia concentration, and redox potential.

Examination of drainflow data enabled polluting drainflow volumes and their associated average nitrate concentrations to be quantified, to form the basis of a design specification for the proposed on-farm strategies. Designs for each strategy were made and limitations on use identified. Approximate costs were calculated and compared to the cost of on-farm anaerobic treatment utilising methanol as the carbon source. This demonstrated that treatment strategies offer a capital cost saving due to reduced design storage capacities, however, operating costs and the additional management expertise required make them less attractive to the farmer. Dilution has potential in areas where excess winter rainfall exceeds 200mm, however, the volume of water that can be diluted is limited. Recirculation requires further investigation, but has potential in areas of low excess winter rainfall and high soil moisture deficits, and where irrigation equipment is already available. Long term storage satisfies all the requirements for on-farm suitability, and would provide an additional environmental benefit of on-farm water conservation, at a cost 25% greater than that for off-farm water treatment alone.

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List of Abbreviations

APHA	American Public Health Association
ASAE	American Society of Agricultural Engineers
ASCE	American Society of Civil Engineers
BCPC	British Crop Protection Council
CAP	Common Agricultural Policy
CEC	Council of the European Community
EA	Environment Agency
EC	European Community
EEC	European Economic Community
EU	European Union
HMSO	Her Majesty's Stationery Office
MAC	Maximum Acceptable Concentration
MAFF	Ministry of Agriculture, Fisheries, and Food
NERC	Natural Environment Research Council
NFU	National Farmers Union
NRA	National Rivers Authority
NSA	Nitrate Sensitive Areas
OECD	Organisation for Economic Co-operation and Development
OFMAC	On-Farm Maximum Acceptable Concentration
WOAD	Welsh Office Agriculture Department
WRC	Water Research Centre

Chapter 1

Introduction : Philosophy of The Nitrate Problem

1.0 Introduction

The essence of the 'nitrate issue' is concerned with the increasing concentrations of nitrate in surface waters, groundwaters, lakes and marine environments (Heathwaite et al., 1993). Heathwaite et al. (1993) conclude that although much of the blame has been directed towards agricultural intensification as the source of nitrogen enrichment, increased nitrogen flux from the atmosphere to the terrestrial environment, together with an increase in the loading from human sources i.e., sewage treatment, are also implicated. This conclusion was reinforced by a report in the Financial Times in October 1993, that stated that rising levels of nitrate in water supplies are due primarily to natural changes in the soil rather than to the use of fertilisers by farmers (Maitland, 1993).

Agriculture, however, remains the main source of nitrate pollution in water, normally accounting for over 60% of total nitrate loss to water (Tunney, 1992). This fact combined with an increasing public awareness of the need for pollution control and the consequences of the Common Agricultural Policy (CAP) having adverse affects on the environment, has lead to the political will to regulate the farming community. Coincidentally, Fowden (1992) stressed the need for practical farming systems that minimised environmental pollution and degradation, to ensure sustainable agriculture.

The challenge is therefore to establish 'sustainable' systems whilst maintaining and increasing present levels of food production worldwide, as although in Europe there is excess production, the UN estimates the world's population will reach 10 billion by the year 2050 (Brown, 1994).

1.1 Background to the Problem

1.1.1 Nitrate Concentrations in UK Surface Waters

Croll and Hayes (1988) reported a rising trend in nitrate concentrations in UK waters. Croll (1990) later states that many water bodies in the South-East and East Anglia exceed the 50mg/l nitrate drinking water limit (Section 1.1.3), the peak concentration usually occurring in late autumn when the first run-off water reaches the river (Section 1.1.2). More recently, it was reported that most of the rivers in lowland England now have mean nitrate concentrations above 24mg/l nitrate (Johnes and Burt, 1993). This trend in rising nitrate concentrations can be seen in Figure 1.1.

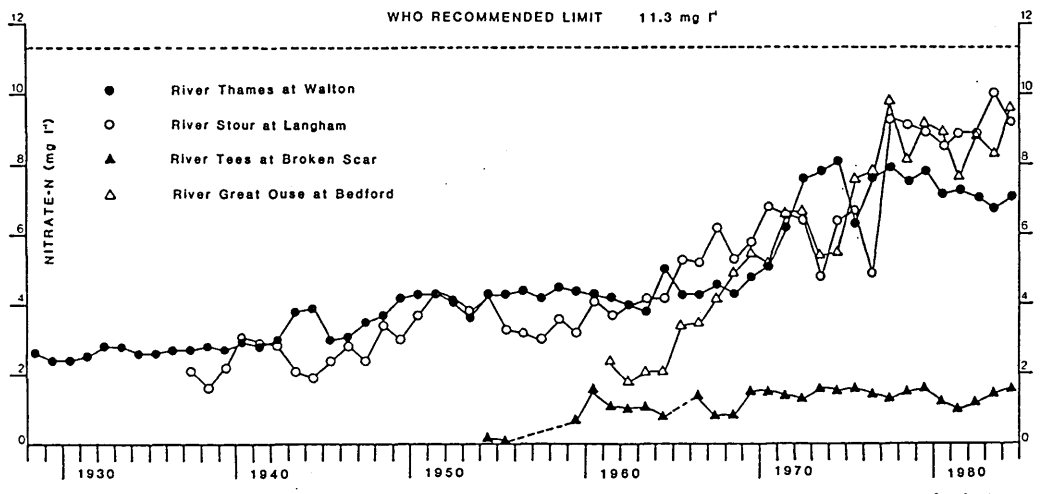

Figure 1.1 Long-Term Trends in Nitrate Concentrations in Four British Rivers (reproduced from Roberts and Marsh (1987) by kind permission of IAHS Press, IH Wallingford, Oxon, UK)

The average annual concentration of nitrate in a number of reservoirs has also been seen to increase. For example, in the Farmoor reservoir which draws its water from the River Thames near Oxford, nitrate concentrations have risen from 9mg/l in 1961 to 27mg/l in 1980 (Croll and Hayes, 1988). In 1995 reservoirs supplied from surface water sources in East Anglia saw large rises in nitrate levels because large abstractions had to be made in late autumn/winter following a drought in the previous winter/summer (Daldorph, 1996).

Recently, the increasing trend in nitrate concentrations has been seen to level out at some monitoring sites. The suggested reason for this is that the effect of the ploughing up of land is now diminishing (Section 1.1.2), however, historical records indicate previous periods in which the peak nitrate concentrations were seen to level out, only to be succeeded by further increases (Croll, 1990). This observation seems to be confirmed by the fact that nitrate concentrations in Anglian Water reservoirs, whilst below the EU limit of 11.3 mg/l NO_3-N, are still continuing to rise (Blantern, 1996).

ENDS (1994) reported that water quality, although improved since 1990, was still worse than it was in 1980, and well short of the objectives set at that time. There is also continued pressure to improve water quality from the European Union (EU)[1], which has stated that the '*application of the subsidiarity principle must not be allowed to lower the high level of existing standards*' (ENDS, 1993b).

There will therefore be an increased need to limit further rises in peak nitrate concentrations, and although the majority, if not all, are groundwater sources and hence beneath free draining catchments, the increase will determine policy which in turn will effect farmers who work artificially drained lands.

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1 Previously the European Community (EC)

1.1.2 Reasons for a Rise in Nitrate Concentrations

The main source of nitrate which has caused nitrate concentration increases in UK waters is agriculture (DOE, 1990b). The reason for the increase is the intensification of farming practices over the last 30 years, so enabling the level of agricultural production to meet market demands. Underlying this increased intensification was the policy of ploughing up existing grassland for use as arable cropland. This returned large quantities of organic matter to the soil, providing a source of organic nitrogen which upon degradation by micro-organisms formed nitrate. This process is called mineralisation, the controlling factors of which will be discussed in Chapter 2.

To bring more land into production, heavy soils i.e., soils containing a high clay content, were drained. Drainage whilst removing the risk of waterlogging and therefore crop stress, compounded the problem in two main ways. Primarily, the drains provide channels through which water containing dissolved nitrate can easily flow from the field into the water course. Secondly, reduced waterlogging allows for increased soil aeration and soil temperature, both of which enhance mineralisation, see Chapter 2. The influence of drainage was noted by Robinson and Armstrong (1988) who state that since it reflects the pattern of intensive farming, the intensity of drainage is broadly correlated with areas that have increasing nitrate concentrations in water supplies.

The characteristics of the catchment have a major influence on the shape of the nitrate concentration graph during the year. If the groundwater contribution to the river flow is substantial, the nitrate concentration peak in autumn will be less obvious. A catchment of predominantly clay soils, particularly where a high proportion contain land drains, will usually show a marked autumn peak concentration when the first substantial re-wetting occurs.

As farming became more productive due to developments in mechanisation and crop husbandry, it became more attractive to large investors. Consequently, land prices rose, and with them a need for consistently good yields of crop to repay investment. At the

same time the Common Agricultural Policy (CAP) of the EU assured farmers of a market and a consistent price for their produce. With this surety from the CAP, investors saw agriculture as an industry that guaranteed a return. The only requirement was that the land produced as much food as possible. This was achieved by increasing nitrogen fertiliser applications.

It is an over-simplification, however, to suggest that reduced nitrogen fertiliser applications will resolve the problem for three reasons. Firstly, it is now recognised that direct loss of nitrate from fertiliser is not generally a major source of the nitrate leaching from the land (Powlson, 1994; Addiscott et al., 1991), the major source being mineralisation of organic matter, a process enhanced by drainage practices and the cultivation of the soil, see Chapter 2. Secondly, if, as stated, nitrogen fertiliser is not the main contributor to the problem then cuts in its use will not reduce the problem immediately. Thirdly, if production levels fall then the effect on rural communities would be the loss of jobs and consequent rural decline, a policy that European politicians would be unlikely to advocate.

1.1.3 Water Legislation and European Union Directives

The EC Council Nitrate Directive concerning the protection of water from pollution by nitrates from agricultural sources was formally adopted by the Council of Environment Ministers, and published as EEC/91/676 (CEC, 1991). The document is recognised as determining the future control policy on nitrate loss from agricultural land (Archer, 1992), as it outlines the requirement of implementing action programmes to reduce nitrate pollution, see Chapter 2.

Concern for increasing nitrate concentration levels in water had previously lead to the EC Directive on the Quality of Water intended for Human Consumption (EEC/80/778) (CEC, 1980), in which the maximum acceptable level of nitrate in drinking water was set at 50mg/l (11.3mg/l nitrate-nitrogen). This compares to the World Health Organisation's (WHO) recommended limit of 100mg/l nitrate (22.6mg/l

nitrate-nitrogen). It is also generally recognised that current EU legislation will be extended to account for the ecological quality of surface waters (ENDS, 1993b).

Nitrate levels in many intensive agricultural areas had been increasing at an average rate of 2mg of nitrate per litre per year, threatening the quality and supply of drinking water. The Nitrate Directive was introduced to reduce and prevent water pollution caused by nitrates from agricultural sources, with a primary aim to reduce and prevent fresh surface or groundwaters from reaching a nitrate concentration of 50mg per litre (Tunney, 1992). This would consequently reduce and prevent problems of eutrophication caused by nitrates particularly in coastal and marine waters where nitrate becomes the limiting nutrient to algae growth.

The Code of Agricultural Practice for the Protection of Water (MAFF/WOAD, 1991) is a Statutory code under Section 116 of The Water Act (DOE, 1989). This means that if the code is not adhered to by the farmer, although not an offence, it could be taken into account in any legal action. Importantly, following the Code is not a defence for a farmer against a charge of causing pollution. This has important implications to farmers on clay catchments where nitrate leaching is as much a consequence of climatological factors as it is agronomic ones. The reasons for this are discussed in Chapter 3.

The 1991 EU Nitrate Directive requires a review of the action programmes be completed every four years. As part of the review, Member States are obliged to submit a report to the European Commission giving details of implementation of the Directive. The first report is due in 1996. If one of the conclusions made by the report is that present action programmes are not stringent enough, and that additional regulatory measures are required, it may have major implications for the agricultural industry, some of which are discussed below, see Section 1.2.2.

1.2 Need for an On-Farm Solution

1.2.1 Environmental Responsibility

In 1990 the government published a White Paper on Environment called *This Common Inheritance* (DOE, 1990a) in which Britain's future environmental strategy was outlined. The summary paper (DOE, 1990b) states that '*we have a moral duty to look after our planet*', and reiterates the government's aim to ensure that water is safe and clean, with controls over pollution being maintained and strengthened where necessary.

More recently (Anon, 1992a) there has been a call by the Royal Commission on Environmental Pollution to establish long term targets for returning lakes and rivers to their original unpolluted condition where an '*environmentally acceptable*' level of species diversity and amenity should be the aim.

The Water Act (DOE, 1989) had introduced legislation which required the agricultural industry to have regard to a statutory code of agricultural practices which minimised the risk of water pollution. With particular reference to the nitrate issue, the government's aim outlined in This Common Inheritance, is to reduce the amount of nitrate leaching from agricultural land. The White Paper (DOE, 1990a) concludes:-

> *'Industry, farmers and individuals all have a responsibility to make sure that we use all our water resources responsibly and cleanly and to look constantly for ways of reducing pollution that could damage them.'*

1.2.2 Economic Costs and Implications for Agriculture

The economic costs associated with the problem of nitrate pollution can be seen in two ways. Primarily, the cost of treating water supplies to reduce the nitrate concentration in drinking waters to below the European Union Directive level of 50mg/l nitrate. Secondly, is the cost of environmental remediation, for example prevention and cleaning of blue-green algae from reservoirs, the problem being a result of excess nitrate and other pollutants in watercourses and rivers.

In 1986 it was estimated by the Nitrate Co-ordination Group that the capital cost of maintaining the public water supply below the EC limit of 50mg/l would cost £199million over the next 20 years (DOE, 1986). In the Anglian Water region alone, the cost would be £70 million over the next 10 years (Croll and Hayes, 1988).

Other costs may not be so explicit. If, as is suggested by Powlson (1994) and Addiscott et al. (1991), nitrate pollution will continue whatever agronomic measures are taken, there are major implications for the agricultural industry. Nitrate pollution prevention measures would need to be so restricting as to make agricultural production unfeasible on some land. The consequences of this are immeasurable. On a local level it has implications on land values, rural employment, and rural economy. On a national level it would increase agricultural commodity prices, and consequently the British consumer would have to pay more for their food.

Indeed, the National River's Authority have explicitly stated, '*The simple fact is that nitrates will continue to rise in groundwater unless farming practice changes*' (NRA, 1994). If, however, nitrate pollution were controllable from some catchments, allowing food production yields to be maintained, those catchments would gain in importance as areas of agricultural practice. It is suggested that control measures can be enforced on artificially drained land, as the nitrate is concentrated into drain lines and trenches, from which it could be controlled and perhaps treated, see Chapter 3.

1.2.3 Polluter Pays

The water supply companies have introduced water blending and treatment programmes to help them comply with the drinking water requirement. Meeting the limit by blending high nitrate water with low nitrate water, or by chemically purifying it, is an additional expense that the water industry feels should be incurred by the polluter and not the user of the resource.

If, as suggested above, nitrate pollution levels continue to increase at the rate they are, then present pollution treatment methods e.g. blending, will be undermined. More complex, and consequently expensive treatment methods will therefore be required. This will place an additional cost burden upon the water industry, which with its privatisation in 1989 now has an increased emphasis placed on cost cutting. It is therefore suggested that the water industry may in the future look to the agricultural industry to pay for the pollution resulting from its activities.

Indeed, the Chairman of Wessex Water Plc insisted that the agricultural industry '*is responsible on the use of pesticides and responsible on all environmental issues*' (Hood, 1994). Whether that responsibility will be extended to paying for any consequences of environmental pollution has yet to be seen, especially whilst the water industry is required to spread sewage sludge on agricultural land.

It is evident that a cost is incurred for the resulting consequences of nitrate pollution. This cost is ultimately incurred by society as a whole be it presently through increased water costs, or perhaps in future through higher food costs due to a reduction in crop yields. As society has caused the pollution, the polluter must ultimately be every member of that society, and therefore we should either be prepared to pay the cost of maintaining our environment, or alternatively look at methods that can reduce nitrate pollution.

1.3 Summary

The Department of Environment (DOE, 1988) has stated:

> '*The problem of high nitrate levels is a difficult one to resolve both because of the need to take into account the interests of the water undertakers, farmers and consumers and because of the complexity of the problem*'.

Previously, it had recommended (DOE, 1986) that the:

> '*DOE and MAFF should continue to explore the practicability of protection policies together with water and farming interests*'.

How then do we both protect our water resource whilst satisfying the farming interests? Powlson (1994) suggests that although many of the principles underlying the nitrate problem are now understood, translating them into practical management systems that will decrease leaching to the extent necessary under current legislation presents a formidable challenge.

Development of novel techniques to both reduce the risk and quantity of nitrate pollution, whilst enabling farming practice to continue normally, would meet that challenge. This thesis outlines an approach to that challenge, describing detailed specific investigations, the results and conclusions of which are discussed with reference to the objectives of the study, and concludes by defining strategies based upon the findings of the study as a whole.

Chapter 2

Review of Nitrogen Pollution and On-Farm Control

2.0 Introduction

Nitrogen (N) is needed for photosynthesis which, in turn, is essential for crop growth. Differences between crops, and between amounts of N applied and taken up by crops, can result in varied N residues in soil after most arable crops have been harvested. Although these residues can provide some of the next crop's needs, a portion is normally leached (MAFF, 1993). It is this phenomenon that may lead to nitrate concentrations in water draining from agricultural lands exceeding an acceptable limit, and why an on-farm control method is desired.

2.1 Nitrogen Pollution and Prevention Policy

2.1.1 Nitrogen in Soils : Sources and Fate

In most soils, in excess of 90% of the nitrogen is present in organic forms (Vinten and Smith, 1993). This organic nitrogen is made up of a range of compounds, derived from biological materials e.g., roots, microflora, fauna, and leaf litter. However, the relatively small amount of nitrogen present in inorganic form, as ammonium and nitrate, is significantly greater in agricultural soils, especially those under intensive agricultural management (Addiscott et al., 1991; MAFF, 1993; Powlson, 1994).

The possible sources and losses of nitrogen were considered by Wild and Cameron (1980) who produced the following equation for the mineral N (NH_4^+ and NO_3) budget:

Inputs Outputs

$$N_a + N_f + N_m = N_{pl} + N_g + N_i + N_l + N_r + N_d$$

where the subscripts for N indicate:-

a	atmospheric deposition
f	fertiliser and manure
m	mineralisation
pl	plant uptake
g	gaseous loss by denitrification
i	immobilisation
l	leaching loss
r	runoff
d	increase in the soil

This equation reveals the complexity of the mineral N budget, with its importance upon nitrate leaching losses to surface waters being illustrated in Figure 2.1. Some elements of the budget are difficult to measure or predict, consequently research has been undertaken to improve understanding, and enable quantification. The result of these studies are summarised below.

2.1.1.1 Atmospheric Deposition

Rain contains nitrogen in the forms of ammonium and nitrate. Substantial inputs of N to the crop/soil system have also been observed from dry deposition of gases such as ammonia and oxides of nitrogen. It has been estimated (Goulding, 1990) that 50-60 kg N/ha enters cereal growing systems annually, and although these inputs are partly offset by gaseous losses, a net input of 40 kg N/ha will result.

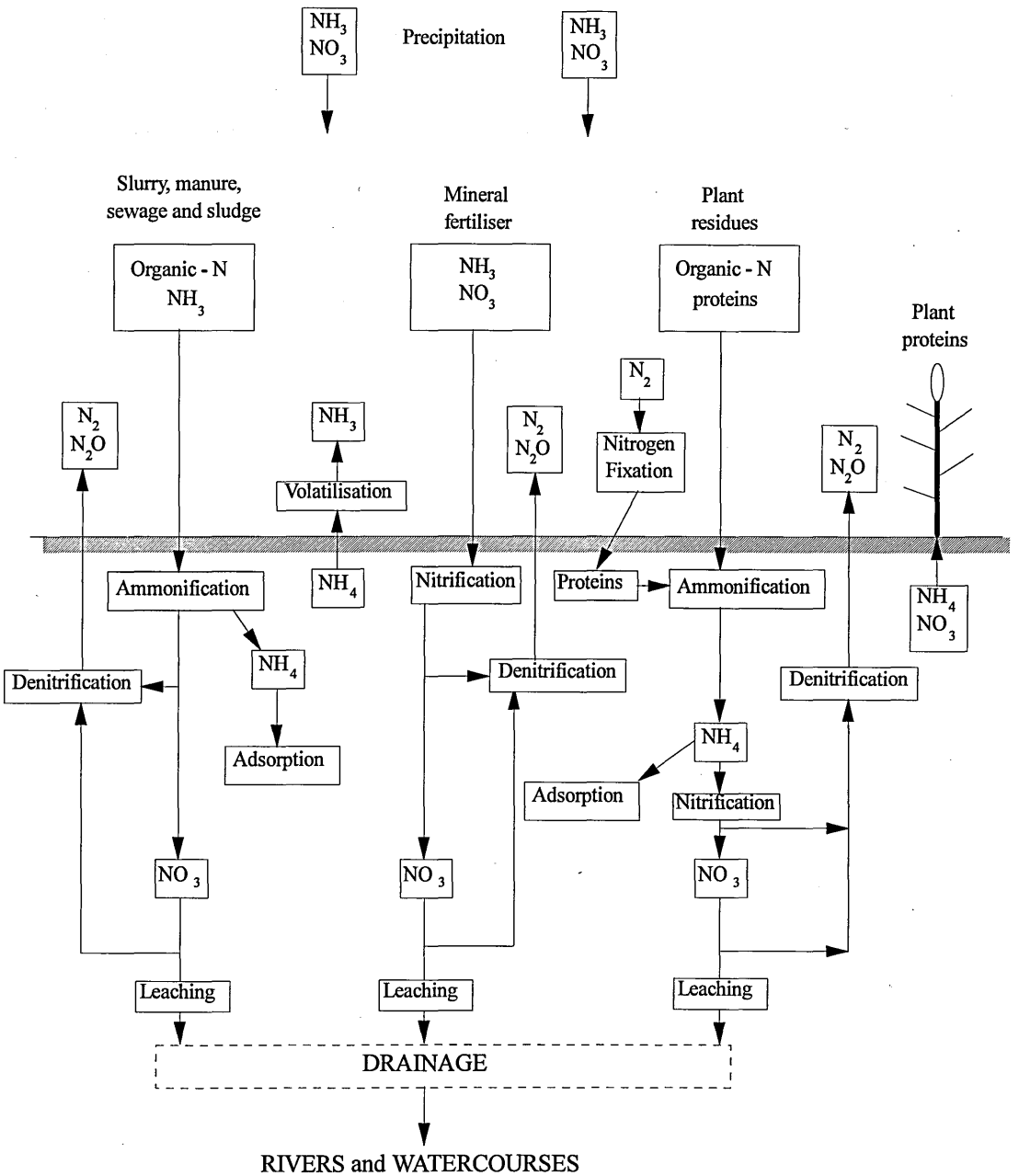

Figure 2.1 Sources and Pathways of Nitrate into Surface Water (adapted from Freeze and Cherry, 1979)

2.1.1.2 Mineralisation, Immobilisation, and Denitrification

A bacterial cell in soil can obtain nutrients from either organic sources such as amino acids, or from inorganic sources such as ammonium ions, but it must assimilate elements in a ratio approximately equal to that required for elemental composition of its own cell (Wood, 1989). For example, carbon and nitrogen must be assimilated in the ratio of approximately 5 : 1, and if a microbe is to utilise all of the carbon in a substrate such as straw with a high C : N ratio of 90 : 1, then it must obtain extra nitrogen from inorganic sources e.g. nitrate, to balance with, and satisfy, its own C : N ratio. The uptake and *assimilation* of inorganic nutrients by bacteria during the decomposition of organic materials is termed *immobilisation* (Wood, 1989).

During decomposition of organic material by micro-organisms some of the carbon is released as carbon dioxide (CO_2) by respiration; therefore as less of the carbon is assimilated, less nitrogen is required to meet the required C : N ratio (Wood, 1989). The excess nitrogen, relative to carbon in the substrate, is released into the soil in the form of ammonium (NH_4^+). The release of inorganic materials during the decomposition of organic materials is termed *mineralisation* (Wood, 1989).

The importance of both mineralisation and immobilisation upon nitrate levels in the soil can be illustrated using the example of decomposition of straw following incorporation. The high C : N ratio of straw (50 to 100 : 1) normally results in a short term net fall in the amount of plant-available nitrogen i.e., immobilisation. However, the long term effect of continued straw incorporation is greater mineralisation (Powlson et al., 1987). This is important because a microbial oxidation reaction converts ammonium to nitrate, the release of nitrate being termed *nitrification.*

Denitrification is a further important microbial process that occurs in soils, see Figure 2.1. Facultative anaerobic bacteria in the absence of oxygen use oxidised forms of nitrogen as alternative electron acceptors, those bacteria requiring specific reductase enzymes for each stage of nitrate reduction. Enzymes are either assimilatory i.e., cell

synthesis, or dissimilatory i.e., energy production. In this study it is important to differentiate between the two nitrate reduction processes as the biological treatment of water to remove nitrate is through dissimilatory nitrate reduction (see Section 2.2).

Dissimilatory nitrate reductase, the enzyme responsible for the first stage of nitrate reduction i.e., nitrate (NO_3) to nitrite (NO_2), is repressed in the prescence of oxygen and enhanced in the absence of oxygen. This mechanism allows oxygen to be used when available, also sparing NO_3 for assimilation (Wood, 1989). Dissimilatory nitrate reduction is particularly associated with the production of gaseous products nitrous oxide (N_2O), nitric oxide (NO), and nitrogen gas (N_2). The stages of dissimilatory nitrate reduction are as follows:

$$NO_3 \text{ --> } NO_2 \text{ --> } (NO) \text{ --> } N_2O \text{ --> } N_2$$

It is certain that nitrate reduction goes through the nitrite stage, but nitrite does not normally accumulate and the most commonly detected products are nitrous oxide and nitrogen gas. Nitric Oxide is rarely detected and is thought to remain enzyme bound during the reduction (Harris, 1988).

In soils, the extent and the rate of the above processes are affected by many factors, including soil temperature, moisture content, pH, and in particular, the C : N ratio of the organic material in the soil. An understanding of this final relationship is important for effective agricultural management of nitrate levels in soils.

2.1.1.3 Agricultural Management of Nitrogen

A major aspect of research has been to determine, and so limit, the effect of several agricultural management practices on nitrate losses. Those practices include inorganic fertiliser use, the application of organic manures, crop cover, cultivation practices, and grassland management.

Studies on clay soils at Brimstone Farm in Oxfordshire over a 14 year period have showed that significant leaching of nitrate in the spring period, after nitrate application, was rare, and by far the most nitrate leaching occurred during winter (Goss et al., 1993). Inorganic N fertiliser loss has also been quantified using ^{15}N-labelled fertiliser. This has enabled the relatively small amounts of fertiliser residue left in the soil to be measured, even against the background of the large amount of native soil N. The proportion not recovered in either crop or soil varied between years from 2% to over 35% (Powlson et al., 1992), and is dependent upon factors including soil type, crop management, and climate.

Crop cover is the main factor determining the quantity of the nitrate present in soil in autumn and early winter, the presence of one decreasing the amount vulnerable to leaching (Catt et al., 1992; Powlson, 1994). Early sowing may, however, be impractical and an alternative management practice would be to use a winter cover crop to provide a means of decreasing leaching by absorbing N. However, this is not always the case, especially if sowing in late autumn or if germination is delayed because of dry soil conditions.

The incorporation of cover crops has also raised the question of its mineralisation contributing to leaching losses, especially since the Brimstone Farm work also indicated increased nitrate leaching in the winter following cover crop incorporation (Catt et al., 1992).

In continuous arable cropping, both type and timing of cultivation have a significant effect on mineralisation and, hence nitrate leaching (Stokes et al., 1992; Goss et al., 1993). Work by Leeds-Harrison et al. (1992) concluded that careful management of structured, heavy clay soils might result in a reduction of nitrate pollution.

Addition of organic manures to either grassland or arable soil in autumn can greatly increase the amount of nitrate formed by mineralisation (Powlson, 1994). Measurements of nitrate in the profile of soils with a long history of organic manures

invariably show greater quantities than in soils given inorganic fertilisers (Glendining et al., 1992; Wadman and Neeteson, 1992; Chambers and Smith, 1992).

Ungrazed grassland is unlikely to result in substantial nitrate leaching (Addiscott et al., 1991); however, the ploughing up of grassland or grass/legume leys in autumn is recognised as leading to large leaching losses (Macdonald et al., 1989; Powlson, 1994).

2.1.2 Losses of Nitrate from Agricultural Land

Agriculture is the main source of nitrate in drinking water (NRA, 1992; Tunney, 1992; MAFF, 1993). The loss of nitrate from agricultural land is, however, a complicated process. Nitrate loss, or "leaching", will occur throughout a water catchment area. It is principally a "diffuse" source of pollution; however, on clay catchments where water draining from the land is collated at a drain outfall, pollution could be considered as "point-source" (see Section 3.2.1).

Nitrate concentration is influenced by, among other things, the amount of rainfall, or more specifically the amount of water passing through the soil during the winter. The problem is greatest in drier regions of the UK, whereas areas of high rainfall tend to have lower nitrate concentrations due to increased dilution (MAFF, 1993). Nitrate losses are, however, primarily dependent upon the type of farming system operated, see Chapter 3.

Archer (1992) stated that nitrate concentrations in water are equivalent to average annual losses of nitrate, and are directly related to excess winter rainfall. For example, if excess winter rainfall is 350mm, nitrate leaching of 40kg/ha of nitrate-nitrogen would produce nitrate concentrations in excess of the 11.3mg/l nitrate-nitrogen (50mg/l nitrate), the EU limit for drinking water.

In a catchment of predominantly clay soils, and where a high proportion contain land drains, a very marked autumn peak nitrate concentration is observed in rivers and

watercourses, when the first substantial re-wetting of soil occurs (MAFF, 1993). This means that any assessment of the effects of farming on nitrate concentrations in rivers and watercourses must take into account peak concentrations, rather than averages as is usually the case with groundwaters (Lord et al., 1993).

Significantly, the Brimstone Farm work (Harris et al., 1984; Harris et al., 1988; Goss et al., 1988a; Goss et al., 1993) has illustrated the unpredictable and ephemeral nature of nitrate losses from drained clay agricultural land. Importantly, it has also indicated that no agricultural management practice prescribed to minimise those losses can be said to prevent all losses. The inability to predict with any accuracy and/or control nitrate pollution, highlights the need for an alternative strategy for nitrate pollution prevention. The provision of an on-farm method of protecting rivers and watercourses from nitrate pollution events, some of which could well exceed acceptable nitrate concentrations, could form part of this strategy.

2.1.3 Pollution Prevention Policy

In 1991, the European Directive (EEC/91/676) to protect water from pollution by nitrates derived from agricultural sources was adopted (CEC, 1991). Its primary objective is to reduce and prevent fresh surface waters from reaching a nitrate concentration of 50 mg/l (Tunney, 1992), coincidentally reducing and preventing problems of eutrophication caused by nitrates.

In response to this requirement placed on member states of the European Union several courses of action have had to be implemented (Section 1.1.3). Initial reports (ENDS, 1993a) on the success of government initiatives have suggested that without reductions in the area of land under intensive agriculture, nitrate losses will not be significantly reduced. The implication is that land would need to be withdrawn from agricultural production, the effect of which will be to reduce its economic value. Compensation measures for loss of agricultural production have been provided, however, farms outside

designated Nitrate Sensitive Areas (NSA) receive no payments whilst still being subject to investigation and possible prosecution by water authorities.

Nitrate leaching is a natural process and consequently some loss each year is inevitable. The magnitude and timing of those losses are, however, unpredictable. If surface waters are to be protected, and maintenance of current agricultural production levels is desired, alternative solutions, other than a reduction of inputs and/or reduction of productive land, will need to be found. One such method could be the microbial reduction of nitrate to produce nitrogen gas, the control parameters for this phenomenon being outlined below.

2.2 Anaerobic Nitrate Reduction : Review of Control Parameters and Products

The form of nitrate reduction used in biological water treatment processes is called dissimilatory nitrate reduction or nitrate respiration, as nitrate (NO_3) is used instead of oxygen (O_2) as a terminal electron acceptor by respiring heterotrophic micro-organisms (Gauntlett and Craft, 1979). As stated in Section 2.1.1.2, the term dissimilatory nitrate reduction is used to differentiate it from the more general term of denitrification, commonly associated with nitrate reduction in soils, which encompasses all nitrogen transformation processes including assimilatory reduction of nitrate by organisms to form amino acids.

Environmental control parameters influencing nitrate reduction include the supply of metabolizable carbon which provides an energy source, the ratio of soluble carbon atoms to soluble nitrogen atoms (C : N), aeration, pH, and temperature. These control parameters not only control the rates of nitrate reduction they also control the nature of the gaseous products of nitrate respiration, in particular the ratio of nitrous oxide (N_2O) to nitrogen gas (N_2). The parameters are discussed below.

2.2.1 Aeration

Nitrate reduction is essentially an anaerobic process, which predominantly occurs only when no free oxygen is present in the environment. Low free oxygen levels are therefore a pre-requisite for nitrate reduction in a water treatment system.

Redox potential or Eh (electropotential) has been found to be an efficient and sensitive measurement for determining aeration status (Fluhler et al., 1976, Meek and Grass, 1975). In particular, nitrate reduction varies in response to aeration primarily due to variations in the redox potential. In general, dissimilatory nitrate reductase, the enzyme responsible for the first stage of nitrate reduction, is repressed in the prescence of oxygen and de-repressed in the absence of oxygen (Wood, 1989).

Sprent (1987) states that the Eh below which nitrate respiration, and hence reduction, occurs in soils is 400mV. However, it is suggested that this is only the figure for the start of nitrate reduction cascade i.e., NO_3 to NO_2 (Section 2.1.1.2), and that complete nitrate reduction from NO_3 to N_2 only readily occurs below 200mV (Patrick and Mahapatra, 1968).

The instability and irreproducibility of redox data, indicate that measurement of redox potentials down to the last millivolt in natural systems has little significance (Bohn,1971). Redox potentials should therefore be considered qualitative rather than quantitative measurements (Bohn, 1971), and hence could be used to interpret the aeration status of the environment. The apparatus required to monitor Eh is outlined in Chapter 4.

2.2.2 Temperature

The rate of microbial activity and subsequent nitrate reduction is directly influenced by ambient temperature. A minimum temperature for nitrate reduction in soils has been

widely reported with the range varying between -2°C (Dorland and Beauchamp, 1991) and 5°C (Standford et al., 1975; Paul and Clark, 1989).

Rates of nitrate reduction increase rapidly up to 25°C, followed by slower rises up to 65°C (Sprent, 1987); however temperature seems to have a greater effect in soils than aquatic systems (Knowles, 1982), possibly because temperature fluctuations in soils are greater (Sprent, 1987). Temperature has also been shown to influence the nitrous oxide/nitrogen production ratio, see Section 2.2.6 below.

For water treatment systems, the majority of temperature dependence work has been on sewage effluents using biological filters for attached growth, and activated sludge tanks for suspended growth systems (Gauntlett and Craft, 1979). For the purpose of this investigation, only attached growth systems are considered appropriate because they satisfy the principal requirements of the proposed on-farm treatment system (Section 3.1).

Gauntlett and Craft (1979) when investigating attached growth treatment systems suggest them to be less temperature dependent than suspended growth systems. For attached growth systems, they reported a doubling of nitrate reduction rates between 10°C and 20°C, confirming previous work (Sutton, 1975; Harremoes and Riemer, 1977) in which an approximate doubling of nitrate reduction for every 10°C temperature rise was observed. It has been suggested that below 10°C (St.Amant and McCarty, 1969; WRC, 1974) the performance of nitrate reducing treatment systems is seriously impaired.

Observations at lower temperatures coincided with an increase of carbon in the effluent (Gauntlett and Craft ,1979), which suggests a slowing of the metabolic rate at the lower temperatures. They conclude that the effect of temperature is sufficient to influence significantly the size of nitrate reduction unit required to perform a given duty. This last point identifies the need to investigate performance if parameters e.g. carbon source, are changed.

Dawson and Murphy (1972) investigated the temperature dependency of nitrate reduction in water treatment and found at 5°C the rate to be only 1/5 of the rate determined at 20°C. An important observation made by the authors was that following an initial acclimatisation period of 8 days at 5°C significant nitrate reduction rates were possible. The respective figure at 27°C was 5 hours. Within these acclimatisation periods approximately 25% of nitrate was reduced, followed by linear nitrate removal until nitrate became limiting i.e., exhausted. The difference in length of acclimatisation periods was partially explained by the inoculum being grown at 27°C, and consequently only a small fraction of the original inoculum probably being adaptable at 5°C.

Significantly, Dawson and Murphy (1972) found that at 5°C the microbial populations were between 2%-10% of those normally utilised in large scale plants. They suggest that this finding will make large scale microbial nitrate reduction at low temperature a viable option. It may also show that although fewer species of micro-organisms can adapt to low temperatures, those that do can readily reduce nitrate because the environment is non-competitive in that there is an over-supply of both carbon and nitrogen.

The importance of temperature as a control parameter is evident. Previous studies indicate that high ambient temperatures enhance nitrate reduction, however, it is the potential to reduce nitrate at the non-optimum temperature of 10°C which is an objective of this study.

2.2.3 Carbon Source

The role of nitrate in nitrate reduction is primarily that of a hydrogen acceptor for energy-yielding oxidative reactions of micro-organisms which are facultative in the sense that they are able to substitute nitrate or nitrite for oxygen as an acceptor of hydrogen (Delwiche and Bryan, 1976). It is therefore necessary that a form of organic material in the role of a hydrogen donor be present for nitrate reduction to occur (Finsen and Sampson, 1959).

Bremner and Shaw (1958b) found the rate of denitrification in soils to be dependent upon the amount and type of organic material present. Bremner and Shaw (1958a) had investigated soils amended with ground straw and glucose, and later Myers and McGarity (1971) used sucrose to instigate nitrate reduction. Couto et al. (1985) observed the effect of a seasonal water table on plateau soils, concluding that nitrate reduction was only observed in horizons where there was a carbon source that was readily utilisable by the microbial population.

Many carbon sources have been used in the treatment of nitrogenous waters. In wastewater treatment systems, the inherent organic carbon content of the sewage is utilised (Michael and Jewell, 1975; McCarty and Smith, 1986; Horan, 1989; Lamb et al., 1991), or supplemented to improve performance (Finsen and Sampson, 1959; Young and McCarty, 1967; Smith et al., 1972).

For treatment systems in which the main process is nitrate reduction, methanol predominates as the carbon source because of its cost, ease of use, and chemical simplicity. Its chemical simplicity enables a more efficient and rapid utilisation, optimising nitrate reduction and operating efficiency (Gauntlett and Craft, 1979; McCarty et al., 1969; Tamblyn and Sword, 1969; Sheffield, 1969). Alternative sources have included acetate, lactate, ethanol, molasses, and glucose which all produced similar performance efficiencies to methanol (McCarty et al., 1969). Skrinde and Bhagat (1982) investigated the use of industrial wastes as carbon sources including silage liquor, dairy whey, and brewery yeast concluding that they were suitable alternative carbon sources with comparable nitrate reduction efficiencies to methanol.

The efficiency of carbon utilisation is measured using the consumptive ratio, defined as the ratio of the quantity of carbon source required to accomplish nitrate reduction to the calculated stoichiometric quantity required. Lower alcohols such as methanol have a low consumptive ratio and so tend to be oxidised rather than synthesised into microbial cells (McCarty et al., 1969). It is for this reason that methanol has been promoted for use in attached growth water treatment systems where development of microbial

biomass is a major operational problem (Tamblyn and Sword, 1969; Sheffield, 1969; English et al., 1974; Gauntlett and Craft, 1979). Gauntlett and Craft (1979) also identified a major potential disadvantage of using methanol, that of possible toxic effects of residual methanol in treated water.

Alternative high energy sources are polysaccharides e.g. molasses and glucose which improve nitrate reduction performance in the following ways. They are significantly easier to metabolize than cellulosic forms of carbon e.g. straw, making them more readily utilisable by the microbial population. Secondly, they are soluble making it easier to achieve a uniform distribution of metabolic energy throughout the microbial population, the effect of which will be to optimise microbial activity and therefore nitrate reduction performance. At low temperatures, Jacobson and Alexander (1980) state that methanol did not stimulate microbial activity as well as glucose, suggesting glucose may be a more appropriate carbon source in low temperature studies.

Selecting an appropriate carbon source is an integral aspect of the design of a treatment system. The carbon source selected effects cost, management strategy, performance efficiency and capability. Consequently, identification of an appropriate carbon source for use in the present study is an objective.

2.2.4 Carbon to Nitrogen Ratio

The effect of carbon to nitrogen (C : N) ratio has been discussed previously in the context of microbial processes within the soil, in particular, mineralisation and immobilisation. As nitrate reduction within water treatment systems is accomplished by heterotrophic bacteria, i.e., bacteria that derive carbon from organic compounds, it is also a process strongly dependent on carbon availability.

The ratio of available carbon to nitrate-nitrogen is used as an important performance parameter in nitrate reducing water treatment systems. If the ratio is too high the microbial population may be unable to utilise all the available carbon, resulting in

carbon being lost as waste in the effluent. Conversely, if the C : N ratio is too low there is insufficient carbon available for utilisation by the micro-organisms and the environment is said to be carbon limiting, with the result that optimal nitrate reduction may not be achieved.

Early work investigated the effect of C : N ratio on dissimilatory nitrate reduction rates in soils. Bremner and Shaw (1958a) identified an optimum C : N ratio for as 3 : 1, in soils amended with glucose and finely ground straw. Bowman and Focht (1974) found C : N ratios around 2 gave the greatest rates of nitrate reduction, and high glucose concentrations appeared to inhibit nitrate reduction. For water treatment utilising methanol, an optimum C : N ratio of 2.6 : 1 at 20°C was determined using a bacterial disc system by Davies and Pretorius (1975).

Stochiometry i.e., the numerical proportions in which substances react with one another, allows the quantity of carbon required for nitrate reduction in water treatment systems to be estimated for a known concentration of nitrate and dissolved oxygen (which has to be removed to establish anaerobic conditions), and from consideration of the quantity of carbon required for microbial synthesis into biomass. McCarty et al. (1969) developed a theoretical equation for nitrate reduction using methanol as the carbon source (Section 6.7.1). The equation was used by several authors (St.Amant and McCarty, 1969; Tamblyn and Sword, 1969; Gauntlett and Craft, 1979) investigating nitrate reducing water treatment systems, and can be adapted for alternative carbon sources e.g. glucose.

Lowengart et al. (1993) stated the stoichiometry of nitrate reduction required a C : N of 1.5 : 1. However, when using straw the ratio of carbon metabolised and nitrogen immobilised for the production of microbial biomass was found to be 12 : 1. The finding indicates the reduced effectiveness of using organic cellulosic materials when compared to a soluble carbon source e.g. glucose. If organic materials were used, either a larger size of treatment zone would be needed to achieve the same rate of water treatment, or if the treatment zone was the same size, the time needed to treat the same volume of water would be longer, hence requiring a larger water storage capacity.

2.2.5 pH

Nitrate reduction is influenced by pH with the majority of work suggesting that for complete reduction of nitrate to nitrogen gas, the optimum pH is 7-8 (Bremner and Shaw, 1958b; Nommik, 1956; Wiljer and Delwiche, 1954). However, Sprent (1987) states that complete reduction of NO_3 to N_2 can also occur within the range of 3.5 to 11.

Acid values above a pH of 3.5 result in suppression of the reduction of N_2O to N_2 gas, and thus it can be said that pH affects the product of nitrate reduction rather than the process itself (Fillery, 1983). Cooper and Smith (1963) concluded that N_2O is a major factor in nitrate reduction in alkaline (pH 7.9) soils as well as acid (pH 6.1) soils, which suggests that the production of N_2O could be limited by maintaining a near neutral environment.

The complete reduction of $2NO_3$ to N_2 generates OH, which may cause environmental pH to rise (Sprent, 1987), and implies that the maintenance of an optimal pH range maybe a practical consideration for nitrate reducing water treatment systems.

2.2.6 Nitrous Oxide

Nitrous oxide is the first intermediary gaseous product following the reduction of nitrate (Payne, 1981). It is also an environmentally damaging substance due to its capacity to degrade atmospheric ozone (Betlach and Tiedje, 1981). The minimisation of N_2O production should therefore be a consideration when designing a nitrate reducing water treatment system.

Nitrous oxide is produced when its reduction is suppressed because of unfavourable environmental control parameters. The controlling parameters are oxygen availability (aeration), pH, and temperature (Focht, 1974; Delwiche and Bryan, 1976). Focht (1974) concludes that the percentage of N_2O with relation to N_2 is not greatly affected by temperature changes, and that aeration and pH are the parameters causing the greatest

variability in N_2O production. The influence of pH on N_2O production is discussed in Section 2.2.5, and the particular sensitivity of N_2O reduction to oxygen is reported by Betlach and Tiedje (1981). Indeed, the production of N_2O was used as a good indicator to differentiate the aeration conditions of soils (Fluhler et al., 1976).

Blackmer and Bremner (1978) suggested nitrate concentration as a fourth control parameter on the production of N_2O, with low concentrations delaying the reduction of N_2O to N_2. However, because the work investigated nitrate reduction in soil, the relevance of the finding for a water treatment system, in which nitrate is freely available, is open to question.

Myers and McGarity (1971) suggested that 10% of the nitrogen in the system observed was lost as N_2O. However, this seems conservative when compared with findings by Payne (1981) who suggested that even after longer incubation times of 8 days at 28°C, nearly 40% of gas evolved was N_2O.

Keeney et al. (1979) reported that soils incubated at 5°C and 15°C produced a higher proportion of gas as N_2O rather than N_2 when compared with soils incubated at 25°C, although total gas production declined at the lower temperatures. It is suggested however, that this may be attributable to a decrease in N_2O reduction, rather than an increase in its rate of formation.

From the above, aeration can be said to be the most significant controlling parameter on N_2O production. Focht (1974) suggests, if conditions were anaerobic, less N_2O would be found since its rate of conversion to N_2 increases faster than does its rate of formation. Consequently, maintenance of an anaerobic environment will ensure that complete nitrate reduction to N_2 gas will prevail.

2.2.7 Clogging

A major operational problem with attached growth water treatment systems is the build up of microbial biomass and the consequent clogging of the porous media (Cunningham et al., 1991; Eighmy et al., 1992; Hijnen and Van der Kooij, 1992). It is a phenomenon associated with the plugging of rock cores in petroleum reservoirs (Shaw et al., 1985), controlled drainage (Section 2.3.2), and also studies investigating the reduction of permeability in soils following prolonged submergence (Allison, 1947; McCalla, 1950; Johnson, 1957; Mitchell and Nevo, 1964; Avnimelech and Nevo, 1964; Wood and Bassett, 1975).

For soil, Allison (1947) showed a generalised infiltration rate curve as a function of many factors including bacterial growth. This finding was reaffirmed by McCalla (1950) who showed that sterile soils did not exhibit a loss in hydraulic conductivity with time, and demonstrated that when refrigerated i.e., when microbial activity was suppressed, permeability losses were a fraction of those found at room temperature.

A more direct approach to the problem of biological clogging was made by Mitchell and Nevo (1964) and Avnimelech and Nevo (1964). These authors found that an increase in the concentration of polysaccharide slimes, which are components of the microbial biomass, correlated with a decrease in hydraulic conductivity. Mitchell and Nevo (1964) also suggest that anaerobic bacteria are not as efficient in utilising dead biomass as aerobic bacteria, the resulting accumulation of biomass plugging the interstitial pore spaces.

It had been previously demonstrated that the addition of organic materials caused a decline in infiltration rates when a soil was kept flooded, but improved when soils were subject to a cyclical wetting and drying regime (Johnson, 1957). This finding and those of Mitchell and Nevo (1964) indicate that the action of aerobic bacteria could be used to remediate clogged systems.

In water treatment systems, microbial growth has been reduced by using methanol as the carbon source because its microbial utilization is stoichiometrically more efficient than other carbon sources e.g. glucose (McCarty et al.,1969). Eighmy et al., (1992) suggest the mechanical remediation technique of wet harrowing for sand filters employed in sewage treatment works, the advantage being it both cleans microbial biomass and encourages microbial growth to the depth of harrowing. The rate of clogging in sand filters was modelled by Hijnen and Van der Kooij (1992) following experiments using acetate as the carbon source. They conclude that biological clogging can be avoided for more than one year when the concentration of carbon in treated water is less than 0.01mg/l.

The problem of clogging can only be totally resolved by using indiscriminate biocides such as mercuric chloride. As this would also destroy the nitrate reducing microbial population, it cannot be considered an option. Since some level of biomass will always be present, it is necessary to manage attached growth systems. Possible management strategies include wetting and drying, wet harrowing, both discussed above, and optimising porous media design to maintain permeability.

Inert porous media design was investigated by Young and Dahab (1983), who showed the importance of media type, size, and shape on anaerobic treatment efficiency, and concluded that proper selection of media would affect reactor costs substantially. Porous media are investigated as a major part of this study.

2.3 Review of On-Farm Pollution Control Methods

2.3.1 Buffer Zones

Buffer zones, or riparian strips, have been promoted as an opportunity for protecting watercourses, whilst providing both environmental and amenity benefits (Haycock and Burt, 1992; Haycock, 1991). In passing from field to watercourse through the natural

environment that constitutes a riparian strip, nutrients are filtered by a combination of microbial processes and uptake by vegetation. Indeed, Cooper (1993) in his review of agriculturally derived surface water pollutants suggested that the loss of riparian zones has in fact accelerated agricultural pollution of watercourses.

Use of buffer zones for pollution control in agricultural catchments that are comprised of drained land will be restricted, as the presence of subsurface drains provides the major flow pathway through the riparian areas, hence by-passing the natural ecosystem and any pollutant removal processes. Muscott et al. (1993) discussed the problem, suggesting that on drained clay soils the effect on pollution would be minimal unless some additional measures were undertaken.

Investigations examining the processes and mechanisms within riparian zones has highlighted a natural phenomenon which has a significant effect on their nitrate reducing capabilities. Relic river or palaeo-channels were identified by Fustec et al. (1991) as able to remove nitrate from groundwater. Haycock (1991) observed rapid nitrate reduction in the first 2m of a 15m floodplain, further examination of which indicated a carbon-rich palaeo-channel. These floodplain sediments continued to retain nitrate in winter which suggests that high levels of nitrate reduction could be achieved when temperatures were low. Indeed, Burt and Haycock (1993) state that the maintenance of a subsurface environment within which denitrification rates can be optimised is the most essential factor effecting absorption of nitrate by riparian zones in winter, and not type of vegetation.

2.3.2 Controlled Drainage

Drainage has previously been identified (Section 2.1.2) as a contributory factor in the increased nitrate pollution of rivers and watercourses. Controlled drainage of agricultural land has therefore been suggested (Gilliam et al., 1979) as a method of pollution prevention.

Stopping drain flows reduces nitrate pollution in two ways. Primarily, it reduces drainage volume, hence preventing the transport of nitrate which has become dissolved in water percolating through the soil. Secondly, because water can no longer freely drain from the land, the watertable rises, the soil becomes saturated and anaerobic conditions soon promote microbial nitrate reduction (Section 2.2.1).

Gilliam et al. (1979) undertook field trials using watertable control to induce nitrate reduction in North Carolina Coastal plain soils. The trials indicated a reduction in nitrate being transported from the field; however, the work was done on sandy loam/loamy sand soils. This implies a greater ability to control the watertable than would be the case if the method were used on clay soils in the UK.

Several other disadvantages of the method arise when considering its application in the UK. The comparable temperature difference between soils in the UK and North Carolina during winter would effect the nitrate reducing capability of the method for two reasons. Primarily, the reduced temperature in UK soils would slow microbial activity, hence reducing microbial nitrate reduction. Secondly, carbon degradation in the soil would be slowed reducing the energy available for nitrate reducing microbes.

Controlled drainage would also effect crop management practices if used throughout winter, with the soil being allowed to drain in Spring. Both the rate at which the soil dries and consequently soil temperatures increase will be slower in clay soils than in sandy soils, effecting nutrient uptake by crops and therefore growth. Problems of access to the land by machinery will also be compounded due to the prolonged waterlogging of the soil. A further management problem associated with the use of this method is bio-fouling (Section 2.2.7).

2.3.3 Ponds

Ponds have been suggested as a means of nitrate pollution control by several authors (Hermann, 1962; Sheffield, 1969; Cooper and Knight, 1990). Two approaches to

reducing nitrate concentrations using ponds (reservoirs) were considered, microbial nitrate reduction and dilution or blending (Croll, 1990).

Significant reductions in nitrate were observed by Hermann (1962) where high nitrate wastes were treated in stabilization ponds. However, these waters contained a high organic load and the mean ambient temperature was 27°C. Sheffield (1969) proposed that the high nitrate water pass through a 4 stage system of algae and aquatic plant (hyacinth) ponds. Results indicated a 44% reduction in total-N, with an average ambient temperature of 20°C. The work also examined the addition of methanol to the hyacinth ponds as a supplementary carbon (energy) source, and indicated a resulting increase in nitrogen reduction rates.

Cooper and Knight (1990) discussed the nutrient trapping efficiency of small detention reservoirs, going on to conclude that they could be used for managing intensive agricultural runoff and downstream water quality. The nutrient trapping efficiency for nitrate-nitrogen was found to be 82% for a five year study period. This efficiency was a function of phytoplankton growth phenomena and seasonal temperature fluctuations, with biological activity being important in the warmer months. During periods of low temperature however, phytoplankton growth was slower and fewer nutrients were removed from the water column. It was also observed that excessive nutrient and sediment concentrations overloaded the pond, reducing its trapping efficiency. As the highest concentration of nitrate-nitrogen measured entering the pond was 2.19mg/l, a value significantly lower than those measured in UK clay catchments (Goss et al., 1993), the implication is that additional management strategies would need to be considered to ensure effective nitrate concentration reductions in winter.

Strategies considered included increasing pond capacity, so allowing storage of all runoff water, and hence dilution. Dilution, also termed blending, is presently used by water companies (Croll, 1990) where it is estimated 50% of input nitrate may be lost in reservoir, see Table 2.1. This though required a retention time of months, as it depended on both concentration dilution and microbial nitrate reduction at the

water-sediment interface. For on-farm use, due consideration of factors such as pond depth and area of water-sediment interface will be needed to optimise both storage capacity and anaerobic nitrate reduction.

Table 2.1 Nitrate Attenuation During Reservoir Storage (after Croll, 1990)

Reservoir	Residence Time (months)	Average Nitrate Concentration	
		Inflow (NO_3 mg/l)	Outflow (NO_3 mg/l)
Alton	10	45	12
Ardleigh	4	52	24
Covenham	10	43	22
Grafham	10	46	18
Pitsford	13	44	30
Rutland	29	44	16

Widespread use of a storage strategy depends on the availability of suitable sites, and an acceptance that there would be an economic cost resulting from the both the loss of productive land, and in constructing and maintaining a pond, especially if the pond capacity meant the farmer had to conform to mandatory planning regulations. This factor is also identified by Cooper (1993) who states that in-stream and reservoir techniques are limited because of economic realities. Similarly, the calculation of sufficient storage capacity to provide dilution may result in uneconomic or unfeasible designs, and over-design may result in costly maintenance of an under used structure.

Environmentalists are also asking farmers to re-construct ponds as they provide habitat for wildlife and flora (NRA, 1993). This pressure may lead to grants and subsidies in addition to already existing schemes e.g., set-a-side, to help reduce the economic disadvantages of pond construction. The option would have further support if it were perceived to provide additional protection to watercourses from nutrient pollution.

Water companies are presently encouraging farmer's in drought susceptible areas of the country to abstract and store water in winter for use in irrigation during summer (Naish, 1994). Such a scheme could be extended in areas where there is a high nitrate pollution risk, enabling development of an on-farm strategy to protect rivers and watercourses.

Evidence suggests that farm ponds would provide a means of on-farm pollution control; however many questions remain regarding feasibility with respect to potential to reduce nitrate levels in waters entering the pond in winter. Consequently it would seem prudent to investigate a more effective method of nitrate reduction which could be used in conjunction with ponds for an integrated pollution control strategy.

2.3.3.1 Use of Barley Straw Bales

The suggestion of placing barley straw bales into ponds, to which the drainage water containing nitrate concentrations exceeding acceptable levels is diverted, was considered.

Straw bales had previously been successfully used in reducing algal blooms in reservoirs and watercourses during summer. However, Welch et al. (1990) showed in their study that the presence of straw bales in the Chesterfield Canal reduced filamentous algae, but there was no evidence of significant reductions of major plant nutrients, including nitrate, the concentration of which was well above that suggested as growth limiting to algae. Further to this, Newman and Barrett (1993) suggested that algal control is a result of the production of antibiotics by the fungal flora, and the release of straw cell wall components during microbial decomposition. They also considered the possibility of in situ microbial production of antibiotics, although gave no evidence.

It was speculated that pesticide residues in the straw were responsible for killing off the algae; however, Blantern (1996) stated that recent studies have shown it to be phenolic substances resulting from the degradation of the straw.

2.3.4 Reed Beds

Reed beds have been used to treat wastewater by microbial activity (Bayes et al., 1989; Cooper et al., 1989). Nitrification of ammonia forms nitrate which subsequently is reduced to nitrogen gas through anaerobic microbial activity. Capacity to reduce nitrate is however negligible during winter when low ambient temperatures reduce microbial activity (Bayes et al., 1989).

Similar findings (Cooper et al., 1989) indicated that reed beds could significantly reduce the Biological Oxygen Demand (BOD) and the soluble solids (SS) of the wastewater, but reductions in ammonia, and by association nitrate, were negligible.

Reed beds promote aerobic environments by virtue of oxygen being passed from the atmosphere to the rhizosphere via the leaves and stems of the reeds through the hollow rhizomes and out through the roots. It is therefore suggested that an anaerobic environment required for nitrate reduction would not prevail if drainage water were applied to a reed bed because the lack of any nitrification would provide a surplus of oxygen in the environment.

2.3.5 Irrigation Management

Irrigation management has been suggested as a method of reducing nitrate concentrations in groundwater (Martin and Watts, 1982). Briefly, the method described uses pumped groundwater for irrigation, the groundwater having leached from the same agricultural land, and illustrates that when concentrations exceed 20mg/l , and under certain conditions, more nitrate could be extracted from the irrigation water than is leached to groundwater. In effect the re-cycling of the water provides a further chance for crop uptake of the nitrate.

Although there is evidence (Meek et al., 1968; Couto et al., 1985) to suggest that nitrate reduction does not occur at depth in the soil profile due to a lack of carbon (energy) for

the microbial population, it has been further suggested that water percolating through the soil transports soluble carbon as well as nitrate (Gilliam et al., 1978), enabling nitrate reduction to occur at the lower depths.

The extension of irrigation management as a nitrate pollution control method to drained clay soils in the UK is limited. The use of irrigation water suggests application at a time of soil moisture deficit i.e., high ambient temperatures and crop growth, with a consequent high crop uptake of nitrate.

During winter the effect of crop uptake would be negligible reducing the possibility of nitrate extraction from re-cycled drainage water. However, it is not sufficiently known if significant quantities of nitrate could be extracted from percolating drainage water by absorption, the potential for which was observed by Sitton (1991).

Early laboratory experiments (see Section 5.1) illustrated that significant reductions in nitrate concentration were not sustainable when high nitrate water (100mg/l NO_3) was passed through columns of soil. It was therefore concluded that without the amendment of a readily utilisable carbon (RUC) source, soil could not be used as a medium to support significant nitrate reduction.

2.3.6 Methanol Reactors

The use of methanol reactors for the treatment of high nitrate concentration waters has been discussed by many authors (Young and McCarty, 1967; McCarty et al., 1969; St.Amant and McCarty, 1969; Jeris and Owens, 1975; Michael and Jewell, 1975; Switzenbaum, 1983; Young and Dahab, 1983; McCarty and Smith, 1986; Richard, 1989; Horan, 1989; Rogalla et al., 1990; Henze and Harremoes, 1990), and the concept is extensively reviewed by Henze and Harremoes (1983). Possibly the most comprehensive study was by Gauntlett and Craft (1979) whose work investigated the major parameters (Section 2.2) that would effect the performance of methanol reactors under UK conditions.

The body of work provided major advances in the knowledge and understanding of microbiological nitrate reduction, with the method satisfactorily working at a broad range of temperatures and nitrate concentrations.

The work also indicated the limitations of the method for use as a low cost, minimum management, nitrate pollution prevention method. Investment in capital equipment, on-going maintenance costs, and the use of methanol as a carbon source, combined with the need for intensive management on behalf of the farmer, make the method less attractive to farmers with small farms. Of greater consideration, however, is whether farmers would require such a sophisticated method for use perhaps only once or twice a year i.e., when the nitrate concentration of drainage exceeds the water quality directive limit.

2.3.7 Biofilters

The use of soil to filter and adsorb nutrients has been discussed above (Section 2.3.5). The capacity to dispose liquid animal waste onto soil is limited as such systems soon become overloaded as they depend on soil fixing and vegetative removal. The method would also not work effectively over-winter, and because vegetative removal is limited in any one season large land areas would be required. This demonstrated to researchers a need to develop a cheap and efficient, all year round, method of liquid animal waste disposal which only requires small land areas.

Erickson et al. (1972) discussed such a novel method for the disposal of liquid animal waste. The method, termed the Barriered Landscape Water Renovation System (BLWRS), involves addition of liquid waste to a layered mound constructed above an impermeable layer. The liquid waste percolates through a thin layer of limestone which filters out phosphate, and percolates into an aerobic zone where nitrification (oxidation) of ammonium to nitrate occurs. The downward movement of the nitrified water continues to the impermeable layer where an anaerobic environment prevails, and if there is sufficient energy, nitrate is reduced by microbes to nitrogen gas. The authors

suggest that the anaerobic layer be amended with molasses, or other cheaper organic materials, if additional carbon is required. The method was shown to work at low temperatures, and only failed when the surface layer froze, preventing percolation of the liquid into the mound.

Use of the method for treating high nitrate drainage waters would have several problems. Of these, the most significant is the method's dependence upon the carbon content of the liquid animal waste providing energy for the microbial processes. Carbon could be added, but use of molasses would be an additional cost. Also, addition of readily utilisable carbon to the mound surface may promote microbial growth, which could cause the top layer of the mound to become clogged so preventing infiltration.

Lowengart et al. (1993) developed a biofilter for the treatment of wastewater's with a high level of ammonium. The system presented used a carbonaceous residue, wheat straw, as a substrate for microbial activity. Micro-organisms degrading such a substrate were expected to take up nitrogen from the water, and a possible sequence of nitrification and denitrification would also prevail. Even though temperatures ranged from 25-30°C, the work illustrated that immobilization of nitrogen was dominant, and nitrification (and subsequent nitrate reduction) only occurred following degradation of the straw to provide sufficient carbon.

The work of Bremner and Shaw (1958a) had previously illustrated the limited ability of straw to be used as a carbon substrate in microbial nitrate reduction because even with a high carbon to nitrogen ratio (25 : 1), the carbon was is in the form of cellulose and hence slow to degrade and release carbon to be utilised by nitrate reducing bacteria.

The work outlined has indicated that for successful nitrate reduction to occur in an on-farm treatment system, it is necessary for carbon to be in a form that is both available and readily utilisable by the microbial population.

2.3.8 Others

2.3.8.1 Groundwater - Underground Treatment

Methods of in-situ treatment of nitrate in groundwater have been proposed by Hiscock (1990), and reviewed by Dahab (1993), who states that the use of such technologies for treatment of nitrate have not gained widespread acceptance. The principal method described involves an addition of a carbon source e.g., methanol or ethanol, into the aquifer via a raw water supply well to stimulate nitrate reducing bacteria. Although the method has been shown to successfully reduce nitrate concentration in groundwater, aquifer plugging/well clogging (Section 2.2.7) is identified as a major operational constraint, and post-treatment filtration and disinfection is required.

2.3.8.2 Bacterial Disc System

An enclosed rotating disc-unit operating under anaerobic conditions and using methanol as a carbon source was investigated by Davies and Pretorius (1975) as a denitrifying system. The use of rotating discs was to overcome the problem of bio-fouling found in conventional filter systems, where the microbial biomass produced as a result of microbial nitrate reduction clogs the porous media used to support the microbial population. The system has several further design advantages over conventional methods, and successfully operated for a range of temperatures, although low temperatures i.e., less than 10°C, decreased nitrate reducing capability.

2.4 Identification of a Possible Solution

The above sections have identified several problems with the treatment of nitrogen in water including clogging, the provision of a carbon source, and low temperature capability. These problems need to be addressed before a proposed system for on-farm treatment of high nitrate drainage waters during winter could be considered.

An additional managerial aspect of the work is the desire that quantifiable nitrate reductions can be a feature of any design. Without this capability the system would be difficult to manage effectively, and so ensure drainage waters met EU water quality requirements.

2.4.1 Concept and Development of Research Aim

In-field zones of high nitrate reducing activity termed 'Hot Spots' have been investigated by several authors (Dowdell and Smith, 1974; Lefflaar, 1979; Smith, 1980; Jacobsen and Alexander, 1980; Sexstone et al., 1985; Parkin, 1987; Seech and Beauchamp, 1988; Christenson et al., 1990; Killham et al. 1993; Priesack and Kisser-Priesack, 1993). These zones are naturally occurring and spatially variable, and have been shown to occur inside large peds of soil.

If the necessary conditions needed to reproduce such zones of high microbial activity were imitated on a larger scale, it may be possible to apply high nitrate drainage water to a zone at the field periphery which would reduce the nitrate concentration before release into a river or watercourse.

The treatment system would incorporate a farm pond that collects and stores drain water, particularly after a storm when high drain flow rates would surpass the through-flow rate of the treatment zone. Ponding would allow greater control over the treatment process, as water would be metered into the treatment zone at a rate that achieved a nitrate concentration in the drainage water below the EU limit. Storage of water would enable a smaller treatment zone to be required, and therefore if a pond already exists and cropped land would not have to be used to construct one, would mean less land area being used for the treatment system.

There are two options as to the level of nitrate reduction required in the treatment zone. Either reduce nitrate concentration in the water to just below the acceptable level i.e., in the range 45-49mg/l, or reduce it to a lower value e.g. 20mg/l, at which point the water

could be used to dilute water with higher nitrate concentrations to below 50mg/l. Further consideration is given to this in Chapter 6.

It is proposed that the treatment zone be buried greater than 1.5m deep where the soil temperature is higher than at the surface. Keen (1931) reported that a soil temperature of 10°C is maintained at a depth of 1.5m over winter. This temperature would support microbial activity during winter when under normal ambient conditions it would be decreased, and nitrate reducing capability reduced.

In the water treatment system proposed the principal of attached growth (Horan, 1989) is utilised and developed. The microbial population usually grows upon an inert material e.g. sand, however in the proposed system the microbes would grow upon a carbonaceous substrate. The microbes degrade the organic material which results in the release of carbon, part of which would be assimilated by the microbes into biomass, and the remainder being available for utilisation by nitrate reducing microbes.

The review of literature has indicated that if anaerobic nitrate reduction is to be considered as a possible treatment option, the following general areas require further investigation:

i) The importance and provision of carbon, including the potential for using organic materials as carbon sources,
ii) The effect of low temperature on the performance of attached growth water treatment
iii) The problem of biological clogging in attached growth water treatment
iv) pH control in water treatment systems utilising organic materials as carbon sources.

In Chapter 3, possible on-farm pollution control methods are described that meet specific on-farm requirements. Once possible solutions have been identified, specific study objectives to meet the study aim are defined.

Chapter 3

On-Farm Nitrate Pollution Prevention : Possible Solutions and Project Objectives

3.0 Introduction

This chapter examines the particular requirements and potential solutions for an engineered on-farm control method for drained clay agricultural catchments. The possible advantages and disadvantages of each proposed solution are discussed, and the suitability of each one assessed. Finally, areas requiring original investigation are highlighted, from which the detailed objectives of the study are defined.

3.1 Requirements of the Proposed Solution

Any proposed solution should satisfy the following requirements:

i) Nitrate pollution control

ii) Low cost

iii) Minimum management

iv) Integration into farming system

v) Operable at low temperature.

3.1.1 Nitrate Pollution Control

Present thinking on nitrate pollution control has been dominated by an examination of the inputs to agricultural systems and the mechanisms that control nitrate leaching (Section 2.1). It is proposed that an engineering solution to the problem be sought based on the philosophy that the problem is one of point source rather than diffuse pollution, see Section 3.2.1 below.

An engineering solution would allow the farmer to control the quantity of nitrate pollution into rivers and watercourses. The benefit of this would be to give both environmental protection, and legal protection for the farmer from what could become a prosecutable offence in the future (Section 1.1.3).

3.1.2 Low Cost

Cost must be kept to a minimum and this will be achieved through reducing the amount of additional resources required to implement a solution. Resources are defined as capital expenditure, maintenance, and running costs which include managerial time and expertise.

Solutions are available (see Section 2.3), which require both high levels of capital expenditure and managerial expertise. It is recognised that any treatment method will require elements of both, but, it is an objective of this study to minimise these requirements.

To do this the farmer should be able to utilise as many of the resources already available on the farm. Such resources include land (e.g. set-a-side), soil, agricultural crops and their residues, and the farm drainage system, including any water storage capability.

3.1.3 Minimum Management

Linked to the capital cost of any automatic treatment control systems, will be the day-to-day running costs. Ideally, once established, the proposed solution should require a minimum amount of management and expertise to operate.

3.1.4 Integration into Farming System

Many agronomic methods have been tested to reduce nitrate pollution. The majority of the methods have, however, required some interference with on-going agricultural

practice, as have present nitrate reduction control methods. This is especially the case for drained land with the proposed use of buffer or riparian zones to protect watercourses (Section 2.3.1).

A requirement of the proposed solution is that it can be fully integrated into present farming practice, with a minimum of interference.

3.1.5 Operable at Low Temperature

Most of the water discharges from farms occur during winter time, when rainfall exceeds evapotranspiration and ambient temperatures are low. Any treatment system proposed must therefore be capable of operating at low temperatures. Soil temperatures at depth remain higher than ambient over the winter period; hence siting any microbial treatment method underground may offer potential benefits.

3.2 Potential for On-Farm Control Methods

3.2.1 Concept of Problem as Point Source Pollution

On clay agricultural catchments containing a high proportion of land drains, high autumn peak nitrate concentrations (up to 50mg/l NO_3-N) can occur in drainage water (Harris and Rose, 1992; MAFF, 1993). The drainage system channels the waterborne nitrate to a single point, that single point being either a ditch or tile drain outlet feeding into a river or watercourse. If the nitrate concentration of the drainage water is unacceptably high, it is possible to intercept and treat that water as point source pollution before discharge into a river or watercourse. On-farm treatment is therefore possible on clay catchments.

3.2.2 Catchment Classification

A hydrological land classification of England and Wales is available, namely the Hydrology of Soil Types (HOST) system (Boorman et al., 1991), to identify where on-farm control may be possible. This classification is based on a number of conceptual models that describe dominant pathways of water movement through the soil and, where appropriate, substrate, calibrated using catchment scale hydrological indices (Boorman and Hollis, 1990).

Cross referencing with maps that indicate climatological and agronomic data would enable drained agricultural catchments with point source nitrate discharge to be identified.

3.2.3 Instrumentation to Monitor Nitrate in Drainage Water

Interception of drain discharge water would only be necessary when nitrate concentrations exceed acceptable levels. Continual nitrate monitoring is therefore necessary to indicate when this problem arises. Until only a few years ago continuous monitoring of drainage water would have proved an impossible task. The development of solid state nitrate sensing Ion Specific Electrodes (ISE) now allows such a capability.

ISE's are now available as off the shelf technology (BPS, 1991). The solid state nature of the technology has meant the instrument has improved stability, making it robust enough for use under field conditions.

3.3 Possible Methods

With point discharge it should be possible to intercept and divert unsatisfactory drain discharges into a pond/reservoir for treatment, or temporary storage, so protecting rivers

and watercourses from nitrate pollution. There are three broad approaches to nitrate concentration reduction:

i) Pond Storage and Dilution

ii) Long Term Storage

iii) Anaerobic Water Treatment.

For any on-farm treatment system using water storage, only the water that exceeded the acceptable limit (Section 6.3) would need to be collected. To enable this, additional control structures and equipment may have to be incorporated into the system. Careful costing of each strategy therefore needs to be undertaken to determine the economical viability of each method.

3.3.1 Pond Storage and Dilution

An on-farm water storage facility would enable the drainage water, which contained unacceptably high nitrate concentrations, to be intercepted before it reached the river or watercourse. Such a provision could act as a "fail-safe" system that would ensure that no drainage water that exceeded the 'on-farm maximum acceptable level' (OFMAC) of nitrate entered a river or watercourse. This facility could be an existing farm pond, or new reservoir, the construction of which would be simplified by the impermeable nature of the clay soil.

Reservoir storage and dilution is a principal method by which water companies meet the Maximum Acceptable Concentration (MAC) required for drinking water. In the context of the water industry the process is termed 'blending' (Croll, 1990). Water containing unacceptably high nitrate concentrations, is blended with water containing low/negligible nitrate concentrations, producing water of an acceptable nitrate concentration that can be released into the environment.

Blending on the farm would be achieved by "bleeding" the high nitrate concentration water stored in the pond/reservoir back into the drainage system, where it would be diluted by drainage water containing low/negligible nitrate concentrations.

The main advantage of dilution is simplicity in terms of both understanding and implementation. Implementation though is only possible if both a pond/reservoir with a large enough capacity can be constructed and/or sufficient water is available for the necessary dilution. Ability to construct is dependent on both the availability and relief of the land, and hence may be a disadvantage of the method in certain circumstances.

Although a water storage facility is common to all the proposed on-farm methods, construction of a pond/reservoir is seen as a disadvantage to the pond dilution method in particular. This is because the size of the pond/reservoir would require land, the employment of professional consultants and contractors, and also the probable use of expensive construction materials. This would substantially increase costs. Such cost would have to be weighed against the cost of yield reductions due to agronomic restrictions, or possible fines resulting from water pollution incidents.

The political will to increase on-farm water storage capacity is now evident. It has been suggested by Naish (1994), President of the National Farmer's Union (NFU) that the storage of surplus water available in winter would ensure a more secure supply for other farming practices, whilst making better use of existing resources. Additional benefits would come from habitat creation, and wildlife and landscape conservation (Naish, 1994), and hence the government should encourage the practice. If a further benefit of pollution control was highlighted, interest in on-farm water storage would be enhanced.

3.3.2 Long Term Storage

When water is stored for a long time i.e., greater than 6 months, natural nitrate reduction occurs at the water-sediment interface. Cooper and Knight (1990) identified the environmental benefits of an on-farm water storage facility for nitrate reduction, and

impoundment of water has been recognised (Wyer and Kay, 1989; Croll, 1990) as one possible solution to the nitrate problem (Section 2.3.3).

3.3.3 Anaerobic Water Treatment

Dilution techniques do not reduce the total nitrate discharge, instead they largely manipulate concentrations. However, nitrate levels could be reduced if treated, and anaerobic treatment would seem to be the most promising method to achieving this. Treatment of the water would also assist in reducing the storage capacity needed, as the intercepted water would not need to be stored for longer than it took to treat.

Anaerobic treatment of waterborne nitrate by micro-organisms is not uncommon. Several methods have been discussed in Section 2.3, and a possible on-farm method incorporating the principles of previously tried methods is outlined below.

The environmental conditions necessary for anaerobic nitrate reduction can be summarised as follows. The micro-organisms must have a continuous supply of readily utilisable carbon (RUC) for energy, the oxidation-reduction potential (redox) needs to be maintained below 200mV, and the pH of the water maintained between 6 and 8. Temperature is also a key environmental control parameter, and although the optimum temperature of approximately 35°C is outside autumn/winter temperatures, adequate reduction may still be possible at lower temperatures.

Two options are available for anaerobic treatment:

i) Point Source Treatment

ii) Treatment Zones.

3.3.3.1 Point Source Treatment

Denitrification reactors have been extensively investigated for use by the water industry as a method for reducing waterborne nitrate concentration levels. Of those reviewed in Section 2.3.6, some authors have reported reactors designed specifically for on-farm use (Sheffield, 1969; Tamblyn and Sword, 1969).

The reactor(s) would be sited adjacent to a pond. Water would be pumped into the reactor from the pond, the flow rate being determined to reduce the nitrate concentration to below the On-Farm Maximum Acceptable Concentration (OFMAC).

The main advantage of a point source treatment method is the ability to control accurately the rate of nitrate reduction by controlling the carbon inputs. Improved control, however, results in increased expenditure on capital equipment. The more sophisticated treatment method also incurs high maintenance and running costs through the use of a soluble carbon source and intensive management. The high cost associated with this method is therefore its main disadvantage.

A further advantage of the method is that the land area required to site the point source treatment plant would be significantly less than that required for either of the dilution methods or the anaerobic treatment zone method discussed below.

3.3.3.2 Treatment Zones

Previous workers (Lefflaar, 1979; Smith, 1980; Myrold and Tiedje, 1985; Parkin, 1987; Seech and Beauchamp, 1988; Christensen et al., 1990) have identified, and in some cases attempted to model, soil zones of high nitrate reduction. These zones have been referred to by Parkin (1987) as 'hot-spots' (Section 2.3.7). Nitrate reduction zones on a spatially larger scale have also been observed by several workers (Fleischer et al., 1991; Fustec et al., 1991; Groffman et al., 1991; Haycock and Burt, 1992). Production of

in-field nitrate reduction zones would require the synthesis of the necessary environmental conditions outlined above and in more detail in Section 2.2.

It would be advantageous if the readily utilisable carbon required for nitrate reduction could be supplied from a degrading farm produced organic material, that would simultaneously act as a support media upon which the micro-organisms could grow. This concept is based on the principle outlined by Hobson and Robertson (1977) who stated that although organic material can supply carbohydrates for microbial growth, degradation is limited to only a number of bacteria. The remainder of the bacterial population depend on these few to provide sugars, the sugars being available because most of the polysaccharide degrading bacteria produce more sugar than they can metabolize themselves.

In previous work the support media has been in the form of an inert material e.g. coarse sand, and carbon was supplied from either a waterborne source e.g. sewage effluent, or added at the required rate using a water soluble carbon source e.g. methanol (Section 2.2.3).

The idea of simultaneously using the support media as the carbon source in the context of attached growth water treatment systems has recently been investigated (Lowengart et al., 1993). This study, however, was done at temperatures of 25-30°C, a factor that would have enhanced microbial activity, with a consequent increase in both organic material degradation and nitrate reduction. Less favourable environmental conditions would prevail on U.K. farms during winter and as such, performance and treatment capacities reported by Lowengart et al. (1993) are not transferable.

A schematic diagram of a possible on-farm solution is shown in Figure 3.1. Water leaving the field through drains would be intercepted and directed into a pond. From the pond, water would flow, under a natural hydraulic head, into the treatment zone containing a farm produced organic material, which simultaneously supplies energy and

bacterial working/growth sites. The treated water would then be discharged into the watercourse.

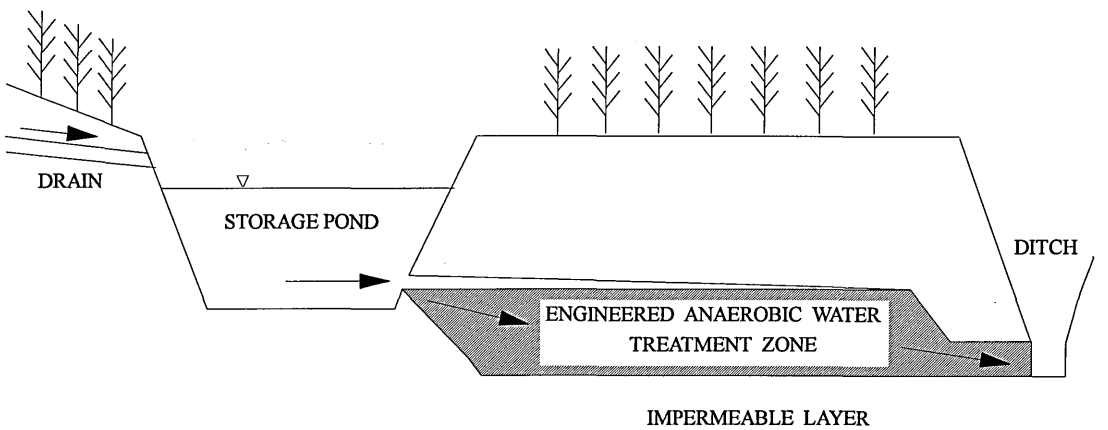

Figure 3.1 Schematic Diagram showing Possible Drain Outflow Diversion for Anaerobic Treatment of Nitrate Polluted Drainage Water

The main advantage of this approach is that it would satisfy most of the requirements for an on-farm solution (Section 3.1). The only requirement not fully satisfied is minimum management. The treatment zone method would be self-sustainable in terms of the system inputs, but due to the complexity of the anaerobic nitrate reduction process, the outputs would need to be regularly monitored to ensure that no environmentally damaging by-product was being released e.g. ammonia. Output monitoring is common to all treatment methods, and therefore overall, the proposed system would be better in terms of self-sustainabilty than other treatment methods.

The main disadvantage of this method is the requirement of land to construct the treatment zone. It is suggested, however, that the potential use of set-a-side land may reduce this as an obstacle. It is therefore the aim of this study to pursue the possibility of using treatment zones under U.K. conditions.

3.4 Definition of Study Objectives : Areas Requiring Original Investigation

The aim of this study was to investigate economic methods of treating drain discharges from agricultural catchments, to reduce unacceptably high nitrate concentrations, so protecting surface water quality. In Chapter 2 several areas were proposed as requiring investigation to ascertain the potential of anaerobic nitrate reduction as a possible treatment process (Section 2.4.1). In this chapter, treatment zones, which utilise the principal of anaerobic nitrate reduction, have been specifically proposed as a potential on-farm control method. In order to fulfil the aim of this study certain objectives therefore need to be met.

Figure 3.2 illustrates that the required reduction in nitrate concentration of the drainage water can be determined from figures for Excess Winter Rainfall, Catchment Area, and Nitrate Leaching Losses. Parameters requiring control and management in anaerobic nitrate reducing water treatment systems are highlighted in the centre of Figure 3.2, i.e., Aeration, Nitrous Oxide, Temperature, Carbon Source, Nitrogen Supply (C:N), Clogging, pH and Ammonia; the diagram also illustrating the relationship between controlling parameters (Section 2.2). Of these, the main control parameter in nitrate reduction is aeration status, as the process is an anaerobic one. Aeration status is also important as it determines the nature and quantity of the gaseous products of nitrate reduction (Section 2.1.1.2). In particular the ratio of Nitrous Oxide (N_2O) to Nitrogen (N_2) gas produced is important as N_2O is an environmental pollutant (Section 2.2.6), and any solution developed should ensure nitrogen gas production prevails. From the literature review a redox potential of 200mV was identified as the threshold below which nitrate reduction to nitrogen gas prevails (Section 2.2.1). Temperature (Section 2.2.2) affects microbial activity and therefore a figure of 10°C was chosen for experimentation to enable the feasibility of anaerobic nitrate reduction in winter to be assessed.

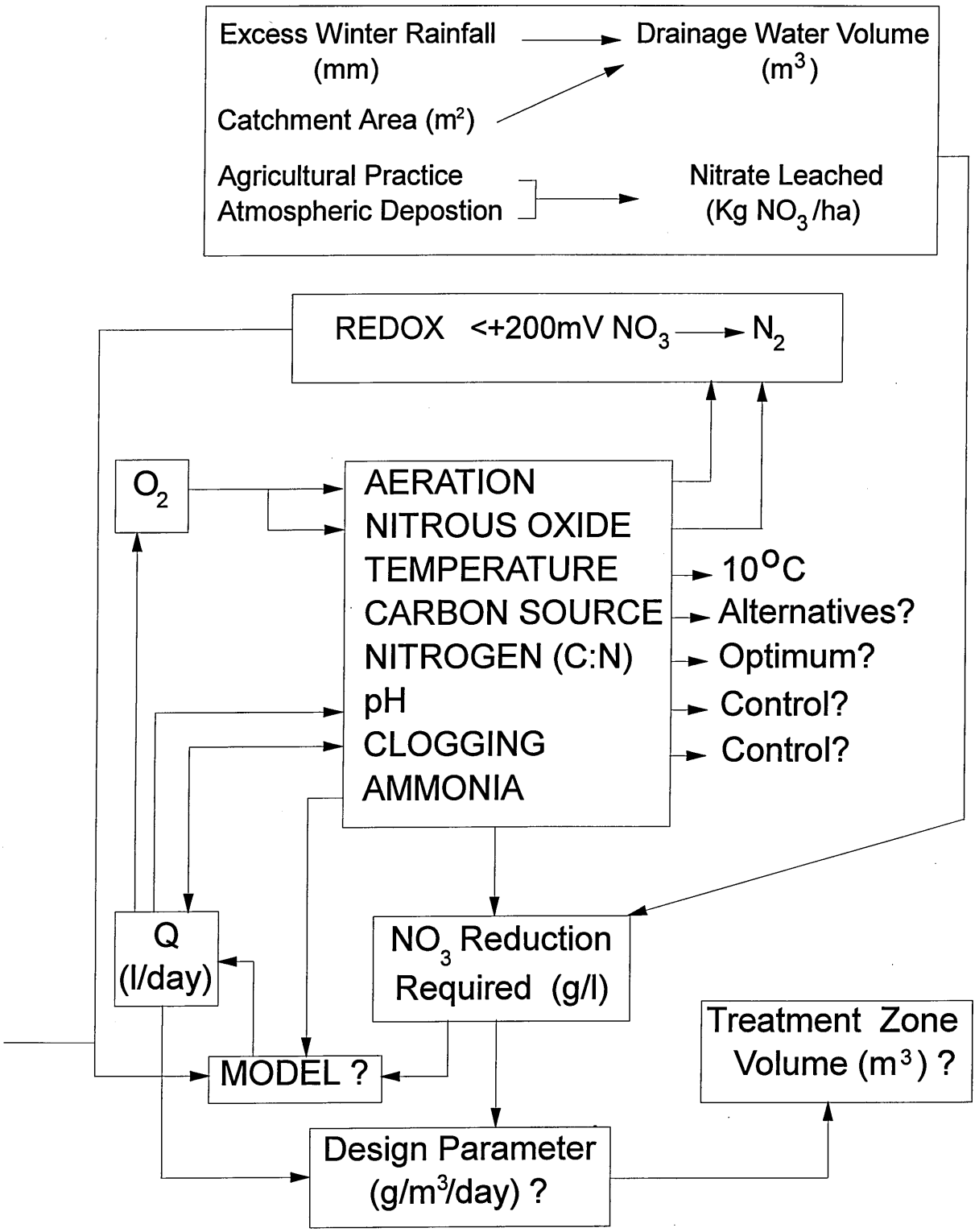

Figure 3.2 Conceptual Framework for Development of Project Objectives

Energy for microbial activity is obtained from carbon (Section 2.2.3). Current industrial carbon sources e.g. methanol, do not fulfil the requirements of on-farm solutions (Section 3.1), and therefore alternative carbon sources require investigation.

Polysaccharides are products of the microbial degradation of organic materials, a possible alternative source of carbon. Hence, the optimum ratio of carbon utilised to nitrate-nitrogen reduced (Section 2.2.4) in water treatment systems operating at low temperatures, which utilise polysaccharides as their carbon source, requires investigation.

If biologically degraded organic materials were to be utilised as carbon sources in water treatment systems, both pH and ammonia, an environmental pollutant, could be a problem (Section 2.2.5). Investigations with and without pH control would enable potential problems to be assessed. Clogging of the porous media as a result of microbial growth and the production of polysacchride slimes is a problem commonly associated with water treatment systems (Section 2.2.7). How the problem could be effectively managed and/or controlled also required investigation.

Aeration status is, however, the most critical control parameter, and as drainage water contains dissolved oxygen, anaerobic nitrate reduction would be controlled by the flow rate (Q) of water into the treatment system. It was therefore an objective of the study to identify a model that describes the relationship between nitrate reduction and flow rate.

A combined nitrate reduction and flow study would enable a design performance parameter to be ascertained for water treatment systems utilising organic materials as the carbon source. Using this design parameter and data on the magnitude of nitrate losses from agricultural catchments, the size, and therefore cost, of on-farm treatment zones could be determined. Figure 3.2 therefore indicates why investigation of nitrate reduction control parameters would enable the potential of treatment zones as on-farm control methods to be assessed. Such investigations have objectives which are now specified.

Objective I

In a pilot-study (Sitton, 1991) nitrate reduction was stimulated in soil by the addition of methanol and by waterlogging i.e., creating conditions suitable to allow the proliferation of the indigenous denitrifying microflora. Sitton (1991), however, also observed nitrate losses in waterlogged soils with no stimulation. It was therefore hypothesised that if water containing a high concentration of nitrate were recirculated through the soil, the soil would act as a nutrient sink in which nitrate concentrations would be reduced primarily by micro-biological activity. As the primary resources available to the farmer are land and soil, a solution which utilises as many on-farm readily available resources as possible is highly desirable. The first objective was therefore:

> To investigate the potential of soil to reduce nitrate without an additional carbon source, above the initial organic carbon content, and with respect to the quantity of inoculum used.

Objective II

Microbiological activity declines at low temperatures, and therefore both nitrate reducing performance and efficiency i.e., the optimum carbon to nitrogen ratio, may be effected. If anaerobic nitrate reduction is to be considered for use as a treatment in late autumn when nitrate leaching predominates investigation of nitrate reduction performance and efficiency at lower temperatures is required. This study would extend previous low temperature studies, e.g. Gauntlett and Craft (1979), who had found for attached growth water treatment systems that it was not possible to quantify the effect of temperature on nitrate reduction rates with any degree of certainty.

A temperature of 10°C was chosen for study, as it represents the average soil temperature at a 1.5 metre depth in a clay soil under grass, in South-East England, from November to December (Keen, 1931). A depth of 1.5 metres was chosen as a

reasonable, practical operating depth for the treatment zone method. Hence, the second study objective to be met was:

> To investigate nitrate reduction performance and efficiency at low temperature, with an additional carbon source added.

Objective III

Hobson and Robertson (1977) state that only a few out of many thousands of types of bacteria in the world can degrade plant polysaccharides to simple metabolic sugars. The other bacteria depend on these few to provide sugars on which they can live, and the sugars are available because most of the polysaccharide degrading bacteria produce more sugar than they can metabolize themselves. This phenomenon, i.e., more sugar (carbon) is produced than is utilised by the degrading bacteria, allows readily utilisable carbon to be made available for nitrate reducing micro-organisms.

Hence, it was hypothesised that degrading organic materials could be used to support a bacterial population that both reduces nitrate and degrades organic materials. Degradation rates of organic materials are reduced at lower temperatures, a phenomenon compounded by the anaerobic conditions which cause decomposition to be both less complete and slower (Jenkinson, 1981). The third objective was therefore:

> To investigate the biodegradation of organic materials at low temperature and under anaerobic conditions, to ascertain the viability of using organic materials as sources of readily utilisable carbon for microbial nitrate reduction during winter.

Objective IV

It was hypothesised that organic materials could be used as the carbon source in anaerobic nitrate reducing water treatment systems operating at low temperatures. Previous work, however, discussed in Section 2.3.7, highlighted an absence of data on nitrate reducing capacities at lower temperatures for anaerobic treatment systems incorporating organic materials. The fourth objective was therefore:

> To investigate nitrate reduction at low temperature using an organic material as the carbon source.

It was hypothesised that degradation of organic materials may affect environmental pH, and hence a study with and without pH control would establish the extent and influence of the phenomenon upon treatment performance.
It was hypothesised that the use of organic materials in water treatment systems would reduce the problem of bio-fouling common in industrial water treatment systems (Section 2.2.7). The studies designed to meet the fourth objective would allow this hypothesis to be tested.

Figure 3.2 indicates that nitrate reduction is controlled by several parameters, however, of these, aeration status is the most critical as the process is an anaerobic one. Aeration is controlled by the flow of water into the treatment system because drainage water contains dissolved oxygen. Hence, a model describing the relationship between flow rate, nitrate reduction, ammonia, and redox could be developed from the experimental data, so providing a management tool for decision making. If pH control was not required i.e., it was an independent variable, then pH would also be incorporated into the model.

An investigation of economic methods of treating nitrate polluted drain discharges would require the design and costing of possible solutions and a comparison with present water industry treatment costs. From the experimental investigations a nitrate

reduction performance parameter would be identified to enable the design of an on-farm treatment zone. However, to design a treatment zone a specification is required. Quantification of both nitrate leaching losses and drainage water volumes requiring treatment, would therefore be required.

3.4.1 Summary

The study objectives are to:

i) Investigate the potential of soil to reduce nitrate without an additional carbon source, with respect to the quantity of inoculum used, and initial organic carbon content.

ii) Investigate nitrate reduction performance and efficiency at low temperature, with an additional carbon source added.

iii) Investigate the biodegradation of organic materials at low temperature and under anaerobic conditions, to ascertain the viability of using organic materials as sources of readily utilisable carbon for microbial nitrate reduction during winter.

iv) Investigate nitrate reduction at low temperature using an organic material as the carbon source.

Experimental studies to meet these objectives are described in Chapter 4.

Chapter 4

Experimental Method Development and Instrumentation

4.0 Introduction

The first section of this chapter describes the methodology and development of the laboratory experiments, with the importance of each to a specific project objective being highlighted. The second section gives a detailed description of instrumentation used for measurement and analysis in the experiments.

It is important to note that the treatments tried within each study were selected on the basis of results from the previous one(s). The work schedule was designed in this manner because aspects of each study had to be shown to be feasible before the next study could proceed. As stated in Chapter 3, the parameters that have been highlighted for investigation include, carbon supply/source, low temperature, biological clogging, and pH.

4.1 Method Development

The following studies were made:

i) Soil Study
ii) Low Temperature Study
iii) Biodegradation of Organic Materials Study
iv) Combined Organic Material and Low Temperature Study.

4.1.1 Soil Study

The objective of this study was to investigate the potential of soil to support anaerobic nitrate reduction without an additional carbon source, relative to the quantity of inoculum used and the initial organic carbon content of the medium.

This objective specifically examines the potential of micro-organisms to reduce the nitrate concentration of water flowing through the soil, that potential being a result of the residual carbon content, and inherent microbial population of the soil. As nitrate reduction is also dependent on temperature, redox and pH, this study was seen as a pilot one in which the only factor being assessed was that of carbon. To this end the other primary factors were maintained constant to ensure that they were not significant in influencing nitrate reduction levels. If satisfactory levels of nitrate reduction were obtained, and those levels were sustainable, then nitrate reduction would be investigated at low temperatures to obtain data to develop a management model.

Previous work by Sitton (1991) indicated that soil be mixed with sand, in a ratio of two parts sand to one part soil, to overcome infiltration and vertical flow problems found when using soil cores that contained a small clay fraction. Sitton (1991) accepted that although this was undesirable in terms of accurately representing the natural soil system, it was nevertheless unavoidable.

This study assessed the sustainability of observed nitrate reduction levels, and the influence of varying sand to soil ratios on the hypothetical sustainable nitrate reduction levels. The soil used in the experiment was also the primary source of bacterial inoculant, assuming that the sand only supported a negligible bacterial population. If variable nitrate reduction levels were observed between the different sand to soil ratios investigated, then as variable amounts of inoculum had been incorporated, bacterial plate counts would be required to gauge the significance of this variable.

If nitrate reduction was found to be significant, a further objective would be to examine the influence of aggregate size on nitrate reduction, as the size of an aggregate would determine the extent of anaerobic environment formed within the aggregate. Once completed, methods of mechanisation to produce the soil grade required to obtain optimal nitrate reduction, could be assessed and if necessary developed. Similarly, methods of mixing the soil with sand to produce the required ratio would need further investigation.

4.1.1.1 Materials and Method

The apparatus consisted of soil columns made of translucent perspex plastic. The design of the columns was based on that of the permeameter cell used in soil hydraulic conductivity tests. To aid disassembly and cleaning, each cell was constructed in three main parts, a base plate, a column section, and a top plate. The three sections were held together using four vertical steel rods that linked the base plate to the top plate. On tightening with wing-nuts the three sections compressed together, the assembly being made watertight by using rubber O-ring seals placed between the mating surfaces.

Figure 4.1 illustrates schematically the construction and final assembly of the permeameter cells used in the experiment. Each cell was 250mm in length with a 94mm inside diameter. Three 10mm holes were drilled down the column's length at intervals of 65mm. A drilled rubber grommet, through which a platinum electrode (Section 4.2.2) had been inserted, was placed in each hole forming a watertight seal. In the top plate a 20mm diameter hole was drilled in which a drilled rubber grommet was placed, and through which an Ag/AgCl/KCl reference electrode (Section 4.2.2) could be inserted.

At the bottom of each cell was a chamber into which the leached solution could collect, aiding water movement to an outlet tap. The chamber had 2mm holes drilled in the upper surface over which a filter was placed. The filter consisted of a layer of fine fibre glass matting and a layer of metal gauze with a hole diameter of <0.5mm. The filter

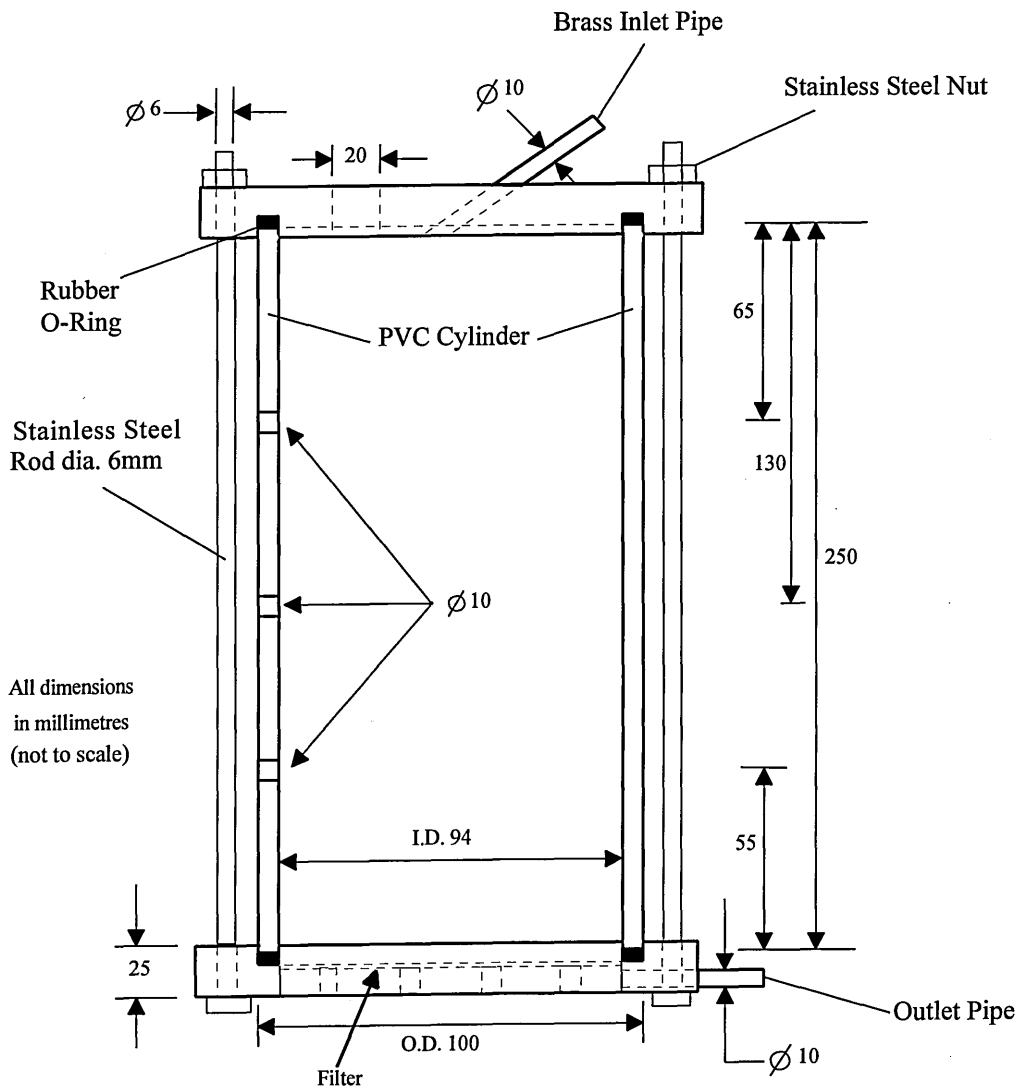

Figure 4.1 Schematic Diagram of Permeameter Cells Used in Laboratory Studies (N.B. Redox Measurement Apparatus Not Shown)

prevented soil fines, washed out of the cell by constant leaching, from blocking the chamber holes and/or outlet tap, and also reduced effluent discolouration.

A soil/sand mixture was used to simulate a soil profile and the required mixture ratio was produced by measuring out a known mass of sandy loam soil, to which was added a known mass of coarse 16/30 (1-0.5mm) washed and graded Lower Greensand sand. As the mixture was added to the cell, it was tamped down several times to ensure an even density of packing. Each cell was filled with the specified soil/sand mixture. A freeboard of 10-20mm was left to ensure that the influent could enter and disperse uniformly at the top of the column. Four ratios of sand to soil were tested, four to one, three to one, two to one, and one to one.

The sandy loam soil used in the pilot study was ground and sieved to eliminate aggregates. A full analysis of the sandy loam soil used is given in Appendix I. The readily available organic matter content of the soil assessed on a basis of a dichromate titration was 3.17% (British Standard, 1961).

A standard nitrate solution was prepared for use as the influent solution using the stock chemical potassium nitrate (KNO_3). The quantity of KNO_3 required to produce a nitrate concentration of 100mg/1 was calculated as follows:

Molecular weight of KNO_3 = 101.10

Molecular weight of NO_3 = 62.01

$$\frac{101.1}{62.01} = 1.63$$

Hence, 163mg KNO_3 added to 1l distilled water will give a nitrate concentration of 100mg/l, and is equivalent to a nitrate-nitrogen (NO_3-N) concentration of 22.6mg/l.

The influent solution was supplied to the top of the cell from a constant head apparatus via plastic tubing. The constant head apparatus in turn was fed from a 25 litre reservoir. This arrangement ensured that all cells under test were held under a constant head whatever the level of solution in the reservoir. Any observed variation in flow would therefore be a result of hydraulic conductivity changes and not hydraulic head. The apparatus assembly can be seen in Figure 4.2.

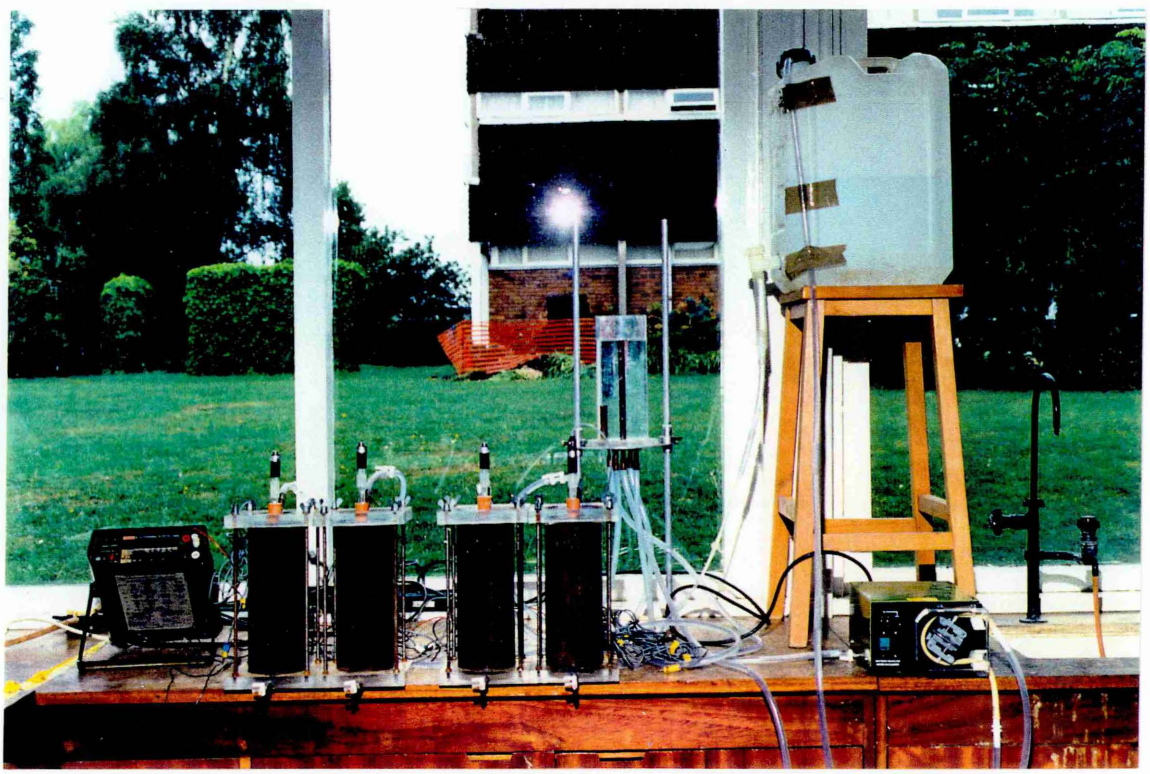

Figure 4.2 Experimental Apparatus Assembly Used in Soil and Low Temperature Laboratory Studies

Prior to the nitrate solution being introduced to the cell, the sand/soil mixture was purged of air by wetting up from below. Initially, the solution was held in the cell for a period of 72 hours to ensure that any free O_2 in the sand/soil was utilised by aerobic bacteria in order to create the necessary anaerobic conditions in which nitrate reduction would prevail.

Following the 72 hour establishment period the outlet tap was opened to allow leaching. The flow rate of leached solution was adjusted to ensure that the redox (Eh) values produced were below the 200mV nitrate reduction threshold (Section 2.2.1). Then every 24 hours, for a period of 7 days, a sample of the leached solution was collected from each cell for analysis.

4.1.2 Low Temperature Study

This study was carried out with an additional carbon source since the soil study showed that significant nitrate reduction was neither feasible nor sustainable without the addition of a readily utilisable form of carbon. The objective was therefore to investigate nitrate reduction efficiency at a low temperature of 10°C.

The literature review highlighted research work, e.g. McCarty et al. (1969), which investigated carbon sources for nitrate reducing treatment systems. This work showed carbohydrates, e.g. glucose, not to be as stoichiometrically efficient as other organic compounds, e.g. methanol and acetate, because of their higher consumptive ratio.

For the low temperature study, however, it was decided to use glucose and not methanol because it would be compatible to the overall aim of the project, namely, to use farm products as energy sources whenever possible. Those products would either have or produce on degradation carbohydrates in the form of glucose. Nitrate reduction using glucose could therefore indicate the potential use of organic materials. Using glucose would also highlight any operational problems that may only be associated with carbohydrates, and not with other organic compounds.

The low temperature study consisted of two sets of experiments:

i) Feasibility of Nitrate Reduction

ii) Efficiency of Nitrate Reduction - Optimum C : N Ratio.

4.1.2.1 Feasibility of Nitrate Reduction

For the feasibility investigations the 2 : 1 sand/soil mixture was used, to ensure a large microbial population was present in the column at the start of experimentation. The literature search had highlighted the work of Bremner and Shaw (1958a), who suggested an optimal C : N ratio of 3 : 1 for nitrate reduction. For the feasibility investigations the 3 : 1 ratio was used to ensure that nitrate reduction was not limited by insufficient carbon being supplied. For a range of flow rates tested (0.51 - 0.01 l/hr) nitrate reduction, if any, was measured.

4.1.2.2 Efficiency of Nitrate Reduction - Optimum C : N ratio

To assess the efficiency of low temperature nitrate reduction the stoichiometry needed to be investigated. Stoichiometry is the process of calculating or determining the relative quantities (equivalents) and atomic weights of the elements participating in any chemical reaction. Stoichiometric equations developed by McCarty et al. (1969) enable the theoretical optimum carbon to nitrogen ratio (C : N) for nitrate reduction in a water treatment system to be calculated, when utilising glucose as the carbon source. The calculation of the theoretical optimum C : N ratio is given below.

Stoichiometric calculation of optimum C : N ratio

In the low temperature study experiments the concentration of nitrate in the influent solution was 100 mg/l nitrate (22.6 mg/l nitrate-nitrogen).

From McCarty et al. (1969):

The reduction of nitrate-nitrogen to nitrogen gas gives a nitrate concentration change of 2.8mg/l NO_3-N as equal to a milliequivalent per litre of chemical change (meq/l) i.e., the quantity of chemical associated with the transfer of one mole of electrons.

Hence, to convert 22.6 mg/l NO_3-N to meq/l :

$$22.6 \div 2.8 = 8 \text{ meq/l} \quad (1)$$

From batch studies (McCarty et al., 1969) the average consumptive ratio, defined as the ratio of the total quantity of an organic material consumed during nitrate reduction to the stoichiometric requirement for nitrate reduction and deoxygenation alone, for sugar is 1.65. However, the ratio of carbon in glucose to carbon in sugar is:

$$0.4 \div 0.42 = 0.95$$

Hence, the consumptive ratio for glucose is: $1.65 \times 0.95 = 1.56$ (2)

$$\text{Consumptive ratio} = \frac{\text{Equivalent decrease in dissolved organic carbon (meq/l)}}{\text{Equivalent decrease in nitrate-nitrogen (meq/l)}}$$

Therefore, the equivalent decrease in dissolved organic carbon for a 22.6 mg/l decrease in NO_3-N is:

$$(1) \times (2) = 1.56 \times 8 = 12.5 \text{ meq/l}$$

From McCarty et al. (1969):
Half reaction for the oxidation of glucose to carbon dioxide gives a concentration change of 3mg/l organic carbon as equal to a milliequivalent per litre. Hence, the mg/l of organic carbon required to oxidise 12.5 meq/l is: $12.5 \times 3 = 37.5$ mg/l

Therefore, the theoretical optimum ratio of carbon in glucose to nitrogen in nitrate for a nitrate reducing water treatment system is:

$$37.5 \text{ (C)} : 22.6 \text{ (N)}$$

i.e., **C : N = 1.65 : 1**

The calculated figure for optimum C : N ratio can be compared and contrasted to a figure derived from a mass balance for dissimilatory nitrate reduction using glucose as the carbon source. The mass balance is given as follows:

$$4NO_3 + C_6H_{12}O_6 \text{ -------> } 2N_2 + 6CO_2 + 6H_2O$$

From this equation the ratio of nitrogen to carbon by mass is determined using the atomic weights of carbon and nitrogen i.e., 12 and 14 respectively.

Hence, the theoretical carbon to nitrogen ratio = 72 : 56 = 1.3 : 1.

This figure would be the optimum if all the carbon supplied was only used to reduce nitrate to nitrogen gas (dissimilatory nitrate reduction). In biological treatment systems, however, some carbon will be used for cell synthesis (assimilatory nitrate reduction). It is this additional carbon requirement that is represented in the greater ratio of 1.65 : 1, calculated above from the equations of McCarty et al. (1969). A greater requirement for assimilable carbon would raise the optimum C : N ratio to a figure nearer 5 : 1, i.e., the approximate ratio at which carbon and nitrogen are assimilated into cell biomass (Section 2.1.1.2).

For this investigation, the calculated theoretical optimum carbon to nitrogen ratio of 1.65 carbon atoms to 1 nitrogen atom (1.65 : 1) was compared to ratios of 2 : 1, 1 : 1, and a control in which no glucose was added.

4.1.2.3 Materials and Method

For both sets of experiments, the apparatus used was the same as outlined for the soil study, and consists of a perspex permeameter cell, into which soil is packed. The methodology of preparing the soil cells is described in Section 4.1.1.1. Due to the problems highlighted when using a sand/soil mixture ratio of 2 : 1 in the feasibility investigation, however, a sand/soil ratio of 4 : 1 was used in the C : N ratio experiments.

This still provided the required microbial inoculum whilst minimising problems of permeability associated with the use of soils containing a high clay fraction.

For the feasibility study, a Rock and Taylor automatic water sampler was used, to allow hourly sampling of the leached solutions (Rock and Taylor, 1991). This regime allowed the sensitivity of nitrate concentration changes to be examined. In the efficiency study, however, because a longer sampling period of 24 hours was used, sampling could be carried out manually.

A standard influent solution of the required carbon to nitrogen ratio was prepared from glucose and potassium nitrate, the quantity required of each for the C : N ratios tested is given in Appendix II. Each treatment was replicated three times and the collected leachate samples analysed for both glucose and nitrate.

A constant temperature of 10°C was maintained by undertaking the experiments inside a temperature controlled room.

4.1.3 Biodegradation of Organic Materials Study

A supplementary organic carbon substrate is required to provide energy for the reactions that result in microbial nitrate reduction (WRC, 1974). As most water draining from agricultural catchments has negligible organic carbon content (Tamblyn and Sword, 1969), it is vital to investigate possible carbon sources which could be used in a nitrate reducing treatment system. This study therefore examined the feasibility of using organic materials as carbon sources for anaerobic nitrate reduction, satisfying the on-farm solution requirements of minimum management, low cost, and use of on-farm resources.

The literature review highlighted that the biodegradation of organic materials is primarily controlled by temperature and aeration status of the environment,

decomposition being less complete and slower under anaerobic conditions than under aerobic conditions. Hence, the study objective was to determine the degradation rates of three organic materials under anaerobic and low temperature conditions, and so ascertain the viability of using organic materials as sources of readily utilisable carbon for microbial nitrate reduction.

4.1.3.1 Materials and Method

The experimental procedure adopted was to measure carbon dioxide (CO_2) evolution resulting from the degradation of the organic material by micro-organisms. Other workers had assessed this degradation under wholly aerobic or anoxic environments. In this experiment degradation was assessed in an anaerobic environment; however, oxygen is present, but only in the form of an inorganic substrate i.e., nitrate (NO_3).

The test compound was placed in a flask containing the inorganic medium, and inoculated with a mixed population of micro-organisms. By measuring the quantity of CO_2 produced and comparing the observed values to theoretical yields calculated from a knowledge of the carbon content of the test compound, a measure of the ultimate biodegradability was made.

McCarty et al. (1969) showed that the yield of cell biomass from the methanol added equated to 30% of the carbon in the methanol. The difference i.e., 70% of the carbon, was used for respiration and the production of CO_2. For glucose, however, the respiration value would only be 55% because the utilisation efficiency of glucose is 79% that of methanol (McCarty et al., 1969) .

By using the same quantity of carbon in all the tests a direct comparison could be made between the degradable compounds. The assumption was made that readily utilisable carbon (RUC) was utilised immediately by bacteria, and therefore the rate of production of carbon dioxide was proportional to the rate of generation of RUC for all organic compounds.

4.1.3.2 Test Materials

The organic materials tested were wheat straw, wheat straw soaked in molasses, and sugar beet. Wheat straw was chosen because it was considered more absorbent than barley straw.

Attempts at coating the straw with liquid molasses were unsuccessful due to the viscous nature of the molasses, as it was impossible to ensure complete saturation and absorption of the molasses by the straw. However, complete coverage and saturation was achieved by soaking the wheat straw in a 20% molasses solution for 24 hours, and then dried in an oven at 60°C for 48 hours. The wheat straw and sugar beet were similarly dried to ensure that an equivalent mass of material was added to the test flasks on a dry weight basis.

Uniform drying of the sugar beet was achieved by chopping it into thin slices prior to being put into the oven. Once dried, all the materials tested were ground and sieved to provide a size range of 0.5-1.0mm. Grading the material ensured the maximum uniform surface area available for microbial attack and degradation.

Degradable carbon contents of the organic materials were assessed using the ash-test method. The ash-test involves the burning of the organic material in an oven at a temperature of 800°C. The residue remaining after burning is the fraction of the organic material that is non-degradable.

The carbon content of sugar beet, straw and molassed straw by ash-test was 95.8%, 94.9% and 94.0% respectively. 5.00 grams of sugar beet granules were added to three test flasks, equivalent to 4.79 grams of carbon. To ensure an equivalent amount of degradable carbon in each test flask with the other test materials, the mass of straw and molassed straw added was 5.05 grams and 5.10 grams respectively.

In addition to the three organic materials tested, a control was used in which no organic material was added, to assess the quantity of CO_2 evolved from the degradation of the microbial inoculant. To enable conclusions to be drawn from the experiment, three replicates of each test were made.

4.1.3.3 Reference Compound

A reference compound was tested to enable comparison between the organic materials and a compound in which all the carbon is readily degradable.

Compounds such as sodium acetate are preferred as reference compounds, because the problem of fermentation is avoided. Glucose, however, was chosen for two reasons. The carbon is readily utilisable, and the performance of sugar beet could be directly compared to glucose, as the sugar beet's soluble solids share the same chemistry. Glucose would also enable the data from this experiment to relate to the performance data acquired in the efficiency of nitrate reduction study (Section 4.1.2.2).

11.975 grams of glucose (4.79g of carbon) was added to each flask, as 40% of glucose is carbon. This resulted in a test medium glucose concentration of 2994 mg/l, which is equivalent to 16.6 mmol/l (molecular mass of glucose = 180).

4.1.3.4 Carbon to Nitrogen Ratio

To enable the results to be related to the previous tests, a carbon to nitrogen ratio of 1.65:1 was used, i.e., theoretically the most efficient ratio for nitrate reduction. 20.944grams of potassium nitrate (KNO_3) were added to the test medium to provide a terminal electron acceptor (oxidant), which resulted in a test medium concentration of 725.75mg/l NO_3-N.

N.B. 4790mg of carbon added

2903mg of nitrate-nitrogen added (725.75mg/l NO_3-N x 4l)

C : N ratio = 4790/2903 = 1.65 : 1

4.1.3.5 Inoculum

The inoculum used in the biodegradation study was pig slurry. The slurry was stored in a 5 litre incubation flask and flushed with O_2 gas to enable the degradation of the volatile fatty acids, and any residual soluble carbon. Maintenance of this aerobic environment ensured the proliferation of the required facultative microbial population.

Stock solutions of calcium chloride dihydrate, magnesium sulphate heptahydrate, ferric chloride hexahydrate, phosphate buffer, and ammonium sulphate were added to the incubation flask to provide nutrients. Initially, the solutions were added on a daily basis, after 200ml of the inoculum was drawn off and 200ml of fresh pig slurry added. After 14 days fresh slurry and stock solutions were then only added weekly.

When the inoculum was required, a sample was decanted, homogenised in a centrifuge at 2000 rpm for 5 minutes to separate out suspended solids, and then allowed to settle to produce a supernatant liquid. `40ml of the supernatant was added to each test flask, i.e, 1% of the flask volume.

4.1.3.6 Calculation of Carbon Dioxide Produced

To assess the biodegradability of the organic compound under test, the quantity of CO_2 that would be produced if all the carbon in the compound was respired is calculated from the following equation (HMSO, 1989):

$$\frac{Mco_2 \times \%C}{Mc \times 100} = \text{mg } CO_2 \text{ evolved per mg of compound}$$

where, Mco_2 = molecular mass of carbon dioxide i.e., 44

Mc = atomic mass of carbon i.e., 12

$\%C$ = percentage of carbon in test compound

4.1.4 Combined Organic Material and Low Temperature Study

It was hypothesised that at a low temperature of 10°C, degrading organic materials could simultaneously provide a source of carbon, and a site upon which the micro-organisms could grow, in a nitrate reducing water treatment system.

The biodegradation experiments had indicated that ground sugar beet could provide a sustainable source of carbon. The initial ability of sugar beet to generate readily utilisable carbon is, however, primarily dependent upon the diffusion rate of the soluble sugar fraction. As size, i.e., surface area, of the sugar beet pieces determines the rate of diffusion of soluble sugar, a preliminary bench study was carried out on four sizes of sugar beet cubes to measure their respective diffusion rates.

This data would help determine which sugar beet cube sizes would be suitable in the principal flow study, the flow study testing the stated hypothesis.

The study therefore consisted of two experiments:

i) Bench Diffusion Study

ii) Flow Study.

4.1.4.1 Bench Diffusion Study

Sugar (carbon) diffusion from sugar beet is optimised in sugar production by shredding the sugar beet, thus reducing the length of diffusion path to a minimum. For this study, however, a release rate of carbon is required that can sustain nitrate reduction over a period of time measured in days rather than seconds. Before a pilot study could be started, the rate of sugar release needed to be determined for a range of sugar beet cube sizes.

Buckets containing 4 litres of distilled water had cubes of sugar beet suspended in them. Four sizes of sugar beet cube were tested, 40mm, 20mm, 10mm, and 5mm. Because the

method of sugar content analysis is destructive (Section 4.3.6), several similar sized cubes of sugar beet were started simultaneously, and following a designated time period, cubes were taken out of the buckets and the sugar content analysed for.

4.1.4.2 Flow Study

The principal objective of this study was to investigate whether a water treatment system using sugar beet cubes as a carbon source would be sustainable in terms of both maintaining constant nitrate reduction at a constant flow rate, and maintaining hydraulic performance i.e., permeability. If the principal was shown to be sustainable, the data set produced would enable a model to be developed describing the nitrate reduction/flow rate relationship. Such a model could be used to investigate the sensitivity of the relationship to different parameters, and eventually lead to the development of a management tool.

An important parameter controlling nitrate reduction is pH. It was postulated that degrading organic materials could decrease environmental pH, however, it was also postulated that because there was a continuous flow of water through the sugar beet, then pH may be maintained at the required environmental pH i.e., 6-8. Two treatments were therefore considered for the experiment, one with pH control, and one without. For the with control treatment, to ensure that pH was maintained at or near neutral, pieces of lime (calcium carbonate) were added to the sugar beet cubes, the lime neutralising any acid produced. The pH of the distilled water used to make-up the nitrate solution was also found to be acidic, and so was buffered to produce a pH of 6.5.

The apparatus used for the flow study was the same as that used in the low temperature study (Section 4.1.1.1), and is illustrated in Figure 4.3. A control was deemed unnecessary for the study because the treatment performances attained were to be compared to that for glucose, which had previously been tested. Redox data would also be collected from the top, middle, and bottom sections of the permeameter cell to enable the aeration status within the permeameter to be monitored.

The findings of the bench diffusion study indicated that the most suitable size of sugar beet cubes for use in the flow study would be 20mm and 40mm. However, because the experiments were being undertaken outside the sugar beet season, an important consideration was to optimise the use of the remaining sugar beet stock. Using 20mm cubes was considered the best option to do this as it would provide a realistic representation of a treatment reactor, thus effectively testing the hypothesis. Sugar beet stock levels allowed for enough 20mm cubes to be cut for three replicates of each treatment. Testing the hypothesis using 40mm cubes could only effectively be achieved by increasing the scale of the experiments. Such a demand would require the use of more sugar beet, which was not readily available, and also the design and construction of new apparatus.

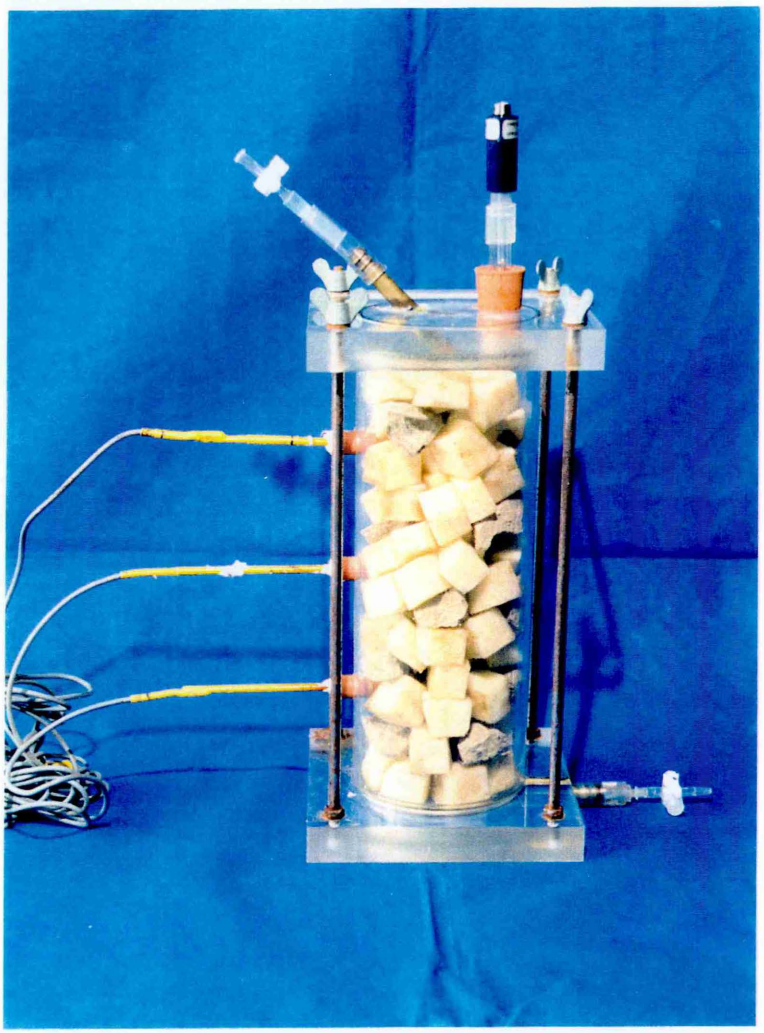

Figure 4.3 Experimental Apparatus Assembly Used in Flow Study showing Sugar Beet and Crushed Limestone Mix

4.2 Instrumentation

The instrumentation used enabled the monitoring of the environmental conditions and quantification of the experimental performance.

4.2.1 Nitrate Analysis and Assessment of Nitrate Concentration Reduction

The technique of nitrate reduction assessment used required a technique of assessing nitrate concentrations in water, described by Focht (1978) as, "Inherently the most direct and simple method of assessing losses of nitrate".

Many methods of nitrate measurement exist from basic field equipment to complex analytical laboratory equipment. It was part of the investigation to assess these methods, different methods being required either in the initial laboratory phase or perhaps later in field experimentation where more robust, stand-alone equipment would be necessary.

Initial investigation highlighted the following possible methods:

i) Ion Specific Electrode

ii) Ultra-Violet (UV) Spectrophotometry.

The stand-alone equipment tested for monitoring the nitrate concentration of drainage water was a solid state ion specific electrode (ISE). This type of electrode has improved stability over the porous membrane type electrode, hence the re-calibration interval can be increased to days instead of hours (BPS, 1991). Without the inherent improvements of the solid-state electrode, the proposed in-field use would be impracticable. The method used was that described in Standard Methods (APHA, 1992).

For increased accuracy and precision, laboratory analysis employed the ultra-violet (UV) spectrophotometric screening method. The method principle, interference's, apparatus requirements, reagents, procedure and calculation are outlined in detail in the

text, Standard Methods (APHA, 1992). For the purposes of this study a brief description of the method is given.

The nitrate ion absorbs strongly in the UV spectrum, with a peak at a wavelength of 202nm. Interference from other inorganic ions and organic matter is compensated for by reading at the 220nm wavelength and subtracting twice the value of the reading taken at the 275nm wavelength where organic matter will be absorbed but not the nitrate ion. The corrected absorbency value for nitrate is used to determine the nitrate concentration by multiplying it by a calibration coefficient, determined from a curve produced using solutions of known nitrate concentration.

Dilution of the samples in the ratio of 1 part sample to 4 parts distilled water was necessary to reduce the nitrate concentrations and corresponding absorbency values to fit within the working range of the UV spectrophotometer. Dilution of the samples meant that the nitrate concentration read had then to be multiplied by 5.

Readings were recorded as nitrate-nitrogen (NO_3-N) in milligram per litre (mg/l). These can be converted to nitrate (NO_3) by multiplying by 4.4, a factor calculated from the ratio of the combined atomic weights of O (16) and N (14) that is to say 62/14.

4.2.2 Redox Potential (Eh) Measurement

The redox method is an effective and sensitive measurement to determine soil aeration status, since Eh is closely related to the presence or absence of gaseous oxygen (Fluhler et al., 1976).

The apparatus used in the experiments is of the design outlined by Obando-Moncayo (1990), and is based on measuring the potential difference between a platinum electrode and silver/silver chloride reference electrode, see Figure 4.4. For a detailed description of the circuitry, specification and calibration techniques, the reader is directed to this text, however for the purposes of this study it is necessary only to note the salient

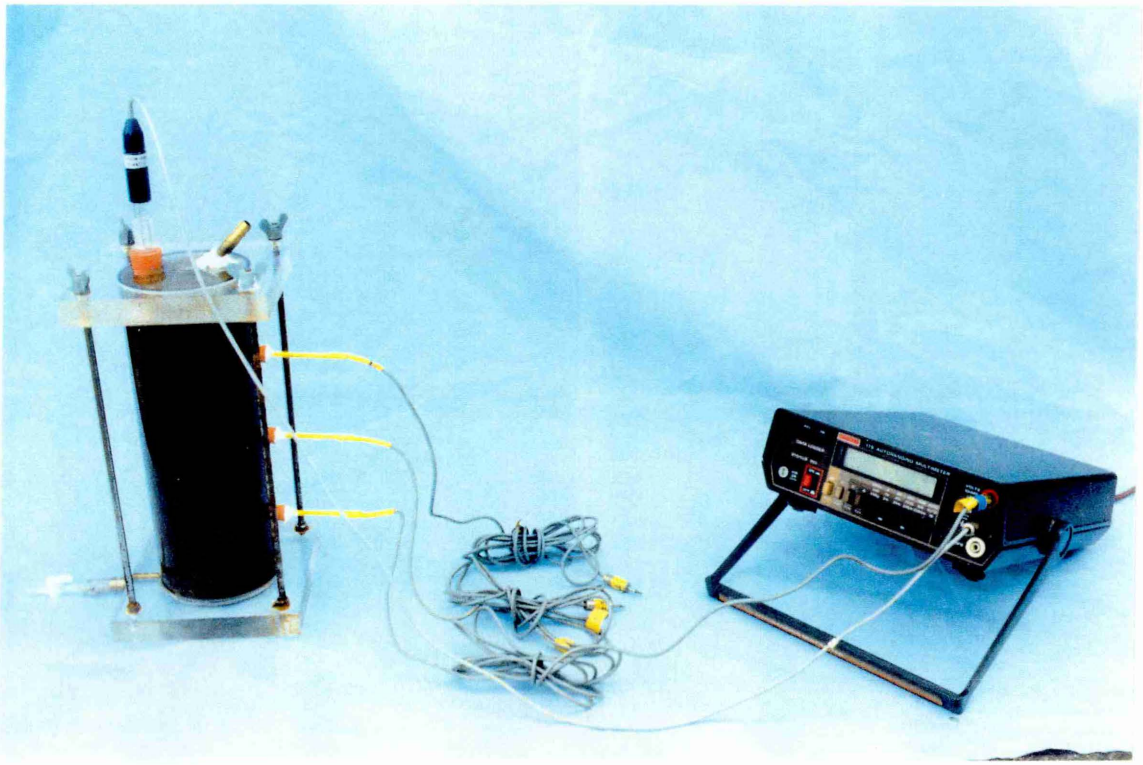

Figure 4.4 Experimental Apparatus Assembly for Redox Potential Measurement

features of the system. The platinum (Pt) electrodes (cathodes) are linked via a fly lead to a Keithley autoranging digital multimeter set to the volts range. The anode required to complete the electrical circuit was a leadless Gallenkamp silver-silver chloride (Ag/AgCl/KCl) reference electrode. This electrode was also attached via a fly lead to the digital multimeter. Prior to installation of the Pt electrodes the tips were slightly abraded to remove any chemical poisoning present.

Taking a series of Eh readings involved connecting the Ag/AgCl/KCl reference electrode of the cell to be measured to the multimeter, and then each Pt probe was connected in turn to the digital multimeter and a reading taken. It should be noted that one reference electrode can be used in conjunction with several platinum electrodes. The distance between electrodes is unimportant (McIntyre, 1967).

The Eh of the solution, is the electrical voltage difference across the Pt and Ag/AgCl/KCl probes and recorded in millivolts (mV). Each reading was adjusted as below:

Eh = E measured (mV) + 207 mV

The +207 mV, is a constant figure related to the potential difference created when using a Ag/AgCl/KCl probe rather than a standard redox hydrogen electrode, i.e., it is the potential of the Ag/AgCl/KCl probe (Obando-Moncayo, 1990).

4.2.3 Glucose Analysis

The method used for glucose analysis is commonly termed as the Trinder method. The glucose level is determined colorimetrically, as a result of enzymatic action. A detailed methodology is outlined in Trinder (1964), however a brief description is given below.

Added to a 1ml aliquot of each water sample taken, was 1.5ml of Godpod and 0.5ml of Phenol. The solution was then incubated at 37°C in a water bath for 10 minutes. Godpod consists of a phosphate buffer, the enzymes to carry out the reaction, and a dye to indicate the colorimetric change. Phenol kills any organisms in the sample that may interfere with the enzyme action.

The samples are read using a UV spectrophotometer set to a wavelength of 515nm. The glucose concentration in millimol per litre (mmol/l) is calculated automatically by the spectrophotometer using a stored calibration curve. The stored calibration curve from 0.05-0.5mmol/l is inputted prior to analysis to account for any variation in chemical and/or more importantly enzyme nature.

It should be noted that the calibration was carried out using distilled water whilst the samples being analysed contained particles of soil/organic matter that interfered with the colorimetry. This problem was resolved by taking a colorimetric reading of the sample

without any reagents added, and subtracting this value from the reading of the same sample with the reagent's added and incubation complete.

4.2.4 Ammonia Analysis

The ammonium ion exists almost entirely as ammonia in solution (Holden, 1970), and at high concentrations (>1mg/l) is an environmental pollutant. Ammonium can be a product of both nitrate reduction and organic material degradation (Section 2.1.1.2). Consequently, there is a requirement that water leaving the nitrate reducing treatment system be analysed to ensure that high levels of ammonia are not entering the environment.

The principle of the analysis is discussed in detail in Standard Methods (APHA, 1992) under the title NH_3-Nesslerization Method. A brief description of the method is, however, given below.

Analysis was carried out using a HACH DR2000 direct reading spectrophotometer. A 25ml aliquot of the test water sample is poured into one of a spectrophotometrically matching pair of crystal glass cells. To this is added 1ml of stabilisation reagent, 1ml of Rochelle salt solution, and 1ml of Nessler Reagent. After the addition of each reagent the solution is shaken to ensure complete reaction. After all the reagents are added, a reaction period of one minute is given after which the sample cell is measured, versus a reagent blank in the second matching cell. The reagent blank is made up using distilled water.

The problem of interference from organic matter in the sample was not as acute as that seen in the glucose analysis, primarily because the colorimetric change of the ammonia analysis is not as subtle as that in the glucose test.

4.2.5 Carbon Dioxide Collection and Analysis

Two methods of carbon dioxide (CO_2) collection and analysis were used for the investigation, headspace sampling and titration, the second method requiring a further modification to the apparatus.

The headspace sampling method (Battersby and Wilson, 1988) was seen as a direct and sophisticated method of assessment in that it used gas chromatography for CO_2 analysis. This technique offered the potential in this experiment to allow the assessment of the range of gaseous products produced including nitrous oxide.

The titration method of CO_2 gas collection and analysis had also to be used in these experiments when the headspace sampling method failed. Further discussion on the failure is made in Chapter 5.

4.2.5.1 Gas Chromatography

The gas chromatography method of carbon dioxide (CO_2) analysis is based on that developed by Sparling (1981) who investigated the activity of soil biota, the measurement of that activity being the production of CO_2 resulting from the degradation of soil organic matter.

In this method, the CO_2 evolved from the biodegradation of the organic material collects in the headspace above the test medium solution, at the top of the biodegradation flask. A known volume of the headspace gas can be drawn off using 50ml syringes. Three-way taps attached to both the top of the flask and syringe, allow for a gas sample to be taken without opening the flask to atmosphere. 1ml samples of the collected gas are injected into the gas chromatograph (GC), which produces a gas signature for the sample. A pre-programmed integrator identifies the part of the gas signature that is the trace curve for CO_2, and calculates the area under that curve. It compares this area to

the area produced for a calibration gas of 10% CO_2, enabling it to calculate the percentage of carbon dioxide in the sample.

With the density of carbon dioxide and the volume of gas in the headspace known, the mass of carbon respired can be calculated from the percentage figure.

4.2.5.2 Titrimetric

The titrimetric method of carbon dioxide (CO_2) collection and analysis used is a modified version of the one developed by Sturm (1973) and is outlined in HMSO (1989). The reader is referred to the HMSO document for the method principle, interference's, procedure, apparatus, reagents, calculation and sources of error. Figure 4.5 illustrates the experimental assembly, and for the purposes of this study a brief description is given.

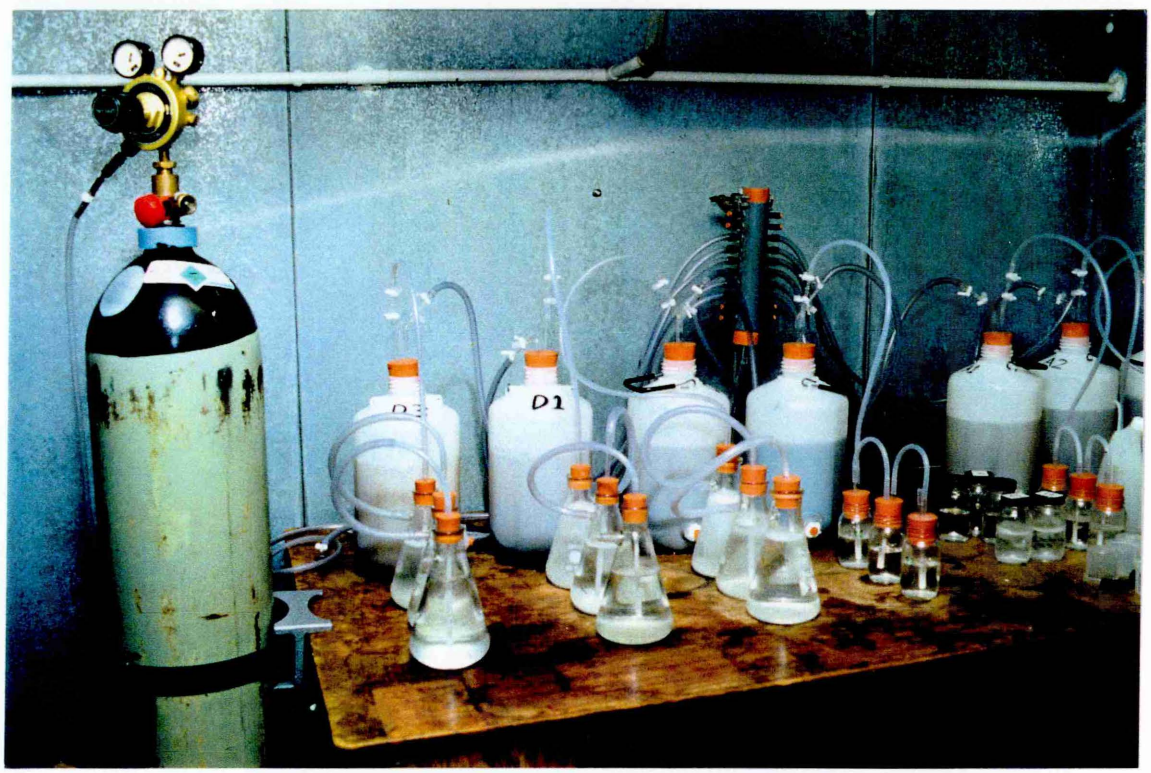

Figure 4.5 Experimental Apparatus Assembly for Biodegradation Study Incorporating the Titration Method of Carbon Dioxide Collection

Carbon dioxide gas evolved from the degradation of organic material was allowed to flow out of the degradation flask through a series of three bubblers containing barium hydroxide ($Ba(OH)_2$) which absorbs the CO_2. A continuous supply of nitrogen gas (N_2) from a pressurised cylinder was used to force the evolved CO_2 out of the biodegradation flasks and through the $Ba(OH)_2$ bubblers. After a measured time period, the proximal bubbler was removed, and the other two bubblers moved one place closer, and a bubbler containing fresh $Ba(OH)_2$ added. A sample of the $Ba(OH)_2$ from the removed bubbler was then titrated against standard hydrochloric acid using phenolphthalein as an indicator.

The volume of HCl required to achieve a colour change is used to calculate the mass of CO_2 absorbed in the $Ba(OH)_2$, enabling the mass of carbon respired to be ascertained. It should be noted that the use of N_2 gas is a departure from the Sturm method which uses oxygen gas (O_2) to ensure aerobic, and not anaerobic, degradation prevails.

4.3.6 Sugar Beet Sugar Measurement

Sugar beet was used as an energy source in some of the experiments and the sugar content was determined using a refractometer. The refractometer actually measures the total soluble solids fraction of the sugar beet juice, however, the sugar (sucrose) content of the total soluble solids is greater than 99%, and thus the total soluble solids reading can be equally considered a reading of sugar content. The method is more familiarly termed the Brix method, with sugar content being measured in %Brix. A hand refractometer was used in this investigation, which enabled measurement to the nearest 0.1%Brix.

The measured sugar content was used to assess the loss of sugar from the sugar beet cubes used in the experiments outlined in Section 4.1.4. The method entails mechanically extracting the juice from the sugar beet matter, requiring the destruction of the sugar beet. The disadvantage of this is that it prevents the sugar beet tested for sugar content then being used in the experiments. The disadvantage is compounded by the

fact that the sugar concentration varies in different parts of the sugar beet root. Although this created a difference error, it was repeated for all samples, and therefore considered acceptable because it would not influence trend observations.

Sugar beet contains sugar in the form of sucrose and not glucose. For the purposes of these investigations, however, the difference in the organic chemistry of the two compounds was considered negligible in affecting any experimental results in which they were being either compared or considered as carbon sources for microbial utilisation.

4.3.7 Soil Bacterial Count Analysis

The method used for determining the bacterial count in soil samples is known as the Total Viable Aerobic Bacterial Count or Plate Count, and is based upon the assumption that a single bacterial colony will originate from a single bacterial cell.

As it is difficult to count more than about 200 bacterial colonies on a single plate (petri dish), a serial dilution of the original sample was made. Using a sterile spatula, 1.0 gram of the soil sample was added to a glass bottle containing 9ml of 1/4 strength Ringers Solution. The suspension was shaken to ensure thorough dispersion of the soil, giving a 1 in 10 dilution. 1ml of this suspension was transferred using an automatic pipette into a further glass bottle containing 9ml of Ringers Solution, thus the original sample was now diluted by 100. A further four serial dilutions were made to get a 1 in 1000000 dilution of the original sample.

Using an automatic pipette, a 1ml sample from each serial dilution was added to a disposable plastic petri dish. Bottled molten yeast extract agar was taken from a water bath at 50°C, poured into each petri dish, and gently swirled to ensure an even distribution throughout the agar. Two replicates of each serial dilution were made. The petri dishes were left to stand for 15 mins to allow the agar to set, placed in polythene bags, and then placed in an incubator at 25°C. After 3 days a count of the colony

forming units (cfu) was made. The petri dishes were returned to the incubator for a further 4 days after which the cfu's were re-counted.

All bottles of Ringer's Solution and yeast extract agar were pre-prepared and sterilised in an autoclave. The plastic tips of the automatic pipette were also autoclaved, being replaced for each manipulation to prevent contamination.

Chapter 5

Experimental Report : Results and Conclusions

5.1 Soil Study

The objective of this study was to investigate the potential of soil to support anaerobic nitrate reduction without an additional carbon source, relative to the quantity of inoculum used and the initial organic carbon content of the medium.

The experimental data is summarised in Table 5.1. Nitrate-nitrogen (NO_3-N) concentration reduction was observed for all four sand to soil ratios over the 7 day (168 hour) experimental period, reduction being highest in the medium with the highest soil content. Figure 5.1 illustrates that nitrate reduction peaked at 48 hours, after which it declined rapidly to very low values.

Table 5.1 Nitrate-Nitrogen Concentration Reductions for Four Sand to Soil Treatments

	Nitrate-Nitrogen Concentration Reduction (mg/l)						
Time (Hours)	24	48	72	96	120	144	168
Sand to Soil Ratio							
1 : 1	3.8	4.73	2.87	2.55	2.18	1.72	1.21
2 : 1	1.66	2.53	0.77	0.69	0.57	0.51	0.53
3 : 1	1.54	2.87	0.77	0.63	0.57	0.36	0.38
4 : 1	0.91	1.58	0.54	0.38	0.36	0.34	0.3

Redox data was also collected and is given in Table 5.2 where the figures are an average of readings taken from the top, middle, and bottom of the cell. The data is illustrated in Figure 5.2, which indicates that the redox potential was below the assumed nitrate reduction threshold of 200mV throughout the experiment, and therefore it was assumed that an anaerobic environment prevailed.

Table 5.2 Redox Potential Data for Four Sand to Soil Treatments

	Redox Potential (mV)							
Time (hours)	0	24	48	72	96	120	144	168
Sand : Soil Ratio								
1 : 1	-53	-103	-104	-155	-155	-164	-162	-179
2 : 1	-31	107	65	47	48	32	30	22
3 : 1	25	48	50	37	23	28	30	35
4 : 1	-78	-101	-133	-33	4	18	23	34

The observed trends in nitrate reduction suggest either an increase in the microbial population or a delay in activity whilst the resident microbial population became adapted to the anaerobic environment. It is postulated that for either case the readily utilisable carbon (RUC) fraction of the soil would have become exhausted after 48 hours, the result being a decline in nitrate reduction.

It is suggested that the differences observed in both initial nitrate reductions and the redox measurements are attributable to differences in organic carbon content of the sand to soil ratios tested. As the organic carbon content of the soil used in the study was 3.94% by titration, the mass of organic carbon for the 4:1, 3:1, 2:1, and 1:1 ratios was 15.76g, 19.70g, 26.28g, and 39.40g respectively. Hence, the micro-organisms would have had more readily utilisable carbon available for nitrate reduction in the 1:1 cell than in the 4:1 cell.

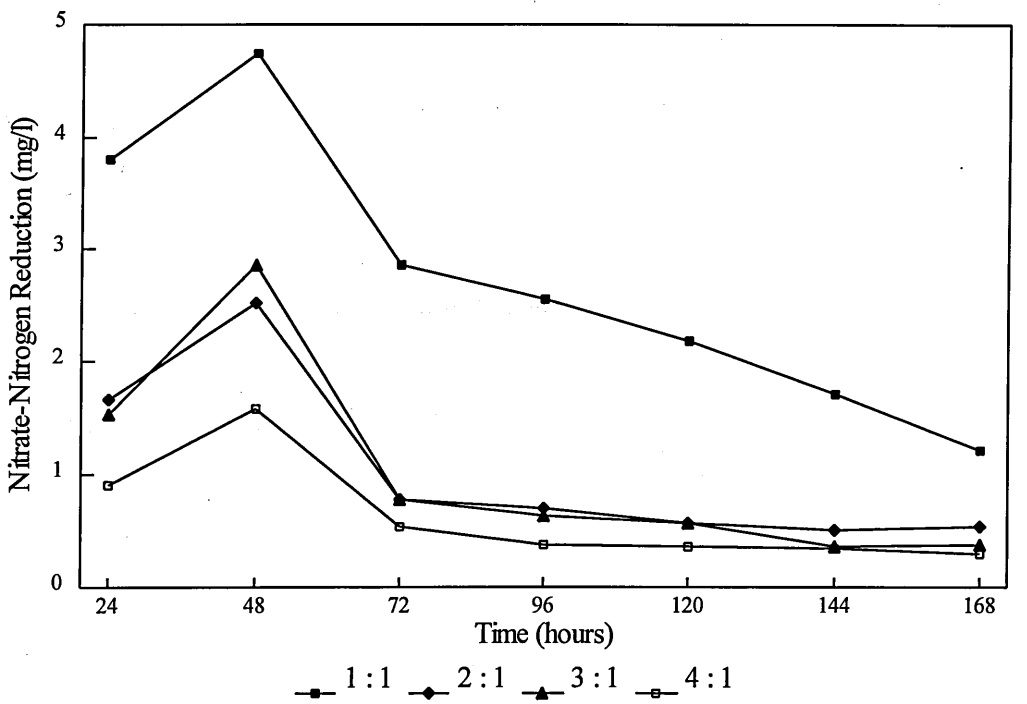

Figure 5.1 Variation in Nitrate-Nitrogen Concentration Reduction with Time for Four Sand to Soil Treatments

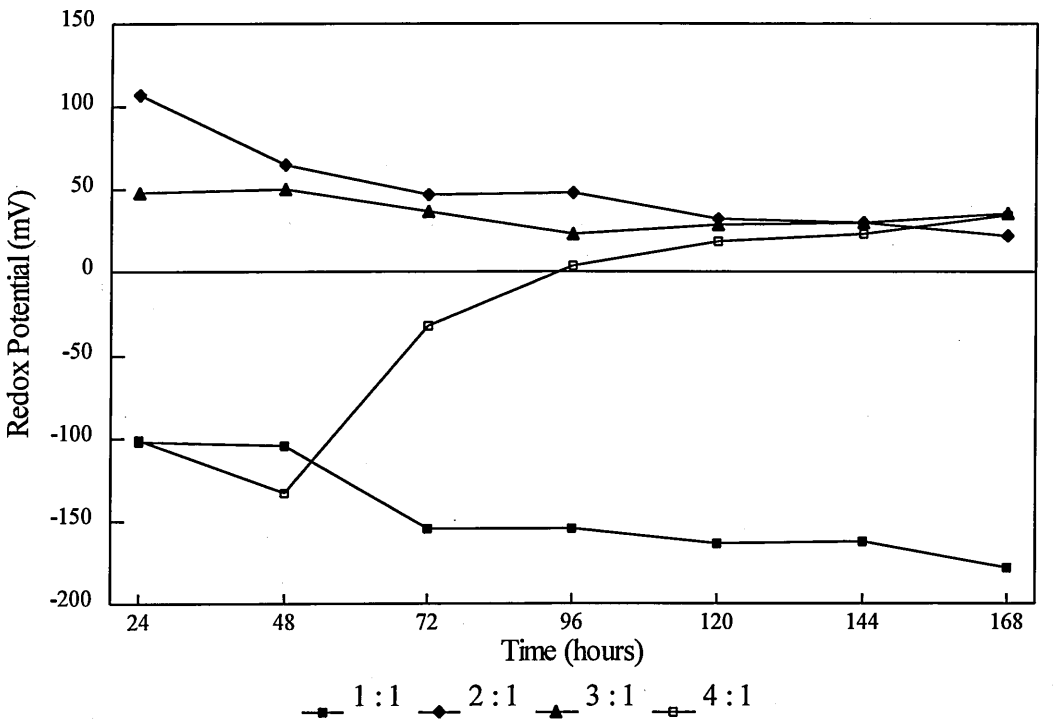

Figure 5.2 Variation in Redox Potential with Time for Four Sand to Soil Treatments

As there was a greater quantity of soil in the 1:1 mixture, it may also be expected that the nitrate reduction would not decline as quickly as for the other mixture ratios. This does not appear to be the case, and it is postulated that a significant proportion of the RUC was utilised for microbial growth and not nitrate reduction.

The lower redox measurements for the 1:1 and 4:1 ratios imply greater reducing conditions prevailed in these treatments over the initial 72 hour period than in the 2:1 and 3:1 treatments. After 72 hours the redox in the 4:1 treatment increased to a similar level to that in the 2:1 and 3:1 treatments. The 1:1 treatment, however, maintained a significantly lower level of redox than the other ratios. It is suggested that differences in redox values may be attributable to variation in the packing density of the medium in the cells, as a total of 2000 grams of sand and soil combined was used for each treatment.

5.1.1 Conclusion

It is significant that even though redox was maintained throughout the experiment below the 200mV threshold required for nitrate reduction, i.e., an anaerobic environment prevailed, nitrate reduction was not sustainable. It was therefore concluded that sustainable nitrate reduction using soil as a medium could only be achieved if an additional carbon source was supplied.

5.2 Low Temperature Study

The objective of this study was to investigate nitrate reduction at low temperature, that is both its feasibility and its efficiency, when an additional carbon source is added. Results of this study are therefore presented in two parts:

i) Feasibility of Nitrate Reduction

ii) Efficiency of Nitrate Reduction - Optimum C : N Ratio.

5.2.1 Feasibility of Nitrate Reduction

The results of the feasibility study are summarised in Table 5.3, presented in order of declining flow rate, and listed in Appendix III. The first eight tests were undertaken over a period of 48 hours, after an initial 24 hour wetting up period. Variability in nitrate reduction over the 48 hour period e.g. Test No.8, suggested that this was too short an experimental time to ascertain trends in nitrate reduction capability. Test No.9 was therefore undertaken to examine if nitrate reduction data collected over 192 hours would allow nitrate reduction efficiency trends to be observed. The results indicate that nitrate reduction is feasible at low temperature; however, significant nitrate reductions were only observed at low flow rates.

Table 5.3 Summary of Experimental Data for Feasibility Study

	Flow Rate (l/hr)			Nitrate-Nitrogen Concentration Reduction (mg/l)		
Test No.	Q max.	Q min.	Q avg.	N red. max.	N red. min.	N red. avg.
1	0.51	0.35	0.42	4.74	2.46	3.56
2	0.46	0.34	0.38	2.46	1.6	2.05
3	0.36	0.272	0.3	12.38	2.4	5.41
4	0.36	0.16	0.26	7.96	1.55	4.54
5	0.22	0.11	0.16	6.4	2.81	4.61
6	0.24	0.1	0.13	7.72	1.23	3.92
7	0.12	0.07	0.09	13.9	6.94	9.63
8	0.06	0.02	0.04	17.03	5.26	12.25
9	0.04	0.01	0.017	21.01	13.22	20.07

All the tests undertaken as part of the feasibility study highlighted a reduction in flow rate over the experimental time. It was postulated that this was due to fine soil particles settling in the small pores of the matrix, which consequently reduced permeability. To

minimise this problem the sand to soil mixture chosen for the efficiency experiments was increased to 4:1, the 2:1 ratio having been chosen in the feasibility study to ensure a high initial microbial population.

An attempt at maintaining a constant flow rate through the cell was made by adjusting the hydraulic head. The experiment failed as a constant flow rate was unobtainable due to an inability to make sensitive head changes with the apparatus. As a result of the problem of fines settling Darcy's law relating head change to flow rate also could not be applied.

5.2.2 Efficiency of Nitrate Reduction - Optimum C : N Ratio

The nitrate reduction data are summarised in Table 5.4, and listed in Appendix III. Data are given for a combination of the three replicates for each of the three carbon to nitrogen (C : N) ratios tested. Significant nitrate reduction did not occur in any of the three treatments when flow rates were above 0.1 l/hr. At flow rates below 0.1 l/hr all the applied nitrate was reduced in both the 1.65 : 1 and 2 : 1 ratio experiments, however, in the 1 : 1 treatment nitrate was still observed in the leachate , see Figure 5.3.

Table 5.4 Nitrate-Nitrogen Concentration Reductions (mg/l) Above and Below a Flow Rate of 0.1 l/hr for Three Carbon to Nitrogen Ratios

C : N Ratios	Flow Rate (l/hr)	
	> 0.1	< 0.1
1 : 1	4	17
1.65 : 1	4	23
2 : 1	7	23
Control (no glucose added)	Readings between -5 i.e., nitrate concentration increased and +5 mg/l	

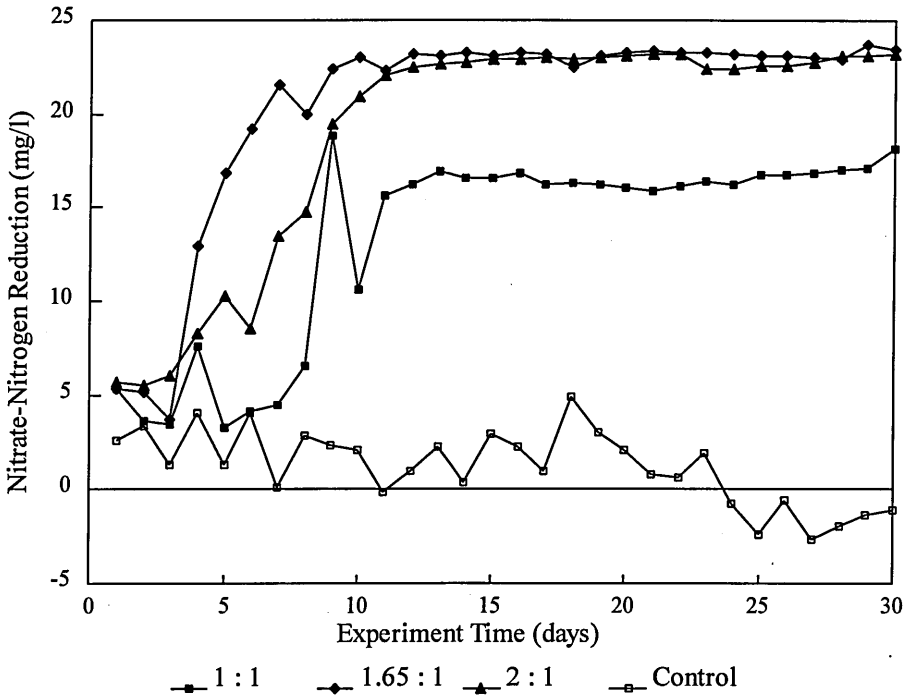

Figure 5.3 Comparison of Nitrate-Nitrogen Concentration Reduction Values Obtained for Three Carbon to Nitrogen Ratios

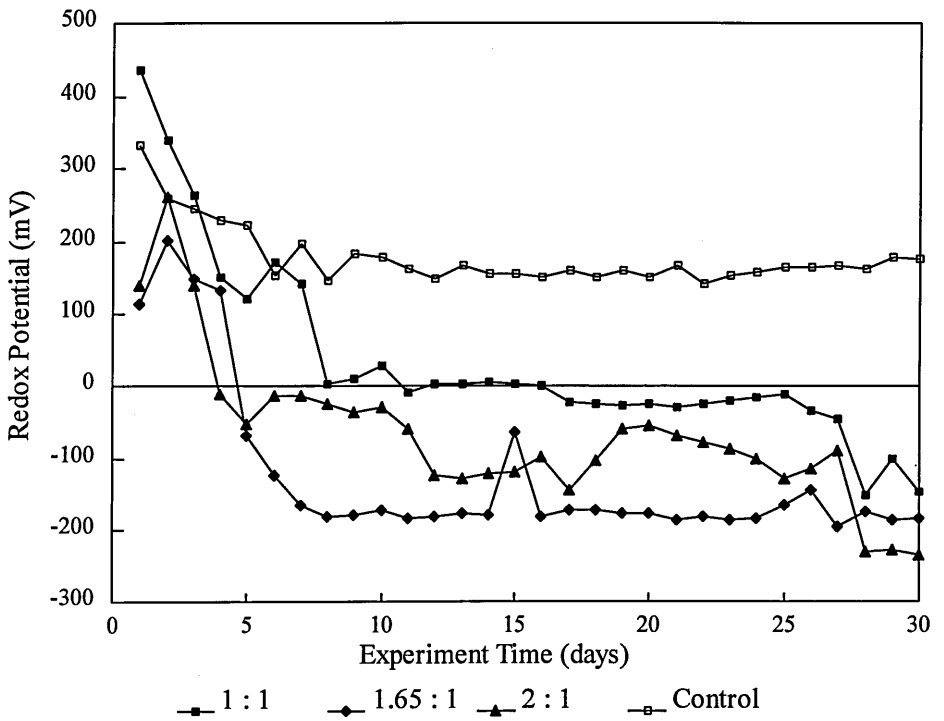

Figure 5.4 Comparison of Redox Potential Values Obtained for Three Carbon to Nitrogen Ratios

Examination of the redox data, summarised in Table 5.5 and illustrated in Figure 5.4, indicates that redox potentials fell below the 200mV nitrate reduction threshold several days after the start of the experiment.

Nitrate availability can be said to have become limiting to microbial activity in the 2 : 1 and 1.65 : 1 treatments where all nitrate in solution was reduced. In the 1 : 1 treatment, however, because nitrate was still observed in the leachate it is suggested that carbon availability was the limiting factor on microbial activity.

Observations of the organic matter content of the leachate appear to confirm that the calculated 1.65 : 1 is the optimum application ratio. In the 1 : 1 and 1.65 : 1 treatments only trace quantities of organics were observed in the leachates from the columns at low flow rates. In the 2 : 1 experiment, however, a high level of organic matter was present in the leachate, suggesting an over-supply of carbon to the microbial population, resulting in an excessive production of metabolites i.e., waste products of cell growth. 1.65 : 1 can therefore be said to be a more efficient ratio than 2 : 1 because all the carbon is utilised to reduce nitrate, and not to produce unwanted metabolites. It is therefore suggested that both the nitrate and organic matter observations confirm the optimum C : N ratio to be 1.65 : 1.

Table 5.5 Time from Start of Experiment for Redox to Fall Below 200mV and 50mV for Three Carbon to Nitrogen Ratios

C : N Ratios	Time (days)	
	< 200mV	< 50mV
1 : 1	4	8
1.65 : 1	3	5
2 : 1	3	4
Control (no glucose added)	6	no reading possible

The flow rates required to obtain a high nitrate reduction are lower than those measured by previous workers, and it is suggested that this is due to two factors. Glucose is not as stoichiometrically efficient a carbon source for microbial utilisation as for example, methanol, and that the lower ambient temperature of 10°C reduced microbial activity.

Comparison of Figures 5.3 and 5.4 indicates that nitrate reduction coincided with redox falling below 200mV, confirming this as the nitrate reduction threshold. Table 5.5 further indicates the time when redox fell below 50mV, a value below which it has been suggested nitrogen gas, and not nitrous oxide, prevails as the product of nitrate reduction (see Section 2.2.6).

The use of de-aired water was considered as an option to promote a reducing environment and so enhance nitrate reduction. If, however, the study results were to be representative of drainage water which contains dissolved oxygen, then solutions used in the experiments should also contain dissolved oxygen. De-aired water was therefore not used. Consequently, at high flow rates there was an increase in the import of dissolved oxygen per unit time, the result being a more oxidising environment, i.e., redox greater than 200mV, and a decline in nitrate reduction. It is postulated that higher flow rates further affect nitrate reduction because they reduce the contact time between nitrate reducing bacteria and nitrate, however, as described in Section 2.2.1 aeration is the dominant controlling parameter.

As flow rate is an important variable affecting nitrate reduction capability in attached growth/flow through water treatment systems it is necessary to use a measure of nitrate reduction performance that combines the nitrate concentration reduction achieved, the volume of water treated, and the volume of treatment bio-reactor. Gauntlett and Craft (1979) expressed performance in terms of grams of nitrate-nitrogen reduced per cubic metre of bio-reactor per day ($g/m^3/d$), describing it as the most useful way of expressing nitrate reduction rates from a design point of view. The average performance in the low temperature studies described was $19g/m^3/d$ at flow rates above 0.1 l/hr (Appendix III).

In the control, the level of nitrate in the leachate was observed to be initially lower (-5mg/l) and then higher (+5mg/l) than the concentration of nitrate in the influent solution (see Table 5.4). It is suggested that the initial nitrate reduction was a result of residual organic carbon in the soil being utilised by the microbial population, and that later increases in nitrate concentrations were a result of two factors. The first is that nitrate previously assimilated by the microbial population was released on death and degradation of bacteria (see Section 2.1.1.2), and secondly residual nitrate in the soil inoculum was leached out. Figure 5.4 illustrates redox fell below the 200mV threshold after 6 days. Significantly, however, nitrate reduction did not increase, re-affirming the need for supplemental carbon.

Figure 5.5 illustrates the characteristic decline in flow rate with time for all three replicates of the 1.65 : 1 treatment, a phenomenon also evident in all other treatments.

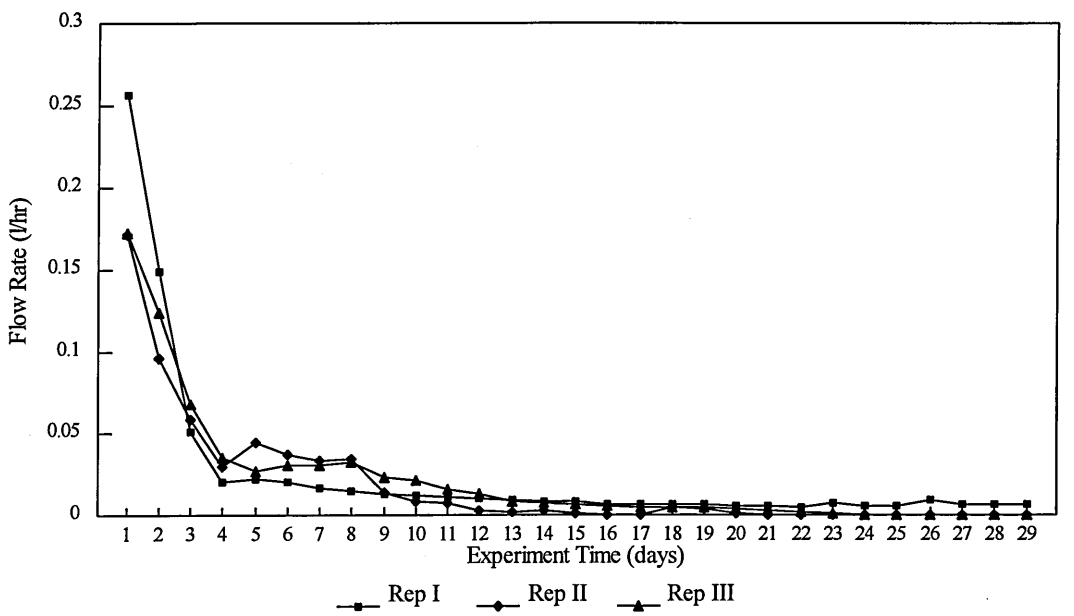

Figure 5.5 Reduction in Flow Rate against Experimental Time (1.65:1 Treatment)

The phenomenon is commonly associated with attached growth water treatment systems, where carbon added for the treatment process promotes the growth of biomass. Consequently, pore spaces become clogged reducing permeability and hence flow rates through the sand/soil medium. Such a system will eventually fail if the clogging problem is not effectively managed. Sugars in particular stimulate the growth of bacterial polysaccharide slimes, and these slimes were evident in these experiments on the top surface of the test cells, see Figure 5.6.

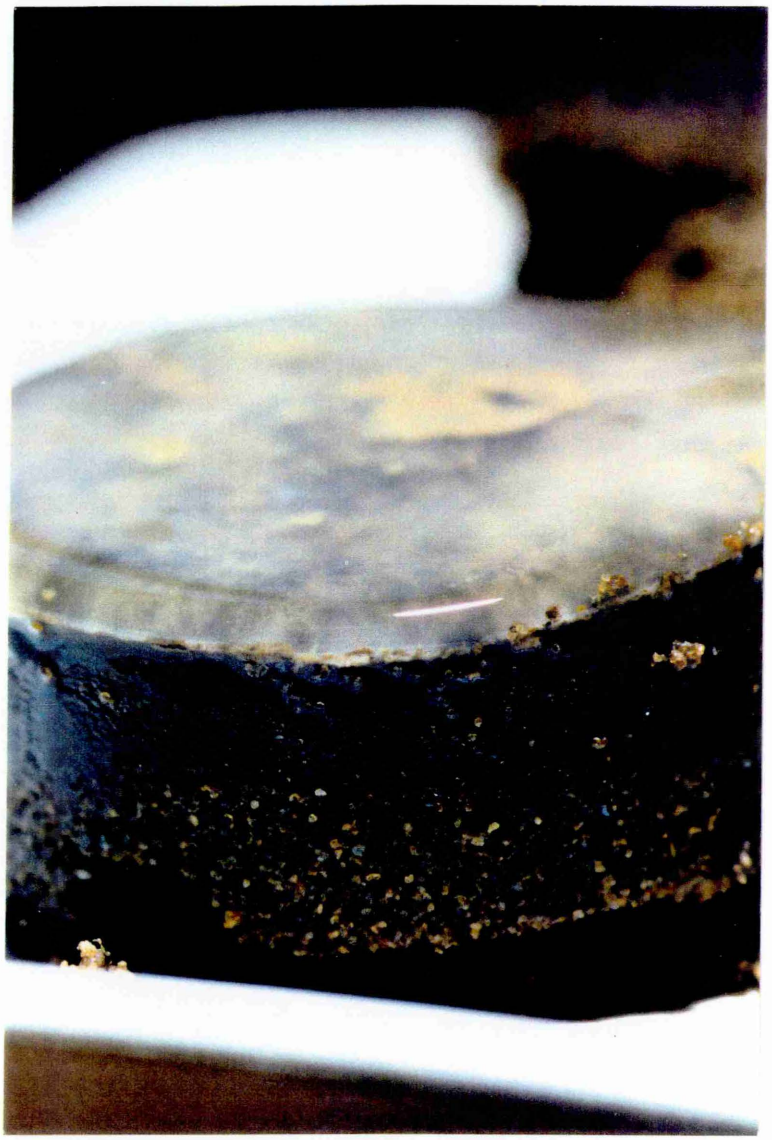

Figure 5.6 Bacterial Slime Layer at Top of Test Cell Soil Column

Bacterial count analysis (see Section 4.2.7) of the slimes and the sand/soil medium taken from increasing depths of the permeameter cells used in the 1 : 1 and 2 : 1 treatments confirm that biological growth and activity predominates in the upper half of the permeameter cells, see Table 5.6. Clogging is discussed in detail in Section 2.2.7, where possible management options are also outlined.

Table 5.6 Bacteria Count Data for 1 : 1 and 2 : 1 (C : N) Treatments

Depth of Sample from Top of Cell (mm)	Bacteria Count Data (cfu/ml x 10^6)					
	1 : 1			2 : 1		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
slime	80	228	74	31	250	100
0-5	39	328	4	16	85	5
5-15	10	6	1	8	7	11
15-130	24	7	3	15	11	8
130-230	9	6	1	7	9	3

5.2.3 Conclusions

Nitrate reduction at low temperatures is feasible; however, complete reduction of nitrate was only observed at flow rates of less than 0.1 l/hr. This is considerably lower than the 180 l/hr reported by Gauntlett and Craft (1979) who utilised methanol as the carbon source. It was postulated that the difference was a result of glucose not being as stoichiometrically efficient a carbon source as methanol, which reduces nitrate reduction performance in two ways. More glucose than methanol is required to achieve the same reduction in nitrate concentration, and because biomass growth is less when using methanol than when using glucose, biological clogging of the porous media is reduced, increasing the area of attached growth. A greater area of attached growth will improve nitrate reduction performance by providing more sites for nitrate reduction.

A nitrate reducing performance parameter was described which combines nitrate-nitrogen concentration reduction achieved, the volume of water treated, and the volume of treatment bio-reactor. Expressing performance in terms of grams of nitrate-nitrogen reduced per cubic metre of bio-reactor per day ($g/m^3/d$) allows comparison of treatment systems from a design point of view. The low temperature studies, using glucose as the carbon source, had an average performance of $19g/m^3/d$, and this value will be compared to that determined for sugar beet in a later study (Section 5.4.2).

The efficiency experiment confirmed the theoretical carbon to nitrogen ratio of 1.65 : 1 as the optimum ratio when utilising glucose as the carbon source in attached growth nitrate reducing water treatment systems.

Redox measurement confirmed the nitrate reduction threshold at 200mV. Measurement of redox could therefore be used to monitor, and so aid control of, the anaerobic environment in later studies.

If attached growth systems using sand/soil mixtures were to be utilised on-farm to treat drainage water, successful management of the clogging phenomenon would be paramount in the feasibility and economics of any system chosen.

5.3 Biodegradation of Organic Materials Study

The objective of this study is to investigate the biodegradation of organic materials at low temperature and under anaerobic conditions, and so ascertain the viability of using organic materials as sources of readily utilisable carbon for microbial nitrate reduction. Results of this study are therefore presented in two sections:

i) Examination of Organic Materials as Possible Carbon Sources

ii) Comparison of Sugar Beet to Glucose.

5.3.1 Examination of Organic Materials as Possible Carbon Sources

Previous researchers had used a headspace sampling method (Section 4.2.5.1) for the assessment of anoxic (methanogenic) degradation. This technique offered the potential in this experiment to allow the assessment of the range of gaseous products produced including nitrous oxide. Unfortunately, consultation with present workers into the feasibility of using the method for anaerobic degradation assessment failed to highlight a major problem, which only became evident once experimentation had begun.

Carbon dioxide (CO_2) gas produced by the micro-organisms degrading the organic material collected in the headspace as expected. It is postulated, however, that before the CO_2 had been drawn off it dissolved, under partial pressure, into the biodegradation flask medium. Comparison of the calculated theoretical carbon dioxide yields to those measured, and a reduction in pH from a starting pH of 6.5, suggest that it was this phenomenon that prevailed, see Table 5.7.

The results, although by no means absolute, still allow a comparison of the relative degradation rates of the materials. The average levels of CO_2 measured for straw, molassed straw, sugar beet and glucose in the headspace experiments were 0.13%, 0.25%, 1.83%, and 3.10% respectively. From this data it is evident that the only organic material tested which was capable of generating significant quantities of readily utilisable carbon (RUC) for microbial nitrate reduction was sugar beet, and hence this was the only organic material tested in further experimentation.

The pH data also indicates a decrease in pH from the initial solution pH of 6.5. It is postulated that this was a result of CO_2 being dissolved under pressure to form carbonic acid ($H_2O + CO_2 = H_2CO_3$), the presence of which decreased the medium pH. This hypothesis is supported by the largest pH decreases occurring in the flask containing glucose, i.e., the flask that would theoretically have produced the most carbon dioxide gas, and consequentially the most carbonic acid.

Table 5.7 Comparison of Measured Carbon Dioxide to Maximum Theoretical Production Using Headspace Sampling Technique

Treatment	Theoretical Max. CO_2 (mg)	CO_2 Measured (mg)	% CO_2	% Glucose Used	Flask Solution pH at Termination
SB1	17563	294	1.67	-	5.98
SB2	17563	316	1.8	-	5.76
SB3	17563	352	2.01	-	5.81
S1	17563	27	0.15	-	6.13
S2	17563	20	0.11	-	6.28
S3	17563	22	0.13	-	6.08
SM1	17563	38	0.22	-	6.09
SM2	17563	50	0.29	-	6.01
SM3	17563	43	0.25	-	5.97
G1	17563	364	2.07	17	5.43
G2	17563	260	1.48	18	5.59
G3	17563	1012	5.76	70	5.31

Replicates: SB - sugar beet, S - straw, SM - straw soaked with molasses, G - glucose

For glucose, analyses were also carried out on the solutions in the flasks to ascertain the quantity of nitrate and glucose reduced. In G3, 70% of the glucose and 74% of the nitrate had been reduced, but only 5.76% of the theoretical carbon dioxide production value had been produced, significantly less than the predicted value of 55%.

Due to the failure of the headspace technique to give absolute results, some of the experiments were repeated with a previously verified titrimetric method of CO_2 analysis (Section 4.2.5.2). This titrimetric method of CO_2 analysis was also applied to glucose flask G3, which had used the failed headspace sampling method, and an additional

18.6% of theoretical CO_2 production was recovered. The total quantity recovered i.e., 24.4% is comparable to that measured in the repeated experiments (Table 5.10), assuming that in addition to the organic carbon being utilised to form biomass, a proportion of the CO_2 would have irreversibly hydrated under pressure to form carbonic acid.

5.3.2 Comparison of Sugar Beet and Glucose

A direct comparison, based on the titrimetric method, of the CO_2 production rates during the degradation of sugar beet and glucose can be made, and is illustrated in Figure 5.7 where G1, G2, and G3 represent the glucose replicates, and SB1, SB2, and SB3 the sugar beet replicates.

Figure 5.7 indicates an initial low production rate of carbon dioxide followed by a gradual increase in production to a maximum point which is only sustained for a limited period and then gradually declines to a very low production rate. The sigmoid curve produced is characteristic (OECD, 1981; HMSO, 1989) for carbon dioxide production resulting from the degradation of organic compounds, and was observed in all cases except G3. It is evident that G1 and G2 behaved similarly, and that there was a significant difference between those two and the sugar beet replicates, which were all similar. This behaviour can be accounted for by the difference in initial soluble carbon content i.e., 4790mg in the glucose replicates and only 1078mg in the sugar beet replicates.

By examining the results it is evident that the pH has a major controlling influence over production rates. This influence is indicated by the premature cessation of CO_2 production for G3, with Figure 5.8 illustrating that the pH for G1 and G2 was alkaline and the pH of G3 was acid. Comparison of Figures 5.7 and 5.8 also indicates that the pH of the medium in G3 had returned to alkaline following resumption of CO_2 production.

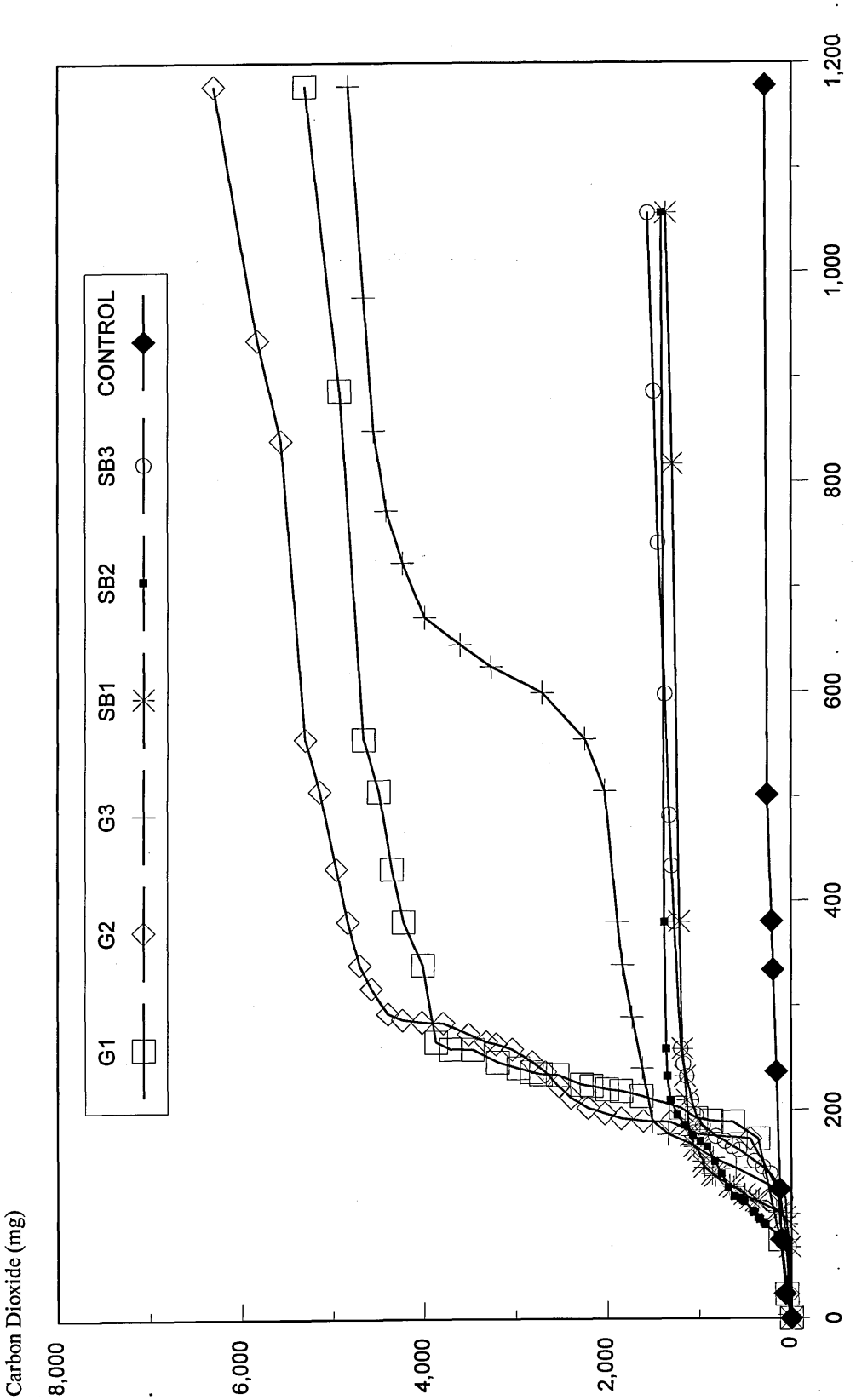

Figure 5.7 Cumulative Mass of Carbon Dioxide Produced against Time (Glucose and Sugar Beet Replicates)

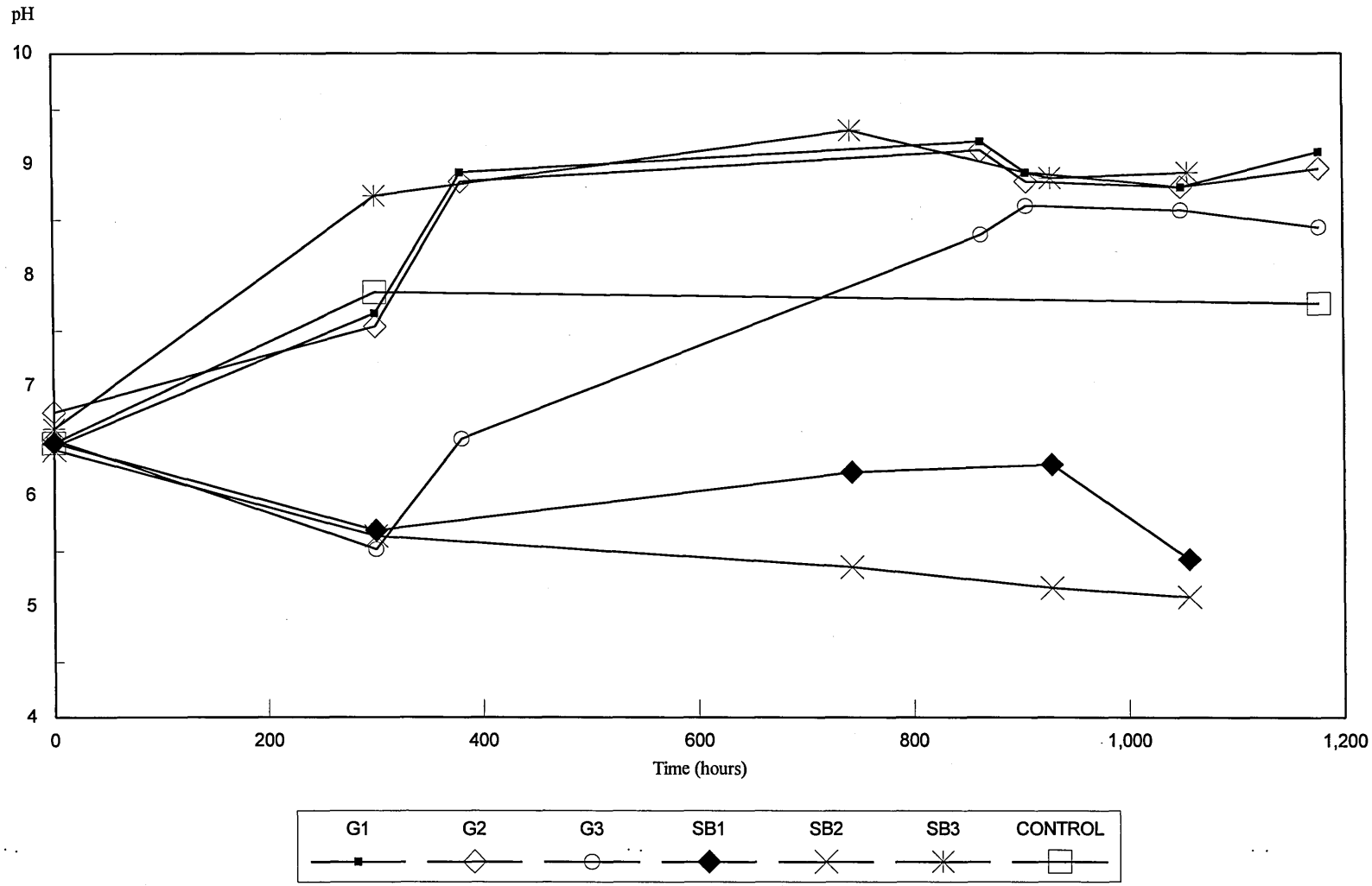

Figure 5.8 Biodegradation Flask Medium pH against Time (Glucose and Sugar Beet Replicates)

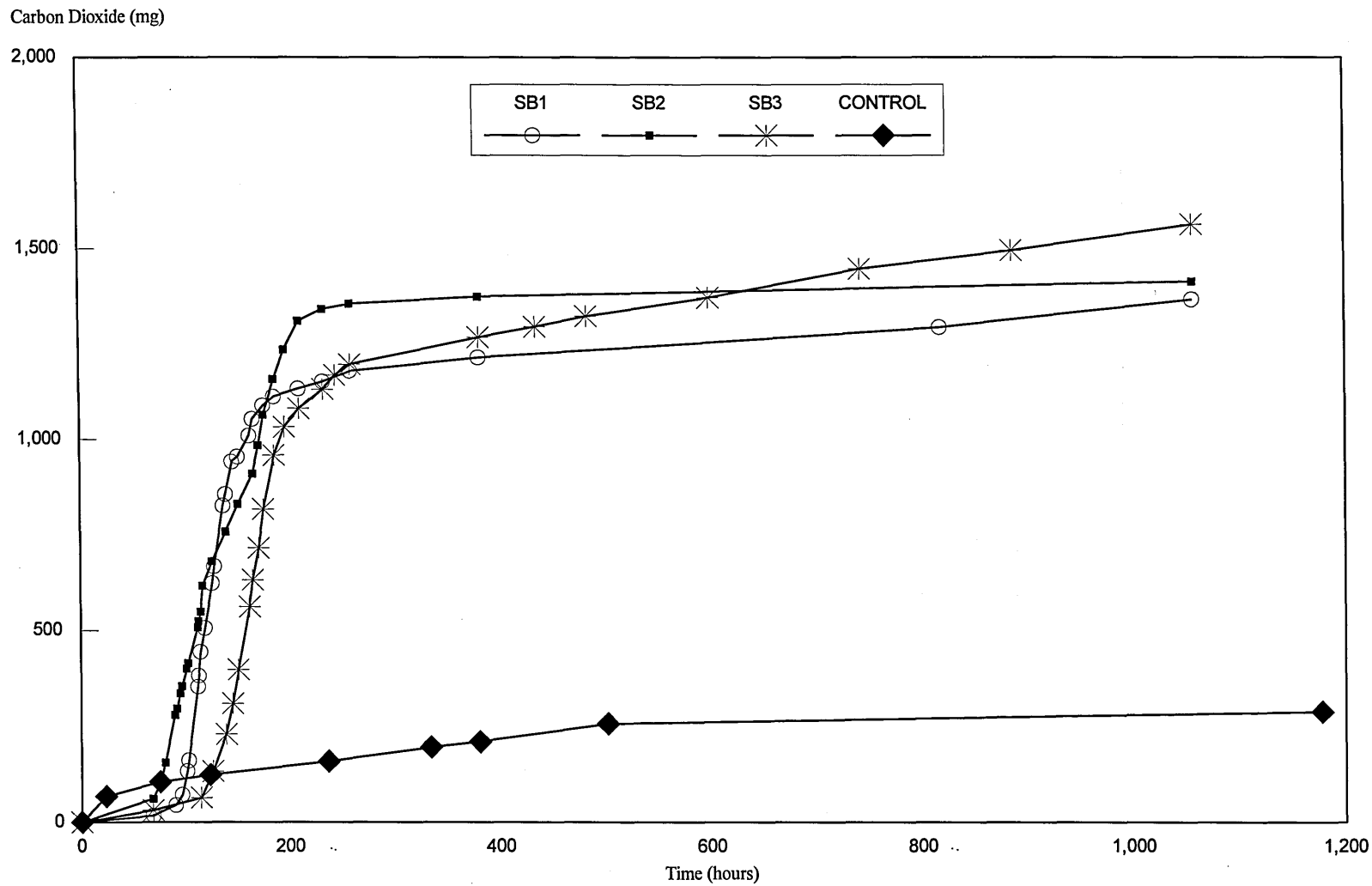

Figure 5.9 Cumulative Mass of Carbon Dioxide Produced against Time (Sugar Beet Replicates)

It is suggested that the reason why SB1 and SB2 still produced a sigmoidal curve, even though they became acidic, is because the initial quantity of readily utilisable carbon in the sugar beet was not great enough to register the cessation observed in G3. This view is supported because G3, even though it had become acidic, produced more CO_2 before stalling, than any of the three sugar beet replicates, see Figure 5.7.

Figure 5.9 (enlarged scale of the sugar beet replicates in Figure 5.7) when compared to Figure 5.8 illustrates that there is a difference in the shape of both the carbon dioxide production curves and the pH for the sugar beet replicates. SB3 has a longer adaption period than SB1 and SB2, and sustains a longer period of logarithmic growth than either SB1 or SB2. The prime difference between the treatments is that SB3 is in an alkaline environment and SB1 and SB2 in an acid one.

pH is also a major indicator of the bio-chemical system that develops within the flask. The flask pH would be expected to rise, if nitrate reduction to N_2 was occurring, due to the release of hydroxyl ions. The data in Table 5.8 shows this phenomenon for G1, G2, and SB3. After 300 hours 83.5%, 82.1%, and 33.5% of the nitrate added had been reduced in G1, G2 and SB3 respectively. In G3, SB1, and SB2, i.e., the flasks with an acidic environment, only 24.4%, 1.8%, and 2.6% of the nitrate had been reduced respectively.

It is postulated that the fall in pH in the sugar beet flasks SB1 and SB2 is due to the sugars being fermented, resulting in the production of organic acids e.g. lactic acid. If fermentation did prevail, lactic acid bacteria common to vegetable matter become prolific acid producers. Fermentation is therefore associated with a lowering of pH.

An explanation for the variation of the pH in G3 is not so obvious. The test procedure was the same for all the glucose flasks even to the point that the inoculum was from the same batch. Why G3 should initially become acidic, suppressing nitrate reduction, is not clear and difficult to explain without physiological data of the bacterial populations.

Table 5.8 Comparison of Nitrate Reduced, Glucose Used and pH after 300 Hours

Flask	Glucose Used (mmol/l)	Nitrate Reduced (mg/l NO_3-N)	pH	
			Start	300 Hours
G1	16.6 (100%)[1]	605 (83.5%)[2]	6.45	7.66
G2	16.6 (100%)	600 (82.1%)	6.76	7.54
G3	8.6 (48.2%)	177 (24.4%)	6.51	5.52
SB1	-	13 (1.8%)	6.48	5.69
SB2	-	19 (2.6%)	6.42	5.64
SB3	-	243 (33.5%)	6.56	8.72
CON		27 (3.7%)	6.48	7.85

[1] Figures in parenthesis give the glucose used as a percentage of initial glucose concentration.

[2] Figures in parenthesis give the nitrate reduced as a percentage of initial nitrate concentration.

When the same parameters were compared for each flask at the termination of the experiment (1200 hours), the remaining glucose in G3 had been utilised, with a consequent decrease in the nitrate concentration and rise in pH, G3 had become alkaline, see Table 5.9.

Comparison of the sugar beet replicates indicates more nitrate in flasks SB1 and SB2 at the termination of the experiment than at 300 hours. If as postulated fermentation prevailed in SB1 and SB2, one of the products could have been amino acids (Wood, 1989). The hydrolysis of amino acids to ammonium (Wild, 1988b), with subsequent oxidation to nitrate, is a possible explanation for the nitrate increase.

On examining Figure 5.7, it is apparent that over the 50-300 hour period, i.e., when the highest rates of CO_2 production occurred, the ratio of CO_2 produced by glucose to that

produced by sugar beet was approximately 4 : 1. In the glucose treatments all the sugar was soluble and therefore readily utilisable by the microbial population. In the sugar beet replicates only 22.5% of the sugar beet is a soluble fraction, the remainder constituting cellular material. The ratio of initial soluble sugar contents between the sugar beet and glucose tests was therefore 4.4 : 1, and hence it can be concluded that the readily utilisable fraction in both systems had been used by 300 hours (12.5 days).

Table 5.9 Comparison of Nitrate Reduced, Glucose Used and pH at Termination of Experiment

Flask	Glucose Used (mmol/l)	Nitrate Reduced (mg/l NO_3-N)	pH	
			300 Hours	Termination
G1	16.6 (100%)[1]	671 (92.6%)[2]	7.66	9.12
G2	16.6 (100%)	656 (90.5%)	7.54	8.97
G3	16.6 (100%)	583 (80.4%)	5.52	8.44
SB1	-	9 (1.2%)	5.69	5.43
SB2	-	-16 (-2.2%)	5.64	5.09
SB3	-	317 (43.7%)	8.72	8.93
CON		42 (5.8%)	7.85	7.75

1 Figures in parenthesis give the glucose used as a percentage of initial glucose concentration.

2 Figures in parenthesis give the nitrate reduced as a percentage of initial nitrate concentration.

Comparison of the measured CO_2 values for glucose and sugar beet given in Table 5.10 indicate that experimental error in collection did not cause the difference between those and predicted values of CO_2 production. This is because the sugar beet replicates produced similar results to the glucose replicates, i.e., only 25-35% of the readily

utilisable carbon fraction was respired. (N.B. Readily utilisable carbon fraction of glucose is 100%, and 22.5% for sugar beet).

In Section 4.2.3.1 it was stated that 55% of the glucose carbon could be evolved as CO_2. This is significantly greater than the 22% and 25% values measured from G1 and G2 respectively in the first 300 hours, see Table 5.10. It is assumed that the remaining 75% was assimilated into microbial biomass and bacterial polysaccharide slimes, such as those observed in the efficiency of nitrate reduction study (Section 5.2.2). Hence, after 300 hours microbial respiration in G1 and G2 had become limited due to carbon substrate exhaustion, and the bacteria would have started to die. The biomass would in turn become a substrate source of carbon, eventually to be released as CO_2. This trend was observed for both G1 and G2, shown in Figure 5.10, where carbon dioxide continues to be produced, but at a slower rate.

Table 5.10 Comparison of Carbon Dioxide Produced at 300 Hours and at Termination of Experiment

Flask	Theoretical Max. CO_2 (mg)	CO_2 Measured at 300 Hours (mg)	CO_2 Measured at Termination (mg)
G1	17563	3939 (22.4%)[1]	5313 (30.3%)[1]
G2	17563	4456 (25.4%)	6306 (35.9%)
G3	17563	1751 (9.9%)	4839 (27.6%)
SB1	17563	1192 (6.8%)	1368 (7.8%)
SB2	17563	1362 (7.8%)	1415 (8.1%)
SB3	17563	1221 (7.0%)	1565 (8.9%)
CON	-	188	288

[1] Figures in parenthesis give the carbon dioxide evolved as a percentage of theoretical maximum.

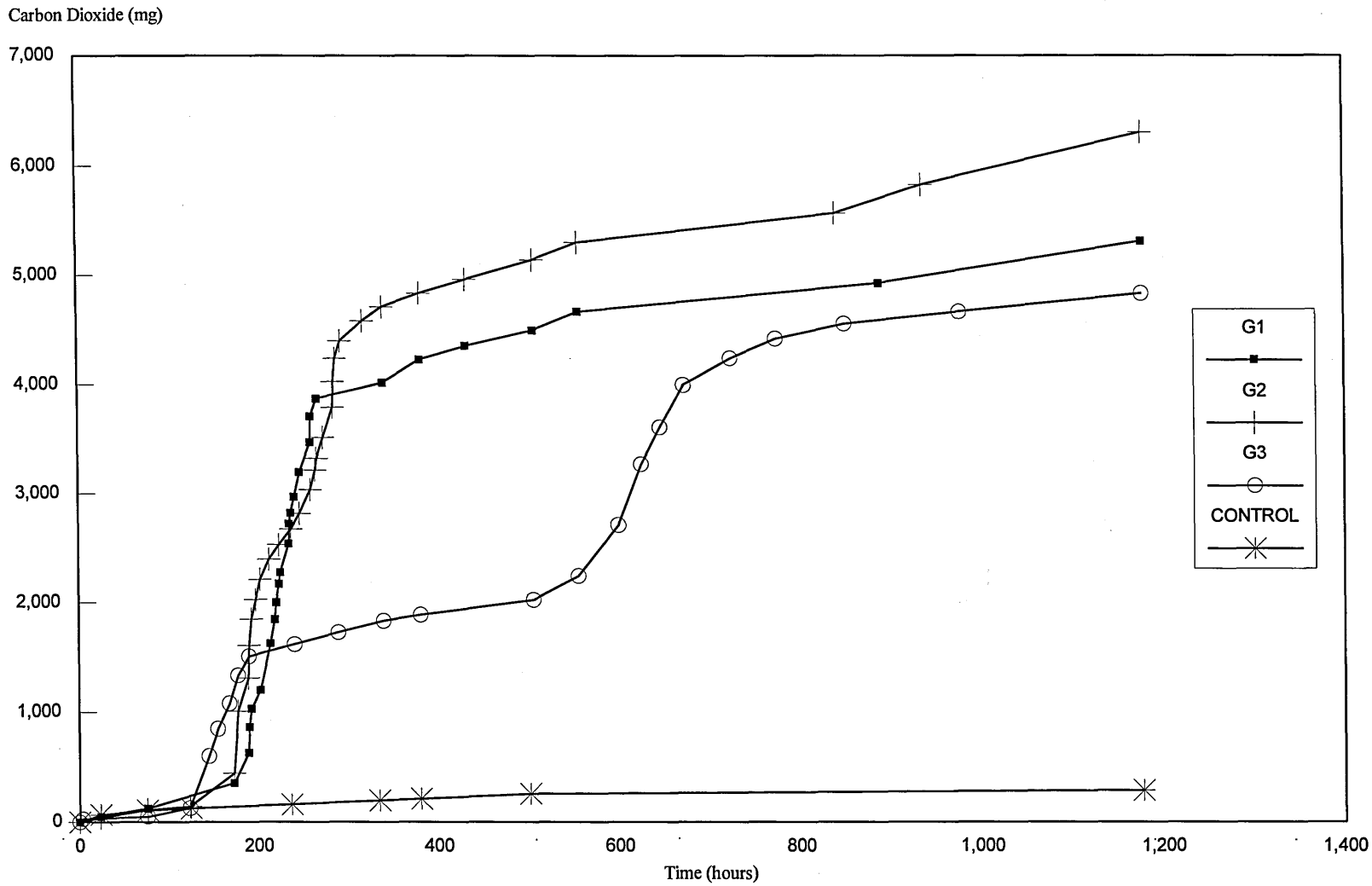

Figure 5.10 Cumulative Mass of Carbon Dioxide Produced against Time (Glucose Replicates)

It is postulated that the reason for the difference in predicted and measured values of CO_2 production for both glucose and sugar beet, is due to the experiments being carried out at the lower temperature of 10°C, compared to those reported by McCarty et al. (1969) which were carried out at 20°C. The development of microbial biomass and slimes at the lower temperatures are, it is suggested, products of a reduced metabolic efficiency.

Further examination of the results suggest that the CO_2 production rates at the end of the experiment, i.e., resulting from the degradation of the biomass in the glucose flasks, are comparable to the final CO_2 production rates measured for sugar beet. This view would be supported if CO_2 production in SB3 were a result of organic material degradation i.e., the 77.5% of the sugar beet that is the non-soluble fraction, and not a result of microbial death and decomposition. Evidence for this came from the production of a pungent odour by SB3 during degradation. Under aerobic conditions micro-organisms normally excrete excess nitrogen from organic sources as ammonium ions. Under anaerobic conditions, however, the formation of foul-smelling amines occurs, e.g. indole and skatole (Harris, 1988). It is postulated that it was this phenomenon that developed, supporting the hypothesis that degradation of the sugar beet and not dead microbial biomass was occurring.

The rate of CO_2 evolution, measured at the end of the experiment, from SB3 is therefore equivalent to the sustainable rate of readily utilisable carbon, the elucidation of which was the main aim of the experiment. The contrasting optimum and final rates of carbon dioxide production are given in Table 5.11.

For the purposes of assessing the potential of using sugar beet as a carbon source it is necessary to contrast the optimum CO_2 evolution rate for glucose to the sustainable CO_2 evolution rate of sugar beet. This is because the glucose value is what would be "sustained" if a continual drip-feed mechanism of adding glucose were used to treat waterborne nitrate. If an average figure of 57.8mg CO_2/hour for glucose is compared with 0.42mg CO_2/hour for sugar beet, assuming that only results from G1, G2, and SB3

are valid, then the maximum availability of readily utilisable carbon from sugar beet would only be 0.7% that of glucose.

Table 5.11 Comparison of Optimum Carbon Dioxide Production Rate and the Rate Measured at Termination of the Experiment

Flask	Optimum Rate of CO_2 Production (mg/hr)	Time at which Optimum CO_2 Production Occurred (hours)	Rate of CO_2 Production at Termination (mg/hr)	Time at which Experiment was Terminated (hours)
G1	60.28	220	1.31	1178
G2	55.27	189	1.97	1178
G3	26.9	177	0.84	1178
SB1	24.15	115	0.24	1056
SB2	15.13	116	0.06	1056
SB3	21.66	165	0.42	1056
CON	2.84	24	0.05	1178

5.3.3 Conclusions

It was observed in the glucose treatment that all the glucose was degraded within 13 days of addition to a nutrient medium, with a subsequent reduction of nitrate concentration from 725mg/l NO_3-N to 125mg/l NO_3-N, due to anaerobic respiration. It is therefore concluded that glucose is an unsustainable source of readily utilisable carbon.

The ratio of assimilated carbon to that dissimilated for glucose was 3 : 1. Previous studies (Gauntlett and Craft, 1979) have measured a ratio of 0.82 : 1. It is postulated

that this difference is a result of the low temperature at which experimentation was carried out, i.e., 10°C, reducing metabolic efficiency.

After 13 days of the experiment, sugar beet had produced 28% of the quantity of carbon dioxide evolved from glucose. As the soluble fraction of the sugar beet was 22.5%, it is postulated that the readily utilisable fraction of the sugar beet had been utilised and therefore biodegradation of the cellulosic component of the sugar beet was occurring.

The presence of an odour with continued CO_2 production from the sugar beet, was ascribed to the degradation of sugar beet and not dead microbial biomass. Hence, sugar beet can be said to be a sustainable source of carbon in an anaerobic environment. The sustainable carbon release rate for sugar beet was measured as 0.42mg of carbon per hour, equivalent to 0.7% of the glucose release rate.

The experiment was not designed to ascertain the state and physiology of microbial populations and biomass, however, it can be concluded that a near neutral environment should prevail to ensure optimum nitrate reducing conditions.

5.4 Combined Organic Material and Low Temperature Study

The objective of this study was to investigate nitrate reduction at low temperature, using sugar beet as the carbon source. The experimental data from the study enabled the development of a model that represents a relationship between nitrate reduction and several environmental control parameters. The results of this study are therefore presented in three sections:

i) Bench Diffusion Study
ii) Flow Study
iii) Model Development.

5.4.1 Bench Diffusion Study

Table 5.12 gives the carbon release rates for the four sizes of sugar beet cube tested, i.e. 5mm, 10mm, 20mm, and 40mm, where the release rate is expressed in terms of grams of carbon released per 100 gram of sugar beet per hour. N.B. Sugar (sucrose) contains 42% carbon.

Table 5.12 Carbon Release Rate (gC/100g/hr) for Four Sizes of Sugar Beet Cube

Time (hrs)	Sugar Beet Cube Size (mm)			
	40	20	10	5
5	0.112	0.152	0.216	0.328
24	0.019	0.021	0.032	0.038
48	0.008	0.014	0.022	0.023
120	0.006	0.01	0.013	0.027
336	0.003	0.008	0.011	-

The data indicates a rapid diffusion of carbon from all four sizes of sugar beet cube following their submersion in water, and is clearly illustrated in Figure 5.11. Between 5 and 24 hours, however, there is a reduction in the release rates by an order of magnitude. Although less dramatic, the rate of carbon release continues to decline for all four sizes over the 24-336 hour period. Carbon is exhausted in the 5mm cubes before 336 hours.

The release rate for 5mm cubes could not be calculated at 336 hours because all the soluble fraction of the sugar had already been released. However, using the release rate after 120 hours and interpolating suggests that the soluble carbon fraction would have been exhausted between 250 and 300 hours.

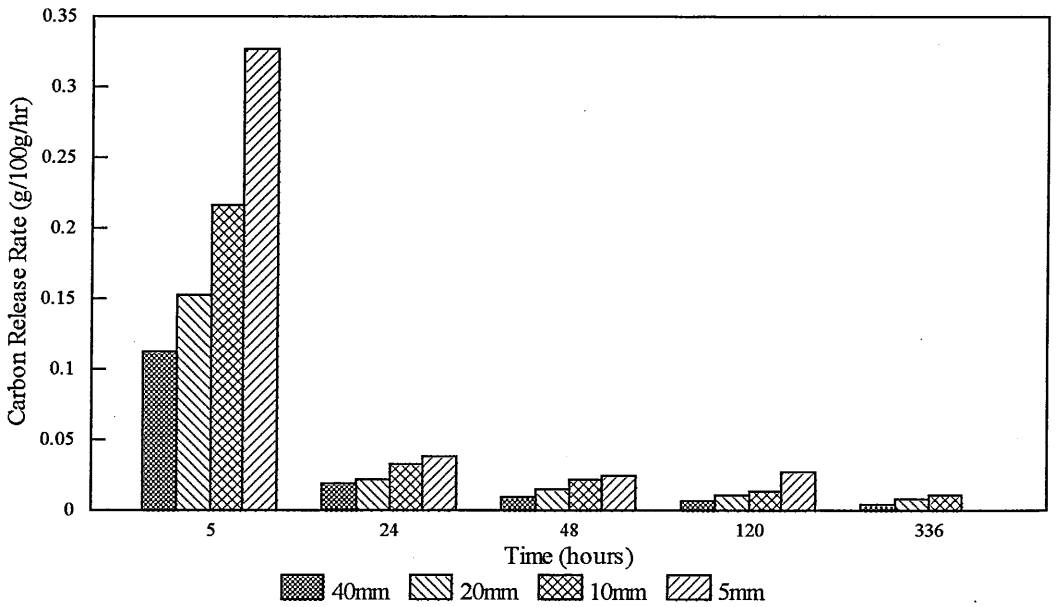

Figure 5.11 Comparison of Soluble Carbon Release Rates for Four Sizes of Sugar Beet Cube

As the initial soluble carbon content of the sugar beet is known (8.4%), and using the final release rates for the 40mm, 20mm, and 10mm cube sizes, the periods of time in which carbon will continue to be released can be extrapolated. These are approximately 85 days, 24 days, and 11 days respectively, and therefore the estimated total release times are 99 days, 38 days, and 25 days respectively.

If sugar beet is to be considered for use as the carbon source in a nitrate reducing water treatment system, it will be necessary to maintain carbon supply over the whole treatment period. It is suggested that the treatment period would be between 30 and 90 days (Section 6.5.3.4) and therefore the total release times indicate the only viable sizes of sugar beet cube to be 20mm and 40mm.

5.4.1.1 Conclusion

Previously, the biodegradation study (Section 5.3) showed that the microbial population will readily utilise the soluble fraction of ground sugar beet pieces to reduce nitrate

under anaerobic conditions. The results of the bench study illustrate that by increasing the size of sugar beet pieces the rate of soluble carbon release decreases, thus increasing the overall release time to several weeks instead of seconds. Comparison of the carbon release times lead to the conclusion that sugar beet pieces of between 20mm and 40mm in size could be used as a carbon source in nitrate reducing treatment systems.

5.4.2 Flow Study

5.4.2.1 Treatment A : Without pH Control

After 72 hours, intense microbial activity was observed within the permeameter cells. Figure 5.12 illustrates the nature of this activity i.e., gases produced as a result of the intense microbial activity accumulated at the top of the permeameter cell creating a froth.

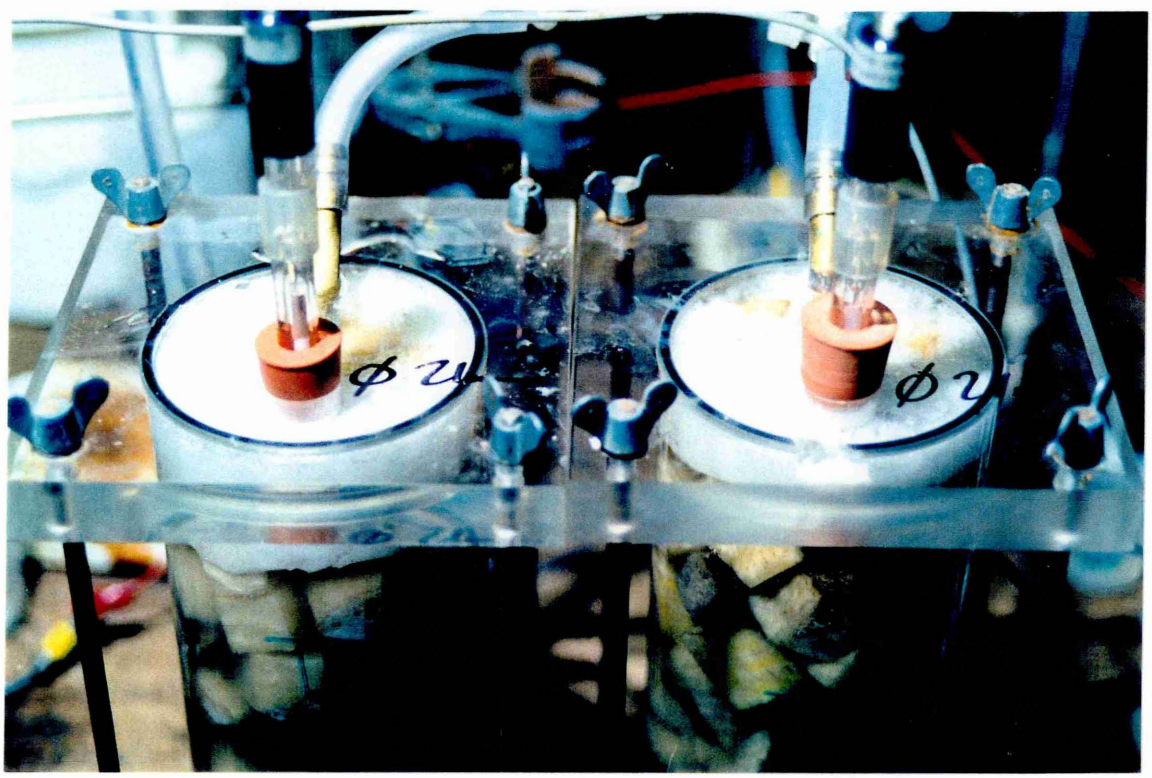

Figure 5.12 Activity Observed after 72 Hours in Flow Study : Without pH Control

The intense microbial activity continued and a concurrent decline in flow rate was measured. Although not quantitatively measured leachate from the cells was observed to be viscous, and it was postulated that this viscosity explained the reduction in flow rate. Flow rates continued to decline until manual adjustment of the outflow taps was required to ensure flow from the test cells continued. It was, however, not long before further manual adjustment was required. Leachate collected at the very low flows was analysed and found to contain nitrate-nitrogen concentrations above 18mg/l NO_3-N. Further analysis of these samples showed them to also have high ammonia (NH_3-N) concentrations, and low pH (4-5). Results for specific flow rates during the flow study without pH control are given in Table 5.13.

Table 5.13 Flow Study : Without pH Control - Sample of Data

Flow Rate (l/hr)	NO_3-N Reduction (mg/l)	NH_3-N in Effluent (mg/l)	Redox (mV)	pH of Effluent
0.54	0.12	5.41	363	4.9
0.39	0.94	5.65	90	4.8
0.2	1.72	6.18	62	4.5
0.1	2.5	7.4	248	4.6
0.09	2.89	7.14	248	4.4
0.08	1.57	6.23	132	4.4
0.07	2.21	7.95	227	4.2
0.06	3.87	6.26	26	4.3
0.05	1.9	6.08	60	4.3

The results suggest that microbial degradation of the organic material produced ammonia and organic acids (Harris, 1988), evident by the presence of ammonia in the leachate and a decrease in environmental pH. It is postulated that the increased acidity of the environment prevented nitrate reduction. However, it is also feasible that any dissimilatory nitrate reduction which occurred may have been countered by the nitrification of ammonia (Section 2.1.1.2) producing further nitrate. Such a

phenomenon may also explain the variations in both the nitrate and redox measurements.

The experiment confirms the importance of pH as an environmental control parameter, and it can be concluded that without pH control organic materials cannot be considered sustainable sources of readily utilisable carbon in nitrate reducing water treatment systems.

5.4.2.2 Treatment B : With pH Control

The variations of nitrate reduction and flow rate over the experiment time are illustrated in Figures 5.13, 5.14, and 5.15. The data sets for the three replicates can be found in Appendix V.

Referring to the figures, all three replicates experienced a variation of both flow rate and nitrate reduction throughout the establishment period of 0-350 hours. Initially, manual adjustment to reduce flow rates was required to allow microbial utilisation of the soluble sugar. However, further reductions in flow were caused by an increase in the viscosity of the solution, and it is postulated that this was a result of the high microbial activity during the establishment phase. Flow rates were therefore increased by manual adjustment of the taps to ensure hydraulic failure, i.e., stalling, did not occur. It can be said that the system 'over-heated' in the initial stages, this phenomenon becoming an important consideration in management of a treatment system utilising sugar beet.

Following the establishment period of approximately 350 hours, flow rates could be set and maintained without further alteration. Further flow rate changes were made, however, to assess the effect of flow rate variation on nitrate reduction and performance. A sample of data from the experiments is given in Tables 5.14, 5.15, 5.16. The data illustrates a relationship between nitrate reduction, flow rate, redox, and indicates a possible relationship with ammonia. A model of this relationship is developed below in Section 5.4.3.

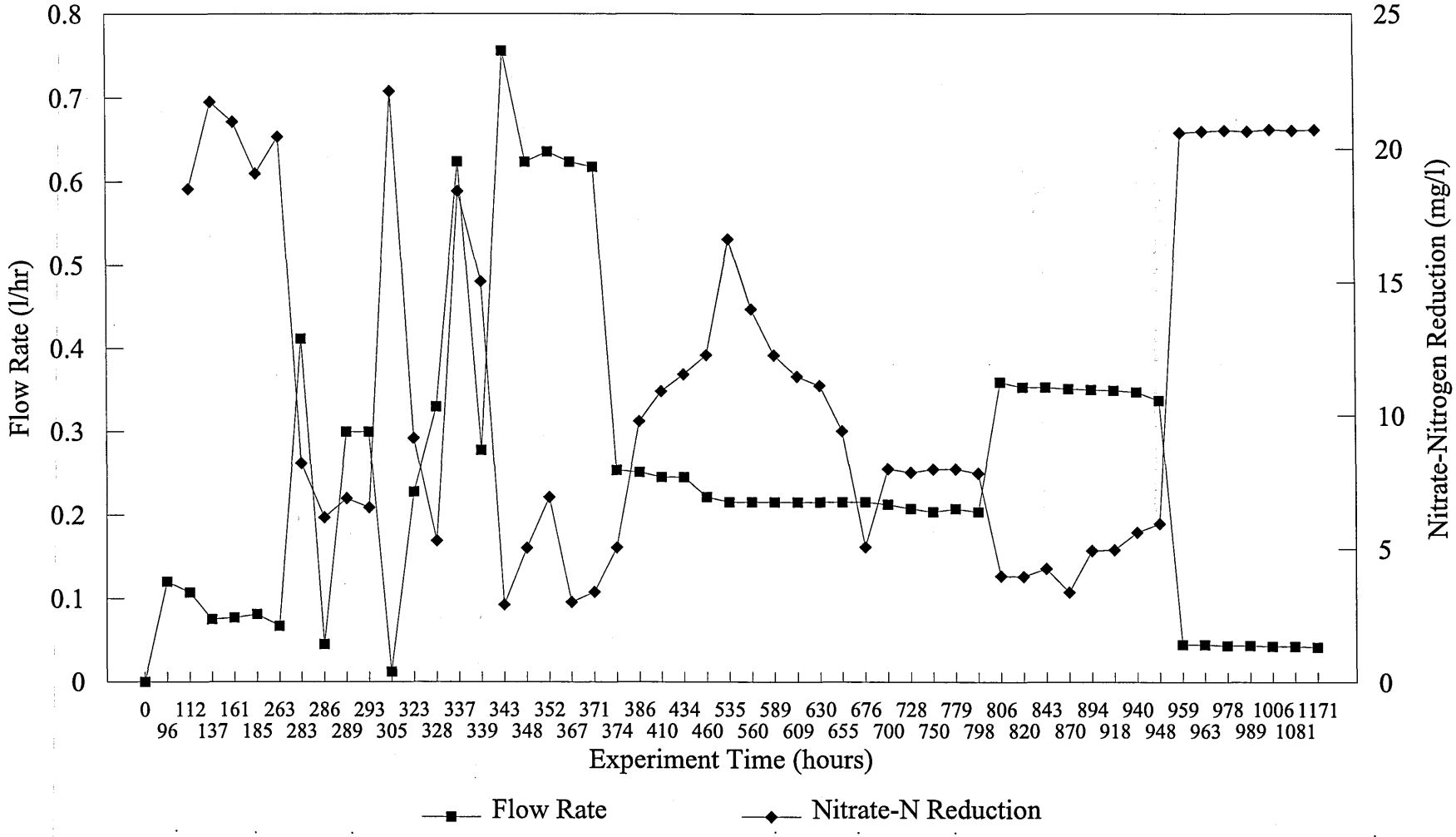

Figure 5.13 Variation of Nitrate-Nitrogen Reduction and Flow Rate against Time - With pH Control : Replicate I

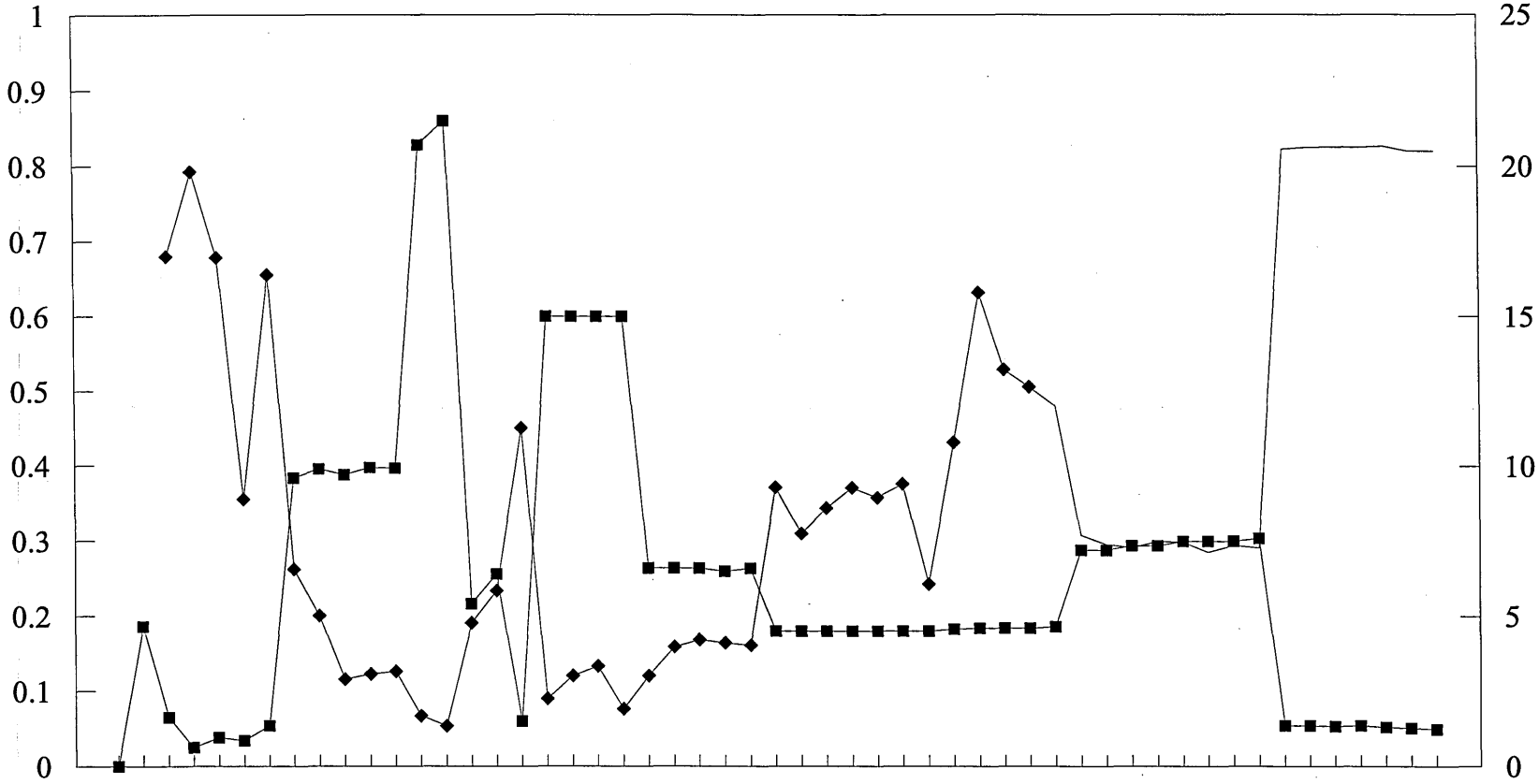

Figure 5.14 Variation of Nitrate-Nitrogen Reduction and Flow Rate against Time - With pH Control : Replicate II

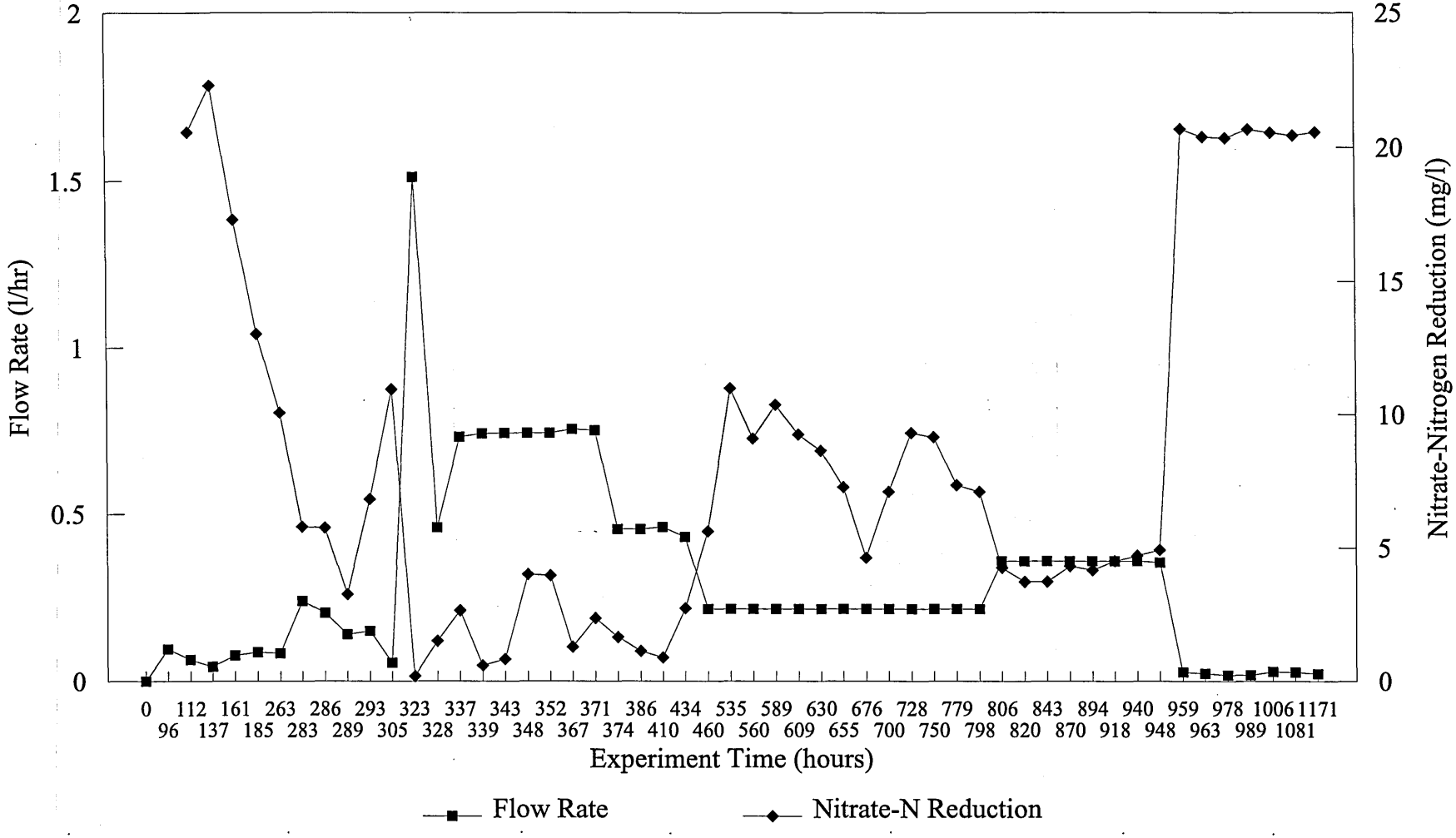

Figure 5.15 Variation of Nitrate-Nitrogen Reduction and Flow Rate against Time - With pH Control : Replicate III

Table 5.14 Flow Study : With pH Control - Sample of Data from Replicate I

Time (hrs)	Flow rate (l/hr)	Nitrate-nitrogen reduction (mg/l)	Redox (mV)	NH_3-N (mg/l)	pH	Performance ($g/m^3/d$)
185	0.08	19	-31	6.24	7.03	18.9
366.5	0.62	3	79	1.7	6.86	22.8
560	0.22	14	-119	3.9	6.47	37
778.5	0.21	8	-53	3	6.42	20.4
939.5	0.35	5.6	210	1.2	6.31	24
1171	0.04	20.7	-76	0.2	7.02	10.6

Table 5.15 Flow Study : With pH Control - Sample of Data from Replicate II

Time (hrs)	Flow rate (l/hr)	Nitrate-nitrogen reduction (mg/l)	Redox (mV)	NH_3-N (mg/l)	pH	Performance ($g/m^3/d$)
185	0.03	8.9	-47	4.75	5.96	3.7
366.5	0.6	3.3	120	0.6	6.14	24.5
560	0.18	7.8	212	2.75	6.69	17.1
778.5	0.18	12.7	137	0.85	6.37	28.5
939.5	0.3	7.4	9	1.4	6.29	27.1
1171	0.05	20.5	-49	0.4	6.57	12.3

Table 5.16 Flow Study : With pH Control - Sample of Data from Replicate III

Time (hrs)	Flow rate (l/hr)	Nitrate-nitrogen reduction (mg/l)	Redox (mV)	NH_3-N (mg/l)	pH	Performance ($g/m^3/d$)
185	0.09	13	-118	7.55	6.9	13.7
366.5	0.76	1.3	72	0.6	5.47	22.8
560	0.22	9.1	-51	6.25	6.61	24.1
778.5	0.22	7.4	46	4.2	6.18	19.4
939.5	0.36	4.7	122	2.45	6.22	20.7
1171	0.04	20.6	-183	1.25	5.88	5.5

After 40 days (960 hours) the flow rates in all three replicates were reduced to 0.045, 0.054, and 0.027 litres per hour respectively. This was done to determine the performance of the system at very low flow rates, and also examine whether at such low flow rates the problem of 'over-heating' would re-occur. All the nitrate was reduced, and flow rates did not decline, proving the system to be hydraulically sustainable. Nitrate exhaustion, however, meant the system had become limiting to microbial activity, and thus was not operating at optimum capacity. Performance in terms of grams of nitrate-nitrogen reduced per cubic metre of sugar beet per day was consequently decreased. Indeed, the data indicates that for flow rates between 0.18-0.3 litres per hour, the average performance attained was 23g/m^3/d (Appendix V).

It was hypothesised that sugar beet cubes could be used as both the attached growth site and source of readily utilisable carbon for anaerobic bacteria in nitrate reducing water treatment systems. Figure 5.16 illustrates the development of bacterial slimes on the sugar beet cubes by the termination of the experiment.

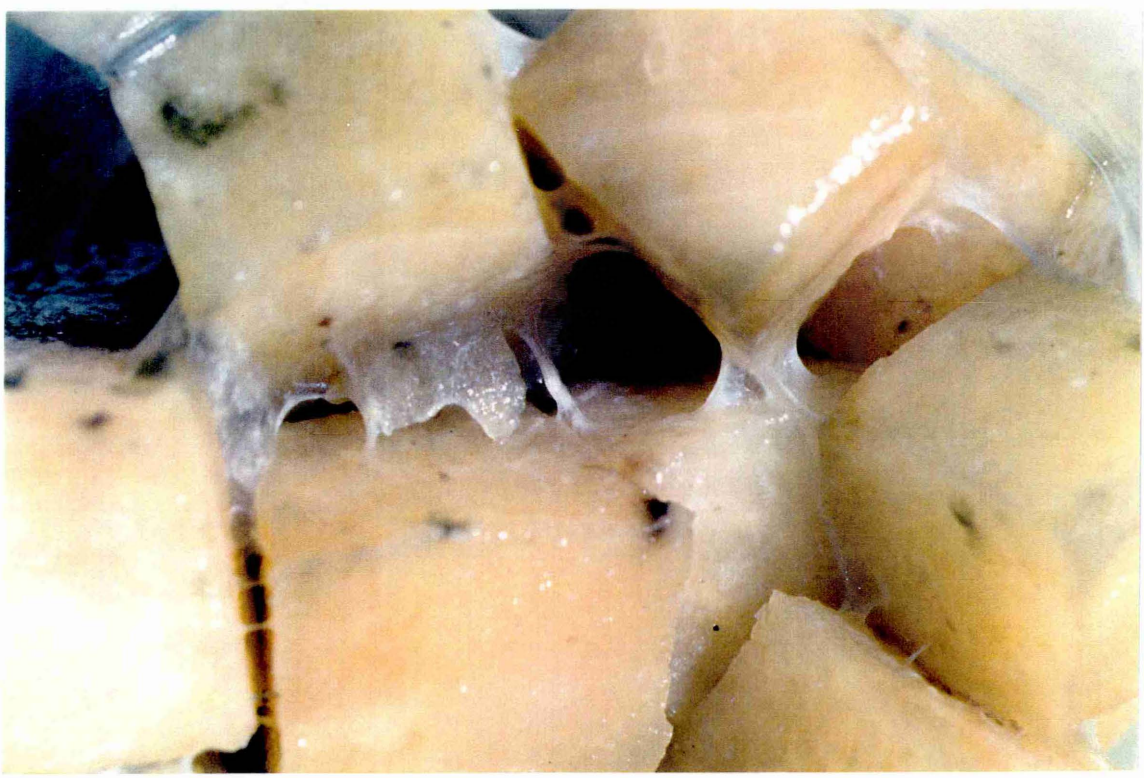

Figure 5.16 Development of Bacterial Slimes on Sugar Beet Cubes

5.4.2.3 Conclusions

Sugar beet has been shown to be a viable and sustainable source of carbon for low temperature biological nitrate reduction in a flow through water treatment systems i.e., readily utilisable carbon is continuously supplied to nitrate reducing bacteria when sugar beet is biologically degraded. Water treatment systems utilising sugar beet as both carbon source and attached growth media are also hydraulically sustainable i.e., the phenomnon of biological clogging commonly associated with flow through water treatment systems does not occur.

By comparing the performance of sugar beet to that of glucose in the low temperature study, i.e., 23g/m^3/d and 19g/m^3/d respectively, it can be concluded that the use of sugar beet as a carbon source in a water treatment system would sustain, and possibly enhance, nitrate reduction at low temperatures.

The problem of biological clogging associated with the previous experiments in which a sand/soil medium was used was not evident, and therefore the use of sugar beet is also sustainable in terms of maintaining hydraulic performance.

The importance of pH as an environmental control parameter was reaffirmed, with the problem of acid production resulting from microbial degradation being resolved by mixing lime pieces with the sugar beet cubes.

A problem of 'over-heating' i.e., high microbial activity, during the establishment phase of the treatment system was observed, and will require careful management to ensure optimal performance is maintained. It is suggested that the problem was a result of the initial high release rate of soluble carbon and therefore could be overcome by using either larger sizes of sugar beet cube e.g. 30mm, thus reducing the initial soluble carbon release rate, or a matrix of 20mm cubes and 40mm cubes.

5.4.3 Model Development

The combined organic material and low temperature study presented an opportunity for developing a model from the experimental data. The model would represent a relationship between flow rate, nitrate reduction, ammonia concentration and redox in a water treatment system utilising sugar beet as the carbon source.

Such a model would allow further information to be attained from the data, including the identification of threshold levels for particular environmental parameters, e.g. ammonia, above which nitrate reduction would be repressed. Control of the treatment system within the identified thresholds would ensure efficient performance, and therefore will be an aid in design.

The model could also be further adapted for use as a management decision making tool. This, however, would require independent validation, i.e., testing with a further data set, and was not possible as part of this research programme because of time restrictions.

5.4.3.1 Regression Analysis

From each experimental replicate of the flow study with pH control (Section 5.4.2.2), 31 data points were obtained following the 350 hour establishment period. The data was collated, i.e, a total of 93 data points, and a model produced using multivariate linear regression analysis.

Multivariate linear regression was used to test the relationship because it gave the option of analysing several independent variables at once. Hence, instead of a straight line regression with a single independent 'x' variable being regressed on a single dependent 'y' variable, there is a multi-dimensional plane where several independent 'x' variables are simultaneously regressed on a dependent 'y' variable. This is important when, as in this case, it is believed that changes in one set of variables directly cause changes in another variable.

The result of the regression analysis provides coefficients for each variable tested and a constant which represents the y-axis intercept. Also given is a value for R squared (R^2) which indicates how closely associated the independent and dependent variables are, or how much variation in the dependent variable can be explained by the combination of the independent variables. The R^2 value is a value between 0 and 100%. The closer the R^2 value is to 100%, the more closely the independent variables are related to the dependent variable.

For this study therefore the independent variables were nitrate reduction (N Red.), ammonia, and redox, and the dependent variable was flow rate (Q). Undertaking the regression analysis this way would enable the equation to be used as a management tool by the farmer i.e., for a stipulated reduction in nitrate concentration the required flow rate through the treatment system could be calculated. The addition of lime to the sugar beet meant that pH was not an independent variable and therefore could not be included in the analysis. The relationship is described below:

Q = 0.471 - (0.0207 x N Red.) - (0.0181 x Ammonia) - (0.000096 x Redox)

(l/hr) (mg/l NO_3-N) (mg/l NH_3-N) (mV)

R^2 = 0.845

5.4.3.2 Sensitivity Analysis

Sensitivity analysis tables (Tables 5.17-5.21) give the nitrate reduction performance expressed in terms of grams of nitrate-nitrogen reduced per cubic metre of water treatment system per day ($g/m^3/d$). The tables were calculated using the flow rate predictions from the model for nominal nitrate-nitrogen concentration reductions of 1, 4, 7, 10, 13, and 20mg/l. A broad range of redox potentials (400, 200, 0, -200, -400mV) and ammonia concentrations (0, 2.5, 5, 7.5, 10mg/l) were used to assess their effect on performance.

Examination of the tables indicates that the treatment system is more sensitive to increases in ammonia concentrations than variations in redox potentials. It is also evident that reductions in nitrate-nitrogen concentrations of greater than 13mg/l NO_3-N reduce treatment efficiency.

5.4.3.3 Conclusions

A regression analysis of the data from the flow study experiment indicated a definite relationship between flow rate and the independent variables nitrate reduction, redox, and ammonia production, with an R^2 of 0.845.

The result also suggests that the relationship can be developed further to be used as a management tool in a nitrate reducing water treatment system. Hence, when a known concentration of nitrate needs to be treated, the maximum limits for both redox and ammonia can be inputted to give the required flow rate of water through the treatment system.

A sensitivity analysis of the model output indicated that treatment system performance is primarily controlled by ammonia concentration rather than variation of redox potential, especially at higher nitrate concentrations. This has important implications for the management of the proposed treatment system, those implications being fully discussed in Chapter 6.

Table 5.17 Sensitivity Analysis : Redox Potential Set at 400mV

400 mV	NH_3-N	0	2.5	5	7.5	10
NO_3-N	$g/m^3/d$					
1		5	5	4	4	3
4		19	16	14	11	9
7		27	23	18	14	10
10		30	24	18	12	6
13		28	20	13	5	-3
20		5	-7	-19	-31	-43

Table 5.18 Sensitivity Analysis : Redox Potential Set at 200mV

200 mV	NH_3-N	0	2.5	5	7.5	10
NO_3-N	$g/m^3/d$					
1		6	5	5	4	3
4		20	17	15	12	10
7		29	24	20	16	12
10		32	26	20	14	8
13		32	24	16	8	0
20		10	-2	-14	-26	-38

Table 5.19 Sensitivity Analysis : Redox Potential Set at 0mV

0 mV	NH_3-N	0	2.5	5	7.5	10
NO_3-N	$g/m^3/d$					
1		6	5	5	4	4
4		21	18	16	13	11
7		30	26	22	18	13
10		35	29	23	17	11
13		35	27	19	11	4
20		15	3	-9	-21	-33

Table 5.20 Sensitivity Analysis : Redox Potential Set at -200mV

-200 mV	NH_3-N	0	2.5	5	7.5	10
NO_3-N	$g/m^3/d$					
1		6	6	5	4	4
4		22	19	17	14	12
7		32	28	24	19	15
10		38	32	26	20	14
13		38	30	23	15	7
20		20	8	-4	-16	-28

Table 5.21 Sensitivity Analysis : Redox Potential Set at -400mV

-400 mV	NH_3-N	0	2.5	5	7.5	10
NO_3-N	$g/m^3/d$					
1		6	6	5	5	4
4		23	20	18	15	13
7		34	30	25	21	17
10		40	34	28	22	16
13		41	34	26	18	10
20		25	13	1	-11	-23

Chapter 6

On-Farm Water Treatment and Management Strategies

6.0 Introduction

The aim of the work was to devise an economic method of treating drain discharges from agricultural catchments, to reduce unacceptably high nitrate concentrations, so protecting surface water quality.

This chapter describes the agricultural catchments where treatment can be used, examines the nature and form of nitrate losses from those catchments, and discusses the design of management strategies to deal with those losses. Those strategies incorporate solutions outlined in Chapter 3 and, following laboratory tests, the use of a nitrate reducing treatment zone utilising sugar beet as the carbon source.

6.1 Location

6.1.1 Catchment Area

The volume of drainage water from a catchment is related to the depth of rain that falls upon the catchment and the catchment area. Other factors affecting drainage volumes include catchment shape, evapotranspiration, and the soil's storage capacity, a factor which will be affected by agricultural practices undertaken within the catchment. As the drainage water volume will directly effect the size of the treatment zone required, quantifying catchment area is an important part of any design exercise.

6.1.2 Soil Type

In Chapter 3 it was identified that for agricultural catchments containing a high proportion of land drains, high peak nitrate concentrations occurred in drainage water, particularly in autumn. It was therefore recommended that on-farm treatment be applicable on catchments of predominantly clay soil, with perched rather than ground watertables where all the water is channelled via subsurface drainage to a single outlet (Section 6.2.2).

6.1.3 Hydrological Data

It was suggested in Chapter 3 that by cross referencing a hydrological land classification system called HOST with maps that indicate climatological and agronomic data, potential problem agricultural catchments could be identified (Section 3.2.2).

Once identified, climate data could also help farmers identify when they need to intercept the drainage water for treatment, thus providing an early warning system. Examination of previously recorded hydrographs for a catchment would indicate how long it takes for discharges at the outfall to rise once rainfall starts to fall upon the catchment. Chemographs giving a graphical representation of the chemical composition of the discharges could similarly be used to identify the critical part of the drain discharges, that is when either highest nitrate concentrations or the highest nitrate loadings occur.

Collection of hydrological data may require, at some cost, expert advice and analysis; however, it may allow the farmer to reduce the scale of the treatment system, as well as manage the drainage water more effectively.

6.2 Integration into Present Farm System

6.2.1 Agricultural Practice

Implementation of the proposed treatment system would mean that present agricultural practice would not need to be changed or interrupted. Farmers will also have the reassurance that their current farming practices would not need to be changed to comply with future regulations and water protection policy. It is this aspect of the proposed system that allows it to be presented as beneficial to both farmer and environmentalist.

6.2.2 Drainage System

Drainage systems which can incorporate the proposed water treatment system will have the following four components: field system, main system, collector and outlet.

The field system gathers the excess water from the land by means of a network of shallow field drains, and where necessary these are supplemented by measures which promote the flow of excess water to these drains e.g. mole drains. The main system receives water from the field system and conveys it to the outlet via a series of ditches, the final ditch to the outlet being termed the collector. The outlet is the terminal point of the whole system where it discharges into a river or watercourse.

An integral component of the treatment system is a capability to store water and therefore a pond/reservoir will either need to be used or constructed. Water storage is required to enable drainage water having a nitrate concentration above the acceptable limit for discharge to be collected. Alternatively, a reservoir of water having a negligible nitrate concentration, could be used to blend with water draining from the land, so reducing the nitrate concentration to below the acceptable limit.

6.2.3 Legal Aspects

The information given below was taken from the booklet Ponds and Conservation, published in 1993 by the National Rivers Authority (NRA) of England and Wales[1].

Generally, although the legal requirements depend largely on the size of the pond to be created and its water supply, the relevant authority should always be consulted first. The NRA require a licence or permission for the following relevant procedures.

6.2.3.1 Abstracting Water

A licence is needed to abstract surface water. Off-stream ponds will need an abstraction licence if they are fed from a surface watercourse, even when the water is returned to the watercourse further downstream. As this is technically an abstraction, the farmer should always check with the NRA Water Resources Department. It should be noted that a minimum of six months should be allowed for the granting of a licence.

6.2.3.2 Ponds/Reservoirs

Structures containing more than 25000m^3 of water above the lowest natural ground level will need a licence from the NRA Water Resources Department Licensing Officer, and will require regular inspection by a Panel Engineer.

Local authorities also require prior notification of any plans to build a pond. Planning permission may then be required for ponds constructed within 25m of a classified road, or where the pond is used for non-agricultural uses e.g. angling.

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1 NRA was superseded by the Environmental Agency in April 1996

6.2.3.3 Land Drainage

For any other work on a watercourse, including diverting a watercourse or the building of a reservoir (if it is attached to a watercourse), and for works within a specified distance of a watercourse (this distance varies regionally), a land drainage consent will be required from the NRA's Flood Defence Department.

6.3 Definition of the On-Farm Maximum Acceptable Concentration (OFMAC)

The definition of the OFMAC has important implications on the design and cost of a nitrate reducing water treatment system. For example, a low OFMAC e.g. 5mg/l NO_3-N would probably result in greater volumes of drainage water requiring management, the implication being an increase in design storage capacity and with it additional cost.

The OFMAC value could be the maximum acceptable concentration (MAC) for drinking water i.e., 11.3mg/l NO_3-N. Chapter 1, however, refers to an increasing need to respect environmental goals. This could mean that the OFMAC is reduced to a lower arbitrary value of 5mg/l NO_3-N, a figure that would also allow for a significant margin of error in the nitrate monitoring instrumentation.

The value used will be determined by the farmers for their particular circumstance, that is the extent of pollution control and safety they wish to imbue on their farming system, as well as the cost of water storage capacity required. For the design exercise below an OFMAC of 11.3mg/l NO_3-N is used i.e., the minimum acceptable level.

6.4 Nitrate Losses from Agricultural Clay Catchments

For the design of effective treatment strategies and options it is necessary to estimate both the magnitude of total nitrate losses from arable clay soil, and also the average concentration of nitrate in the drainage water.

6.4.1 Total Nitrate Losses

An important experiment at Brimstone Experimental Farm in Oxfordshire (Cannel et al., 1984; Harris et al., 1984, Goss et al., 1988a, Goss et al., 1988b) has provided 10 years of data from which estimates of nitrate loss from arable clay lands can be made. A mean annual loss of nitrate-N through all the collectors in tilled, drained land of 41.1kg/ha was reported (Goss et al., 1993), however, the most significant losses, 92%, were through the drainage system between harvest and the spring top dressing of fertilizer.

Goss et al. (1993) also reported empirical equations derived using the Brimstone Farm data to calculate nitrate losses between harvest and the spring top dressing of fertilizer i.e., over-winter losses. The equation relates the amount of nitrate-N leached to the amount of fertilizer added to the seed bed, and is as follows:

$$\textbf{Nitrate Loss (kg N/ha)} = \textbf{21.8} + \textbf{1.09 F}$$

where;

F = autumn fertilizer-N applied (kg N/ha)

Standard Error = 6kg N/ha for the constant and 0.3 for the slope.

The important implication of this equation is that even if no fertilizer is applied in autumn, as recommended in the Code of Good Agricultural Practice, approximately 22kg nitrate-N/ha on average will be leached.

Goss et al. (1993) developed a further regression equation to describe cumulative nitrate loss between the application of the spring top-dressing and harvest. The equation is as follows:

$$\underset{\text{(kg N/ha)}}{\text{Cumulative nitrate loss}} = [\ 0.28 \times \underset{\text{(mm)}}{\text{cumulative rainfall}}\ ] - 1.03$$

In Appendix VI a design example uses the equations to calculate the nitrate leached following a rainfall event from a catchment of known area.

6.4.2 Average Nitrate Concentrations in Drainage Water

It was stated in Section 6.1.1 that the volume of drainage water from a catchment is related to the depth of rain that falls upon the catchment. If, as is suggested above, the average nitrate leaching from an arable clay soil over-winter will be approximately 22kg N/ha, then variations in total excess rainfall will effect average nitrate concentration of the drainage water.

Table 6.1 uses as examples Brimstone Experimental Farm and Silsoe College Farm, and illustrates possible differences in average nitrate concentration, assuming an average loss of nitrate-nitrogen of 22kg/ha.

The figures for average NO_3-N concentrations imply that in areas of low rainfall e.g. Bedfordshire, collection and storage of all the drainage water into a pond for blending/dilution would not be sufficient to ensure nitrate concentrations are reduced below the 11.3mg/l acceptable limit. Hence, alternative treatment systems will be necessary e.g. anaerobic nitrate reduction. These are discussed later in Section 6.5.

Table 6.1 Calculation of Average Nitrate Concentration

Site	MAFF Climate and Drainage Area	Clay Soil Class	Mean Excess Winter Rainfall (mm)	Drainage Volume (m^3/ha)	Average Nitrate-N Concentration (mg/l)
Brimstone Farm, Oxfordshire	31N	Denchworth	210	2100	10.5
Silsoe College Farm, Bedfordshire	28	Evesham	130	1300	16.9

[Rainfall Data from Smith and Trafford (1976)]

6.4.3 Field Studies

Although nitrate losses are unpredictable, nitrate leaching from arable clay catchments has been studied extensively, those studies providing important information that can help design on-farm systems to protect watercourses and rivers.

Arlot and Zimmer (1992) described three possible patterns of nitrate pollution concentration during peak flows following field studies in France. The patterns are characterised by a dilution process during peak flow, and are related to both moisture and nitrate profiles in the soil and around field drains before drainage commences, and are illustrated in Figure 6.1.

Pattern I is characteristic of the first drainflows of late autumn, when nitrate concentrations rise or remain constant throughout the peak discharge. The phenomenon occurs following the build up of nitrate in the soil during autumn as a result of mineralisation of organic-N (Section 2.1.1.2), which is then rapidly flushed out of the soil by preferential (by-pass) flow of water through cracks in the clay. Pattern II and III

are characteristic of typical winter rainfall events, where nitrate concentration drops during peak discharge as a result of dilution by the increased drainage volume. In pattern II nitrate concentrations rise during base flow to a concentration lower than that before the event indicating a net loss of nitrate. Pattern III corresponds with a generally low level of nitrate in the soil profile due to successive leaching over winter and no new inputs of nitrate into the system either through mineralisation or fertiliser applications.

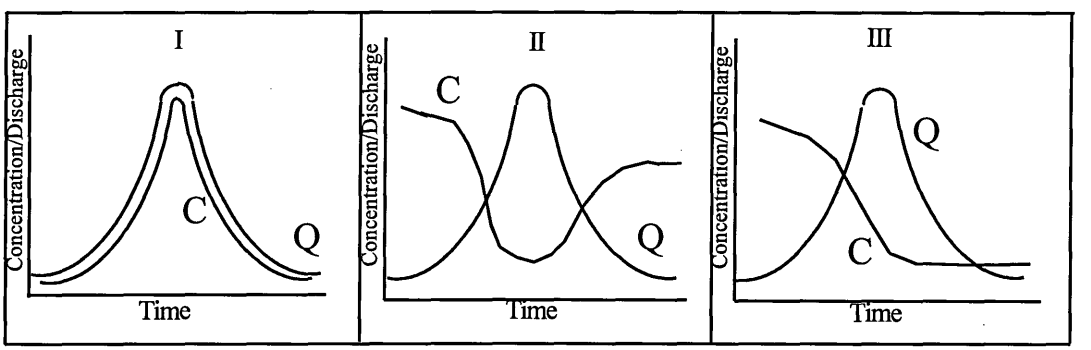


Figure 6.1 Patterns of Nitrate Concentration (C) Evolution During Drainage Events (Q) (after Arlot and Zimmer, 1992)

Harris and Rose (1992) reported experiments which investigated nitrate leaching to surface waters from small agricultural clay catchments. For drained catchments under arable/grassland cropping, nitrate concentrations exceeded 11.3mg/l nitrate-nitrogen for much of the drainage season. In three cropped clay catchments, peaks of nitrate-nitrogen were measured at 55mg/l, 40mg/l, and 20 mg/l NO_3-N. Generally, these peaks were associated with the first drainflows in late autumn following dry summers. However, they also observed high concentrations, 55mg/l NO_3-N, in February when flows were greatest. Harris and Rose (1992) go on to suggest that if this magnitude of nitrate loss occurs in eastern England, where the interaction between rainfall and leaching can lead to high nitrate concentrations when runoff is low, it may prove too difficult to maintain nitrate concentrations below the EC Surface Water

recommendations i.e., that in 95% of samples the concentration should be less than 11.3mg/l nitrate-nitrogen.

The transient nature of both discharge rates and nitrate concentrations described by Arlot and Zimmer (1992) and Harris and Rose (1992) can be illustrated using data collected following three rainfall events in autumn 1993. A summary of the data, taken from the Boot Field experiments at Silsoe College Farm, is given in Tables 6.2, 6.3, and 6.4. N.B. Event 2 data is published in Leeds-Harrison (1995).

Significantly, the data indicates that for all drainflows nitrate-nitrogen concentrations exceeded the EU water directive limit of 11.3mg/l NO_3-N. Hence, although time to peak discharge varied following the onset of drainflow i.e., 10, 44 and 5 hours for Events 1, 2, and 3 respectively, the implication is that it would be necessary to prevent the initial drainflows from entering the watercourse to ensure protection. Data for further events in the 1993-94 drainage season were not available, however, it is suggested that further significant losses would not have occurred as the 31kg nitrate-N/ha leached already exceeds the average over-winter loss of 22kg nitrate-N/ha predicted by Goss et al. (1993). This conclusion is also drawn from the work of Harris and Rose (1992) who observed nitrate concentration peaks to be primarily associated with the first drainflows in late autumn following dry summers.

Importantly, the above conclusion suggests that it would not be necessary to collect all winter drainage volume, as the majority of residual nitrate will be leached by the initial drainflow events in late autumn/early winter. This has important practical implications because it implies that drainflows in late winter/early spring, when nitrate concentrations are below the OFMAC, could possibly be utilised for dilution purposes. It also implies that design storage requirements could be significantly less than the over-winter drainage volumes calculated from mean excess winter rainfall data.

Table 6.2 Drainflow Nitrate-Nitrogen Concentrations

Event 1 : 30th September 1993 - 3rd October 1993

Time since start of drainflow (hours)	Nitrate-nitrogen concentration (mg/l)	Discharge Rate (l/s)	Drainflow (mm)
0	46.3	0.031	0.031
10	44.2	0.096	0.112
12	43.1	0.096	0.112
76	45.6	0.028	0.035

Table 6.3 Drainflow Nitrate-Nitrogen Concentrations

Event 2 : 11th November 1993 - 14th November 1993

Time since start of drainflow (hours)	Nitrate-nitrogen concentration (mg/l)	Drainflow Rate (l/s)	Drainflow (mm)
0	16.2	0.01	0.012
38	22.3	0.05	0.058
44	22.5	0.102	0.119
58	18.1	0.101	0.118
113	n/a	0.012	0.014

Table 6.4 Drainflow Nitrate-Nitrogen Concentrations

Event 3 : 8th December 1993 - 10th December 1993

Time since start of drainflow (hours)	Nitrate-nitrogen concentration (mg/l)	Drainflow Rate (l/s)	Drainflow (mm)
0	14.2	0.021	0.024
5	20.1	0.05	0.058
11	21.7	0.05	0.058
43	18.9	0.011	0.013

The conclusion is further supported by a field study undertaken in the Spring of 1992 by MSc students at Silsoe College (Blackmore et al., 1992). The unpublished work, undertaken on a 30ha clay catchment adjacent to the Bootfield site, indicates that NO_3-N concentrations did not exceed the 11.3mg/l limit following two rainfall events in March. This finding suggests that residual nitrate levels in the soil had already been reduced by autumn and over winter leaching losses. Following a rainfall event at the end of April there was a rise in concentration to a high of 16mg/l NO_3-N; however, it was suggested that this was a result of fertilisers having been applied to the catchment at the end of March.

The above work illustrates the ephemeral and unpredictable nature of nitrate losses from clay catchments, and indicates that high nitrate concentrations can be equally observed at both low and high drainage discharge rates, particularly early in the drainage season.

6.5 Treatment System Design

It was stated in Chapter 3 that there are two possible management strategies. These are either storage and treatment or storage and dilution/blending. Two further options are also now considered, these being storage and recirculation, and storage and treatment. The particular method chosen by the farmer will determine the management strategy taken for controlling the polluted water. The methods were described in Chapter 3; however, for design detail, several questions need to be answered and the implications of those answers discussed. The questions that need to be asked are :

i) What is the average nitrate concentration of the draining water?

ii) What volume of drainage water will need to be collected?

iii) What management strategies can be used?

iv) What is the cost of each strategy?

An important aspect of the management of polluted waters and hence in the decision making of the farmer, is at what point of the drainage system will drainage water be

sampled to assess if it is polluting or not. It could either be where water from a drain outfalls into a ditch, or alternatively where water is taken from the collector drain (ditch) prior to entering a river or watercourse. As this is a hypothetical question for the purposes of this design exercise, the drain collector will be assumed to be the point at which samples would be taken.

6.5.1 Average Nitrate Concentration

As stated in Section 6.4.2 the average nitrate concentration will determine what options and management strategies the farmer can choose for treatment of the drainage water. Average nitrate concentration is calculated as follows:

$$\text{Average nitrate concentration (mg/l)} = \frac{22 \text{ kg N/ha} \times 10^6}{(\text{Mean Excess Winter Rainfall (mm)} / 1000) \times 10^3 \times 10^4}$$

where,

22kg Nitrate-N/ha is the predicted over-winter leaching loss (Goss et al., 1993), and Mean Excess Winter Rainfall values for different areas are taken from Climate and Drainage (Smith and Trafford, 1976).

For example, Bedfordshire and North Oxfordshire have mean excess winter rainfall values of 130mm and 210mm respectively, which produce average nitrate concentrations of 16.9mg/l and 10.5mg/l respectively i.e., one above and one below the acceptable limit (Section 6.3).

6.5.2 Volume of Drainage Water

Several empirical formulae exist for calculating discharge rates following a rainfall event. In particular, Smedema and Rycroft (1983) describe the application of the Rational Formula method to small drained agricultural catchments. Wilson (1992) describes this and other formulae including one developed by Poots and Cochrane (1979) from the Flood Studies Report formula (NERC, 1975). Standard texts including Hudson (1995) and Schwab et al. (1993) describe several other empirical formulae for calculating catchment runoff rates, all of which are satisfactory and chosen on the basis of personal preference.

In this study, however, climate and drainage data (Smith and Trafford, 1976) was used to calculate drainage volumes based on the mean excess winter rainfalls. Bedfordshire and North Oxfordshire are used as examples of areas of low and high mean excess winter rainfall i.e., 130mm (median) and 210mm (median), respectively.

Section 6.4.3 illustrated the transient nature of drainflows and the nitrate concentrations within those flows, and that waters draining from clay agricultural catchments in areas of low mean excess winter rainfall are likely to have nitrate concentrations that exceed the acceptable limit of 11.3mg/l NO_3-N. The data presented above indicated that a drainflow of some 25000m^3 leached an average of 31kg N/ha, and it was concluded that further significant over-winter nitrate losses would not occur, as this figure already exceeded the average nitrate leaching losses predicted by Goss et al. (1993).

6.5.3 Water Management Strategies

The management strategies available have been previously discussed in Chapter 3, and are now reviewed with respect to treating drainflows in Bedfordshire and North Oxfordshire i.e., high and low rainfall areas. Costs are calculated for each option to enable a broad comparison between options to be made.

The Boot Field data indicated that 31kg nitrate-nitrogen per hectare was leached before the end of December i.e., 76% of the average annual loss of 41kg N/ha (Goss et al., 1993). Extrapolating for a 30ha catchment, 930kg nitrate-N was leached (31kg N/ha) following three rainfall events. The respective drainflow volumes for Events 1, 2, and 3 were 17070m^3 , 6240m^3 , 1800m^3 . If all this water were stored, i.e., a total drainage volume of 25110m^3 , the average nitrate concentration would be 37.3mg/l NO_3-N.

The farmer has four options for handling this water:

i) Storage and Dilution

ii) Long Term Storage

iii) Storage and Recirculation

iv) Anaerobic Water Treatment.

6.5.3.1 Option 1 : Storage and Dilution

Drainage water of a high nitrate concentration is diverted into a storage pond until it can be released at a later date to dilute with drainage water with a nitrate concentration below the acceptable concentration e.g. in late Winter/early Spring. The stored water would be metered into the collector to ensure the nitrate concentration of the blended water remained below the acceptable concentration, so protecting the watercourse.

If, as stated above, 25000m^3 of drainflow was collected, and that the average nitrate concentration of that water was 37mg/l, the volume of water with a negligible nitrate concentration required to dilute all that stored water below the OFMAC i.e., 11.3mg/l NO_3-N, would be approximately 82000m^3.

25000m^3 of drainflow is equivalent to 83mm of excess rainfall over 30ha. In areas of high rainfall e.g. North Oxfordshire, the remaining winter excess rainfall, i.e., 127mm, will give an average nitrate concentration of 7.9mg/l NO_3-N assuming, on average, a further 10kg N/ha is leached. The implication is that even in areas where mean excess

metering it back into the collector drain during later drainflows. If dilution were the option chosen by the farmer then a design storage capacity of 25000m^3 is required, as all the initial drainflows would need collection.

The cost of stored water is calculated using a figure of £1.50/m^3 of water stored (Nix, 1996).

Amortisation Cost = £116 per £1000 invested for a 21 year repayment period @ interest rate of 10% (Weatherhead, 1996). N.B. Cost is slightly less than by Discounting because risk is not taken into account.

Capital Costs

Cost of storage capacity of 25000m^3	=	£37500
Cost of control, diversion, and metering devices	=	£ 3000
Total Capital Cost	=	£40500

Variable Costs

Amortisation of Capital = £4698/25110m^3 = 18.7p/m^3.

Other operation costs for long term storage will be a minimum as there is no requirement for the addition of chemicals, or managerial expertise required to operate the system.

As only approximately 3500m^3 can be treated by dilution, alternative strategies are required to manage the remaining water. Those strategies are recirculation, long term storage, or anaerobic nitrate reduction. An additional benefit of both recirculation and anaerobic nitrate reduction is that they provide scope for a reduction in design storage capacity.

6.5.3.2 Option 2 : Long Term Storage

Previously discussed in Section 3.3.2, this is the simplest treatment strategy. Once the high nitrate drain flows are diverted into a storage pond, long term storage i.e., greater than 6 months, will reduce nitrate concentrations naturally (Section 2.3.3). The cost of long term storage would also be less than dilution as metering devices would not be required.

Capital Cost

Cost of storage capacity of 25000m^3	=	£37500
Cost of control and diversion devices	=	£ 2000
Total Capital Cost	=	£39500

Variable Cost

Amortisation of Capital Cost = £4582/25110m^3 = 18.3p/m^3.

Other operation costs for long term storage will be a minimum as there is no requirement for the addition of chemicals, or managerial expertise required to operate the system.

6.5.3.3 Option 3 : Recirculation

It is proposed that the collected water be re-applied to the land at times of soil moisture deficit. It is also suggested that the nitrate in the stored water could be recycled for use as a fertiliser during the growing season using this method.

Although specifically not highlighted as a solution in Chapter 3, recirculation of the water was discussed in Chapter 2 with respect to obtaining a reduction in nitrate concentration. However, now that nitrate-nitrogen leaching losses have been quantified (Section 6.4) it is possible to suggest, and cost, the benefits of recirculation in terms of fertiliser. By re-applying the water to the land in between storms at the same rate of evapotranspiration it will also be possible to reduce the design storage capacity.

By the end of winter, i.e., before the spring top dressing is applied, on average, 22kg N/ha is leached. From a 30ha catchment this equates to the following cost figures:

The cost of ammonium nitrate fertiliser is £125/tonne (Nix, 1996).
Ammonium nitrate contains 34.5% N.

Hence, 1 tonne of N (1000 kg)	=	£362
30ha @ 22 kg N/ha = 660kg N (0.66 tonne N)	=	£234
and,		
Approx. cost of nitrate-nitrogen leached per hectare	=	£8

Two reasons would make it beneficial to irrigate the nitrate-nitrogen leached from 30 ha, i.e., 660kg N, onto a smaller area e.g. 4ha. The first would be to reduce the distance over which water has to be transported, and the second to obtain a fertiliser application rate of 165kg N/ha. This approximates to the recommended fertiliser application rate for winter wheat, and therefore a saving on fertiliser costs equivalent to the cost of fertiliser leached i.e., £234 is also made, an overall saving of £468.

It is suggested that water be reapplied using irrigation equipment. If drip or sprinkler systems were used, water application rates could be more closely matched to soil moisture deficit. Drip or sprinkler systems would be of particular advantage where water was applied to a market garden crop, as this would enable control of both water and fertiliser application rates, and where gross margins would support the higher irrigation equipment costs. The application of smaller irrigation volumes at increased frequencies would also suit the irrigation of crops grown on clay soils i.e., medium Available Water Capacity (AWC) and low infiltration rate.

Irrigation of the water back onto the land has an additional benefit in that it would enable the design storage capacity to be reduced to 17000m^3, that is the volume of the initial, and greatest, drainflow from the example given above.

Capital Costs

Cost of storage capacity of 17000m^3 = £25500

Cost of control and diversion devices	=	£ 2000
Cost of raingun irrigation equipment	=	£20000
Total Capital Cost	=	£47500

Variable Costs

Amortisation of Capital Cost = £5510/25110m^3	=	21.9p/m^3
Estimated Pumping Costs	=	2.0p/m^3
Management Costs	=	2.0p/m^3
Total Variable Cost	=	25.9p/m^3

6.5.3.4 Option 4 : Anaerobic Nitrate Reduction

The feasibility of treating water by microbial nitrate reduction was investigated and reported in Chapters 4 and 5. Those investigations highlighted significant control parameters that have practical implications for on-farm design, and include the following:

a) Provision and Maintenance of an Anaerobic Environment
b) Provision of an Energy Source
c) Water Temperature
d) Maintenance of Permeability
e) Size of Treatment Zone
f) Supply, Processing, and Management of Sugar Beet.

6.5.3.4.1 Provision and Maintenance of an Anaerobic Environment

An anaerobic environment can be provided and maintained by ensuring the treatment zone is waterlogged both prior to and throughout the treatment period. Waterlogging can be achieved by burying the treatment zone to a depth of 1.5m-2m in heavy clay soils and then flooding it. The smeared clay will provide sufficient water-tightness to ensure water levels are maintained. Alternatively, where the subsoil is not heavy clay, the

treatment zone could be hydraulically isolated by lining with butyl, however, this will involve additional cost.

The laboratory investigation into nitrate reduction utilising sugar beet (Section 5.4.2) allowed an equation to be developed (Section 5.4.3) which calculates the flow rate required to reduce the nitrate concentration to the required level i.e., below the OFMAC. It is recognised that this equation needs further validation before it can be reliable enough for design in practice, however, in this section it is used to provide a guide to possible requirements. The equation is as follows:

$$\underset{(l/hr)}{\text{Flow rate}} = 0.471 - (0.0207 \times \underset{(mg/l\ NO_3\text{-}N)}{\text{N Red.}}) - (0.0181 \times \underset{(mg/l\ NH_3\text{-}N)}{\text{Ammonia}}) - (0.000096 \times \underset{(mV)}{\text{Redox}})$$

Example:

For a required nitrate concentration reduction of 10mg/l NO_3-N, and limits of redox and ammonia of 50mV and 5mg/l respectively, the required flow rate of water through the treatment zone would be 0.17 litres per hour.

Methods for controlling flow rate through the anaerobic treatment zone are discussed in Section 6.6.1.

6.5.3.4.2 Provision of an Energy Source

An energy source is required for microbial nitrate reduction. To reduce cost and allow the farmer to utilise on-farm resources, investigations were undertaken to ascertain the feasibility of using sugar beet as an energy source. Those investigations showed that sugar beet was both a suitable and sustainable source of energy, with a problem of acid production resulting from the degradation of the sugar beet being successfully countered using crushed limestone (calcium carbonate). Processing of the sugar beet is discussed below.

The post-treatment management of the sugar beet also needs consideration. It was highlighted that aeration of the sugar beet following a drainage season would allow aerobic degradation of the sugar beet, which in turn would produce more readily utilisable carbon. This may be possible, once the water level has fallen, by exposing the sugar beet to the atmosphere. A cover consisting of large straw bales would simplify this operation.

Additions of fresh sugar beet will be necessary, however, the treatment zone volume would not need to be expanded as consolidation of the sugar beet/limestone will have occurred following draw down.

6.5.3.4.3 Water Temperature

Nitrate reduction is temperature dependent, and so the experimental investigations were undertaken at a low temperature. However, it is recognised that drainage water running off the land during winter may have a significantly lower temperature than the 10°C used in the experiments. Lower temperatures would both directly reduce performance capacity and also effectively act as a coolant as it passed through the treatment zone.

It is suggested that if a treatment zone were located at a depth of 1.5m-2m, heat would conduct from the soil to the treatment zone. The principle of heat conduction from the relatively warmer soil to the water in late autumn/early winter can also help guide water management practice. Heat conduction should occur at the base of the pond, and therefore water diverted to a treatment zone should, where possible, be taken from the bottom of the pond where it will be warmer than water at the top. The temperature of the water could also be increased as it is piped from the pond to the treatment zone. By laying a network of pipes 1.5m-2m underground, heat will be conducted from the soil into the water, raising the water temperature as it passes.

6.5.3.4.4 Maintenance of Permeability

The maintenance of hydraulic permeability is a problem commonly associated with nitrate reducing treatment systems as a result of the clogging of interstitial pores due to the migration of fine material and biomass growth. Use of a matrix of sugar beet and limestone in the experimental investigations illustrated that permeability could be maintained without the need for additional management practices.

It was mentioned above that consolidation of the sugar beet and the limestone was observed following drawdown of the water level. If straw bales were used as a cover, metal gabions could be used to contain and hold the sugar beet/limestone matrix, simultaneously supporting the cover. An additional benefit of using metal gabions is in replenishing with fresh sugar beet. The gabions could be readily pulled out and replaced. Also, if replacement gabions were already prepared, stand-down times for the treatment zone could be reduced.

An alternative option to metal gabions are permeable mesh boxes originally developed for the British Army (Hesco Bastion Ltd, 1992). They store flat but when filled with a bulky material can be stacked one upon each other, making them easy to manage.

6.5.3.4.5 Size of Treatment Zone

In Chapter 5 a performance indicator of grams of nitrate-nitrogen reduced per cubic metre of reactor per day ($g/m^3/d$) was used to express nitrate reduction rates. The investigation using sugar beet indicated a possible sustainable performance of $23 g/m^3/d$. Hence, this value can be used for design purposes to determine the size of the treatment system required to treat expected quantities of nitrate over a specified treatment period.

An example design using the Boot Field data from Tables 6.1, 6.2, and 6.3 is given. The total drainflows for Events 1, 2, and 3 were 56.9mm, 20.8mm and 6mm respectively, with associated average nitrate-nitrogen (NO_3-N) concentrations of 45.6mg/l, 19.52mg/l, and 20.3mg/l. Average nitrate concentrations are used because all the drainflow was above the acceptable nitrate concentration and so would be stored

prior to treatment. This would have resulted in a blending of the water within the pond to an average concentration. Extrapolating for a 30ha catchment the drainflows equate to the following water volumes and nitrate losses:

Event 1 = 17070m^3, 778.39 kg NO_3-N

Event 2 = 6240m^3, 121.81 kg NO_3-N

Event 3 = 1800m^3, 36.54 kg NO_3-N

The period of time between each event, and therefore days available for treatment, was 37, 22 and 3 days respectively. Two management strategies are considered for the example given:

Strategy 1 : Treat All Water Prior to Next Event

If all the water i.e., 25110m^3 were treated, the required treatment zone volume can be calculated as in Table 6.5 below.

Table 6.5 Strategy 1 : Calculation of Treatment Zone Volume for 30ha Catchment

Event No.	No. of days available for treatment	Total nitrate in drainage water (g NO_3-N)	Reduction of nitrate required per day (g NO_3-N)	Treatment zone volume (m^3)	Area of treatment zone 2 metres deep (ha)	% of 30ha catchment used for treatment zone
1	37	778390	21038	915	0.046	0.153
2	22	121810	5537	240	0.012	0.04
3	2	36540	18270	794	0.04	0.13

Therefore;

Treatment zone volume required = 915m^3 (based on largest treatment zone volume required i.e., event No.1)

Possible treatment zone dimensions = 45.8m long x 10m wide x 2m deep.

Strategy 2 : Treat a Proportion of the Drainage Water

If, for example, only half the drainage water resulting from the first event were to be treated i.e., 8535m^3, then the drainage volume from both the second and third events i.e., 8040m^3, could be stored without the requirement for additional storage capacity. As the volume of water requiring treatment is now reduced to 8535m^3 (average nitrate concentration of 45.6 mg/l NO_3-N) only 389.2 kg N would have to be treated, so reducing the area of treatment zone required.

Table 6.6 Strategy 2 : Calculation of Treatment Zone Volume for 30ha Catchment

No. of days available for treatment	Nitrate in water (g NO_3-N)	Reduction of nitrate required per day (g NO_3-N)	Treatment zone volume (m^3)	Area of treatment zone 2 metres deep (ha)	% of 30ha catchment used for treatment zone
37	389200	10519	458	0.023	0.077

Therefore;

Treatment zone volume required = 458m^3

Possible treatment zone dimensions = 22.9m long x 10m wide x 2m deep

Figure 6.2a illustrates in plan and sectional views the layout of the proposed on-farm anaerobic treatment zone method for Strategy 1. Figure 6.2b is an enlarged sectional view of an anaerobic treatment zone.

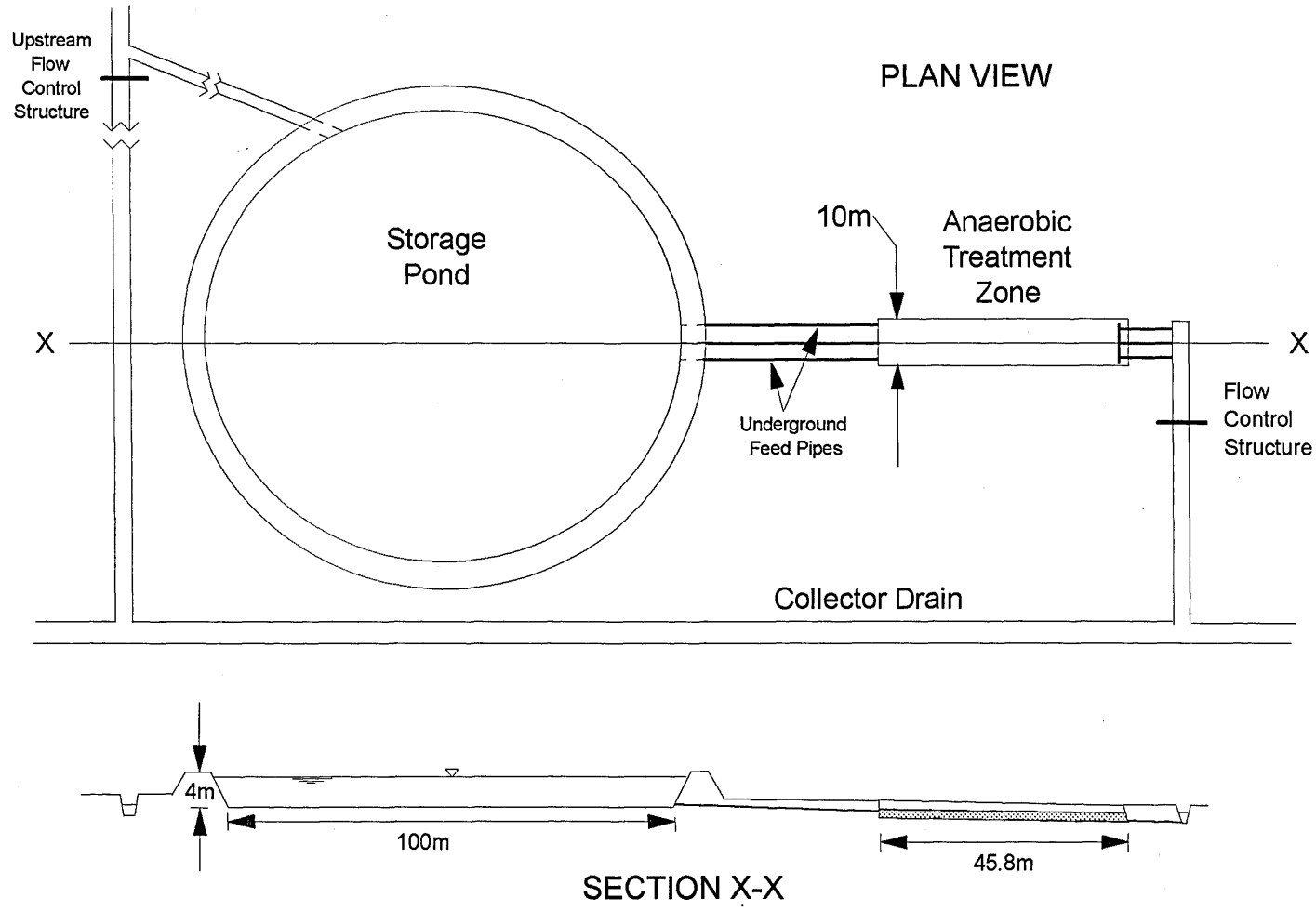
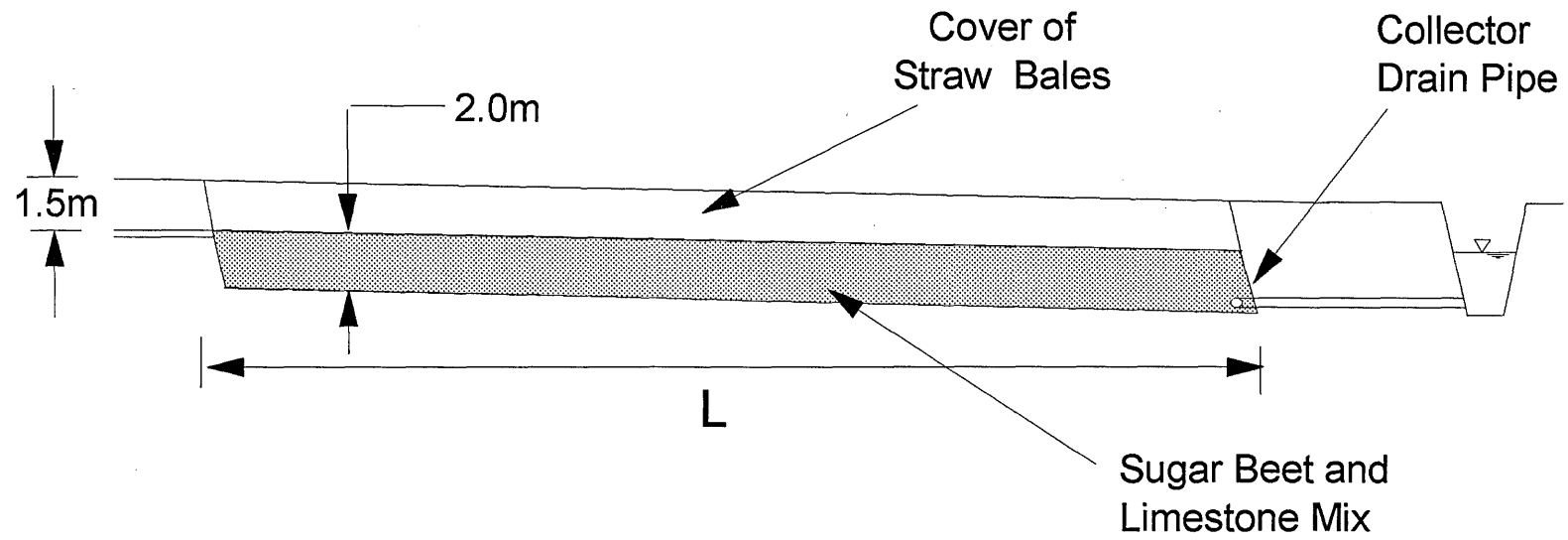


Figure 6.2a Plan and Elevation Views of On-Farm Anaerobic Treatment Zone Method showing Storage Pond (Not to Scale)



Strategy 1 : L = 45.8m
Strategy 2 : L = 22.9m

Figure 6.2b Enlarged Elevation View of Anaerobic Treatment Zone (Not to Scale)

Calculation of Sugar Beet Mass Required :

Treatment zone volume consists of : 50% void space, 25% sugar beet, 25% limestone.

Hence, for the two strategies:

Strategy 1 : Treat all water prior to next event

Treatment zone volume of 915m^3	==>	228.8m^3 of sugar beet
Density of sugar beet = 0.5 tonnes/m^3	==>	114.4 tonnes of sugar beet
Wastage during processing of 25%	==>	28.6 tonnes
Total mass of sugar beet required for a treatment zone volume of 915m^3	==>	143 tonnes

Strategy 2 : Treat a proportion of the drainage water

Treatment zone volume of 458m^3	==>	114.6m^3 of sugar beet
Density of sugar beet = 0.5 tonnes/m^3	==>	57.3 tonnes of sugar beet
Wastage during processing of 25%	==>	14.3 tonnes
Total mass of sugar beet required for a treatment zone volume of 458m^3	==>	71.6 tonnes

6.5.3.4.6 Cost of Anaerobic Treatment Zone Method

An estimate of capital cost and variable cost i.e., operating cost per cubic metre of water treated, for the two anaerobic treatment zone management strategies is made using the following figures:

Cost of water storage construction = £1.50 per cubic metre of water stored.

Cost of sugar beet = £39 per tonne (Nix, 1996).

Cost of crushed limestone = £24 per tonne (Soffe, 1995).

Amortisation Cost = £116 per £1000 invested for a 21 year repayment period @ interest rate of 10% (Weatherhead, 1996).

Strategy 1 : Treat all water prior to next event

Capital Cost

Water storage capacity of 17000m^3	=	£25500
Cost of control and diversion devices	=	£ 2000
Cost of constructing treatment zone	=	£ 1000
Cost of instrumentation	=	£ 1000
(Optional: Cost of electro-mechanical control system	=	£10000)
Capital Cost for Strategy 1	=	£29500 (£39500)

Variable Cost

Cost of replacing sugar beet and limestone:

£5500 (sugar beet)[1] + £3400 (limestone)[2] + £1000 (labour) = £9900

Hence, cost per m^3 of water treated	= (£9900 / 25110m^3)	=	39.4p/m^3
Additional management cost		=	2.0p/m^3
Amortisation of Capital Cost	= (£3422 / 25110m^3)	=	13.6p/m^3
Total Variable Cost for Strategy 1		=	55.0p/m^3

1	143 tonnes of sugar beet @ £39/tonne	=	£5577
2	143 tonnes of crushed limestone @ £24/tonne	=	£3432

Strategy 2 : Treat a proportion of the drainage water

Capital Cost

Water storage capacity of 17000m^3	=	£25500
Cost of control and diversion devices	=	£ 2000
Cost of constructing treatment zone	=	£ 1000
Cost of instrumentation	=	£ 1000
(Optional: Cost of electro-mechanical control system	=	£10000)
Capital Cost for Strategy 2	=	£29500 (£39500)

Variable Cost

Cost of sugar beet and limestone:

£2800 (sugar beet)[1] + £1700 (limestone)[2] + £1000 (labour) = £5500

Hence, cost per m^3 of water treated	= (£5500 / 8535m^3)	=	64.4p/m^3
Additional management cost		=	2.0p/m^3
Amortisation of Capital Cost	= (£3422 / 25110m^3)	=	13.6p/m^3
Total Variable Cost for Strategy 2		=	80.0p/m^3 .

[1]	72 tonnes of sugar beet @ £39/tonne	=	£2808
[2]	72 tonnes of crushed limestone @ £24/tonne	=	£1728

For both strategies the full replacement cost of limestone is quoted. This may be unecessary; however, for this simple statement of costing all possible costs are included for comparison purposes.

When the minimum capital cost of the two anaerobic treatment strategies i.e., £29500 is compared with the minimum cost of storing 25000m^3 of water i.e., £39500, it is evident that a saving can be made primarily due to a reduction in water storage capacity. However, the variable cost is significantly lower, for example with the storage only scheme, assuming pond maintenance costs are the same for both the long term storage and treatment schemes. It is evident therefore that water treatment using sugar beet as the carbon source would cost more in the long-term.

6.5.3.4.7 Supply, Processing, and Management of Sugar Beet

i) Supply

Sugar beet is predominantly grown in areas of arable and mixed agriculture, usually where the farm is within a reasonable distance of a sugar factory. As these areas are predominantly in the east and midlands of England, where nitrate pollution from

agricultural is also prevalent, it is suggested that sugar beet will be available for use by farmers at cost.

Alternatively, farmers could grow their own supply. The average yield of sugar beet is 44 tonnes per hectare (Nix, 1996). Hence, for the two management strategies described in Section 6.5.3.4.5, the area of land needed to produce the quantities of sugar beet required are:

Strategy 1	144 tonnes of sugar beet	= 3.3 ha
Strategy 2	72 tonnes of sugar beet	= 1.65 ha

Using a gross margin for sugar beet of £1340/ha (Nix, 1996), loss of earnings for the land required would be :

Strategy 1	3.3 ha = £4422
Strategy 2	1.65ha = £2211

Hence, the cost of growing the required tonnage of sugar beet would be less than the cost calculated in Section 6.5.3.4.6 i.e., £5500 and £2800 for Strategies 1 and 2 respectively.

It is also suggested that farmers could request to grow the sugar beet on set-a-side land, arguing that the production of crops for use as alternative fuel/energy sources is already permitted under EU regulations.

Sugar beet is planted in spring and harvested the following autumn, and so the farmer would have to ensure sufficient time to harvest/import and process the crop before the onset of the heavy winter rainfalls in November and December.

ii) Processing

Processing of the sugar beet into the required piece size i.e., 20-40mm is another important consideration. Presently no machine exists to process the large volumes of

sugar beet required; however, it is recognised that adaption of machines e.g. turnip choppers or sugar beet handling and cleaning equipment could do this. For any machine developed or adapted, it is necessary that the sugar beet would require chopping without crushing to ensure that significant loss of sugar juice, and therefore soluble carbon, did not occur.

It is also suggested that the food processing industry be investigated, as machines may have already been developed for the production of processed fruits and vegetables e.g. chipped potatoes and pineapple chunks.

iii) Management

Work reported in Chapter 2 indicated that an acclimatisation period may be required for a water treatment zone before treatment could begin. This could be achieved by flooding the treatment zone for several days before treatment began, which will also help to purge the treatment zone of free oxygen, ensuring anaerobic conditions prevail when treatment begins. If, however, rapid acclimatisation was required then microbial activity could be enhanced initially by adding a readily utilisable carbon source e.g. granular sugar.

It was concluded from the experimental investigations that in the initial period the sugar beet could become 'over-heated' i.e., intense microbial activity, producing unstable and unpredictable nitrate losses as well as possible increases in ammonia production. This problem could be resolved by re-circulating the initial flows of treated water, either through the treatment zone, or back into the pond.

Between treatment periods or once the readily utilisable carbon (RUC) fraction had become exhausted, aeration and drying of the sugar beet may enhance microbial degradation of the sugar beet. Aeration would effectively kill off the non-facultative anaerobes, providing the aerobes with a carbon substrate. Aerobic degradation would release RUC because it is more efficient in terms of energy yield and therefore organism

growth, than anaerobic degradation. Hence, the rate of degradation would be increased with consequent greater quantities of RUC being released. If the sugar beet is then re-wetted the larger population of aerobes would die providing more RUC.

A figure of 2 pence per cubic metre of water treated is used in variable cost calculations to cover management costs. Hence, to treat 30000m^3 the annual management cost would be £600.

6.6 Water Management and Control

6.6.1 Water Management

Water can be diverted from the collector into the pond via either a pipe or channel. Before the drainage season begins the gate at the pond intake should be opened, and the collector ditch blocked with a further control gate. All the drainage water would therefore be diverted into the pond and not the watercourse.

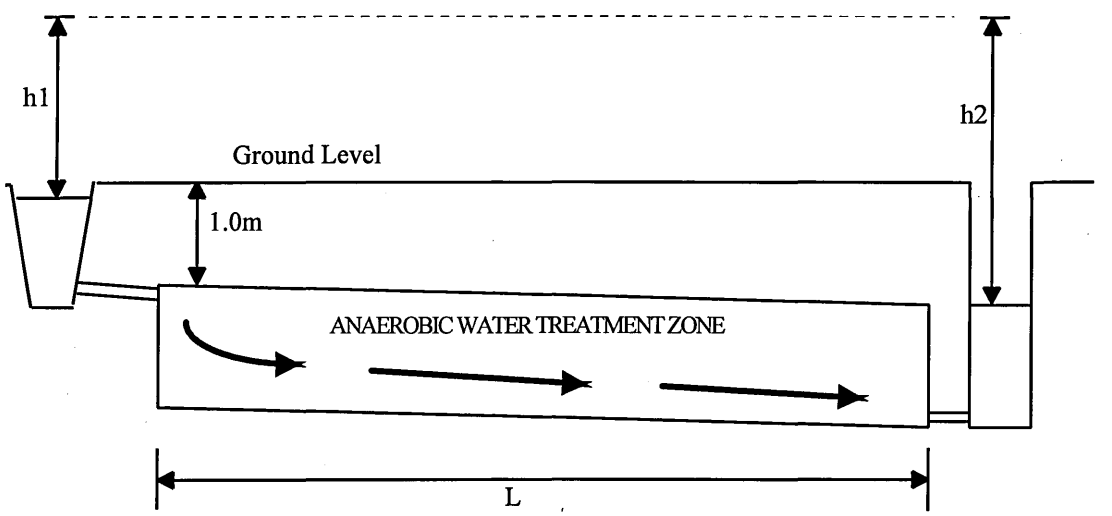
Where required, the outflow from the pond/reservoir could be controlled using a combination of a control gate and flow measurement structure e.g. sharp crested weir. This arrangement would allow the water to be metered at the required rate either back into the collector for blending, or down a pipe into the treatment zone. Automatic control could be achieved using a series of float switches linked to a control board that adjusts the heights of the gate to give the required flow.

Where water is to be diverted into a treatment zone, it is recommended that the offtake be sited at the base of the pond where water temperatures are likely to be higher, so minimising the effect of low temperature on microbial nitrate reduction within the treatment zone.

If the location of the pond provides insufficient head for the water control measures described, water could be pumped both into and out of the pond/reservoir. A portable 5 horsepower pump could be employed for this purpose. Alternatively a tractor p.t.o. driven pump would be equally suitable, and may be preferable during late autumn/winter when demands upon machinery for cultivations and other operations has reduced.

The pond intake control gate would remain open until the storage capacity was reached. If, however, drainage water volume exceeded storage capacity, an overflow structure could be used to feed excess water back onto a grass spillway.

Figure 6.3 illustrates a possible method for controlling the flow rate through an anaerobic water treatment zone by manipulation of the water-level in the outfall ditch.



$$Q = K \cdot \frac{(h2 - h1)}{L} \cdot A$$

Q = Flow Rate through Treatment Zone (m^3/s)
K = Hydraulic Conductivity (m/d)
L = Length of Treatment Zone (m)
A = Cross-sectional area (m^2)
(h2-h1) = Head loss (m)

Figure 6.3 Schematic Diagram showing Possible Method for Controlling Flow Rate Through Anaerobic Treatment Zone

Water levels in the anaerobic treatment zone can be monitored using piezometer tubes, and to make reading easier a graduated stick supported by a float can be used to indicate the water level. Continuous measurement of water level is possible by this method as an array of piezometer tubes will illustrate the water level profile across the treatment zone.

Reservoir design should also be a consideration dependent upon which strategy is chosen. If long term storage is chosen reservoirs should be deep and narrow, hence promoting nitrate reduction at depth whilst reducing evaporation at the surface. For other strategies, where water volumes need to be reduced, evaporation is enhanced by increasing surface area.

6.6.2 Control and Instrumentation

Section 6.4 above highlighted and discussed the variations of nitrate concentrations in water draining from arable clay catchments. By using instrumentation, the farmer would be able to discriminate between drainflows in which nitrate concentrations are either above or below the OFMAC, so giving them the option of only having to treat water that exceeded that limit.

It was mentioned in Chapter 3 that such instrumentation existed as available off-the-shelf technology in the form of a solid state ion specific electrode (ISE). ISE's need to be used in collaboration with a double junction reference electrode, connected to a millivolt (mV) meter. The instrument requires calibration, preferably on a daily basis, however, this would only be required if high accuracy were necessary. For field use, however, with a sufficient safety margin the instrument can be considered as stand alone.

Laboratory trials with an ISE indicated that the sample of water being analysed for nitrate needed to be stationary. A V-notch weir apparatus has already been developed (Cuttle and Mason, 1988) which could present such a stationary sample of drainage

water. It is proposed that the V-notch weir and the ISE be incorporated into the proposed field system by siting it upstream from the entrance to the storage pond/reservoir.

Additionally, the ISE could be connected to a microprocessor which would automatically convert the analogue (mV) signal, using a memory stored calibration curve, to the corresponding nitrate concentration. The nitrate reading would be compared to the OFMAC also stored in the microprocessor memory which, if exceeded, the microprocessor could initiate an electro-mechanical water diversion mechanism. Alternatively, to reduce cost, an alarm could signal when the nitrate concentration in drainage water was exceeding the OFMAC. The farmer would then manually control the diversion of water into a pond or reservoir.

The instrumentation and water capture device are estimated as costing £1000. It is recognised that this may reduce its appeal, especially as it is likely that all drainage water between September to December will need to be stored, making discrimination unnecessary in this important period.

6.6.3 Post-Treatment Management

Once the water has been treated in the nitrate reducing treatment zone, additional measures of ensuring good water quality may need to be employed prior to returning the water to the watercourse. Such measures include either passing the water through reed beds or over a grass treatment plane (buffer strip). Both reed beds and buffer strips are discussed in more detail in Chapter 2, which reveals that they could only be viewed as supplementary measures for improving water quality. It is also recognised that these measures would contract an additional cost on the system, making the overall cost of the option higher than the figures quoted in Section 6.5.3.4.6.

6.7 Comparison of Costs for Alternative On-Farm Pollution Control Methods

To enable a cost comparison to be made with industrial treatment methods, it is necessary to calculate the cost of using methanol as a carbon source in water treatment systems.

6.7.1 Cost of Treatment Utilising Methanol as the Carbon Source

In Section 6.6.3.4.5 above, figures for drainflows and nitrate losses from a 30ha catchment following three rainfall events were used to calculate the treatment zone areas. The same figures are now used to calculate average nitrate-nitrogen concentrations.

They are:

	Average NO_3-N concentration (mg/l)
Event 1 = 17070m^3, 778.39 kg NO_3-N	45.6
Event 2 = 6240m^3, 121.81 kg NO_3-N	19.5
Event 3 = 1800m^3, 36.54 kg NO_3-N	20.3

Methanol concentrations required to treat these nitrate-nitrogen concentrations are calculated using the following formula (McCarty et al., 1969):

$$Cm = 2.47\,(NO_3\text{-}N) + 1.53\,(NO_2\text{-}N) + 0.87\,(DO)$$

Assuming drainage water contains no nitrite (NO_2-N) but has 8mg/l Dissolved Oxygen (DO), then the methanol concentration required to treat each event is:

Event 1	(2.47 x (45.6) + 0.87 (8)) =	120 mg/l
Event 2	(2.47 x (19.5) + 0.87 (8)) =	55 mg/l
Event 3	(2.47 x (20.3) + 0.87 (8)) =	57 mg/l

Volume (litres) of methanol required:

Event 1	$\frac{(120\ mg/l \times 17070000\ l)}{0.7914}$	=	2588 litres
Event 2	$\frac{(55\ mg/l \times 6240000\ l)}{0.7914}$	=	434 litres
Event 3	$\frac{(57\ mg/l \times 1800000\ l)}{0.7914}$	=	130 litres

where; methanol, $\rho = 0.7914$ kg/litre
Hence, total volume of methanol required = 3152 litres.

Capital Cost of Methanol Treatment

Estimated cost of on-farm methanol treatment plant = £30000.
Treatment rates of $1000m^3$/day are possible, however, there would still be a requirement to have a minimum design storage capacity of $17000m^3$ @ £1.50/m^3 = £25500.

Total Capital Costs = £55500.

Variable Cost of Methanol Treatment

Methanol = £1 per litre (SAF Bulk Chemicals, 1996).

Hence, cost of 3152 litres of methanol = (£3152/$25110m^3$)	=	12.6p/m^3
Annual maintenance cost using fluidised sand bed units with methanol dosing = (£5000/$25110m^3$)	=	19.9p/m^3
Amortisation of Capital Cost[(1)] = (£6380/$25110m^3$)	=	25.4p/m^3
Total Variable Cost	=	57.9p/m^3

(1) Amortisation Cost = £116 per £1000 invested for a 21 year repayment period @ interest rate of 10% (Weatherhead, 1996).

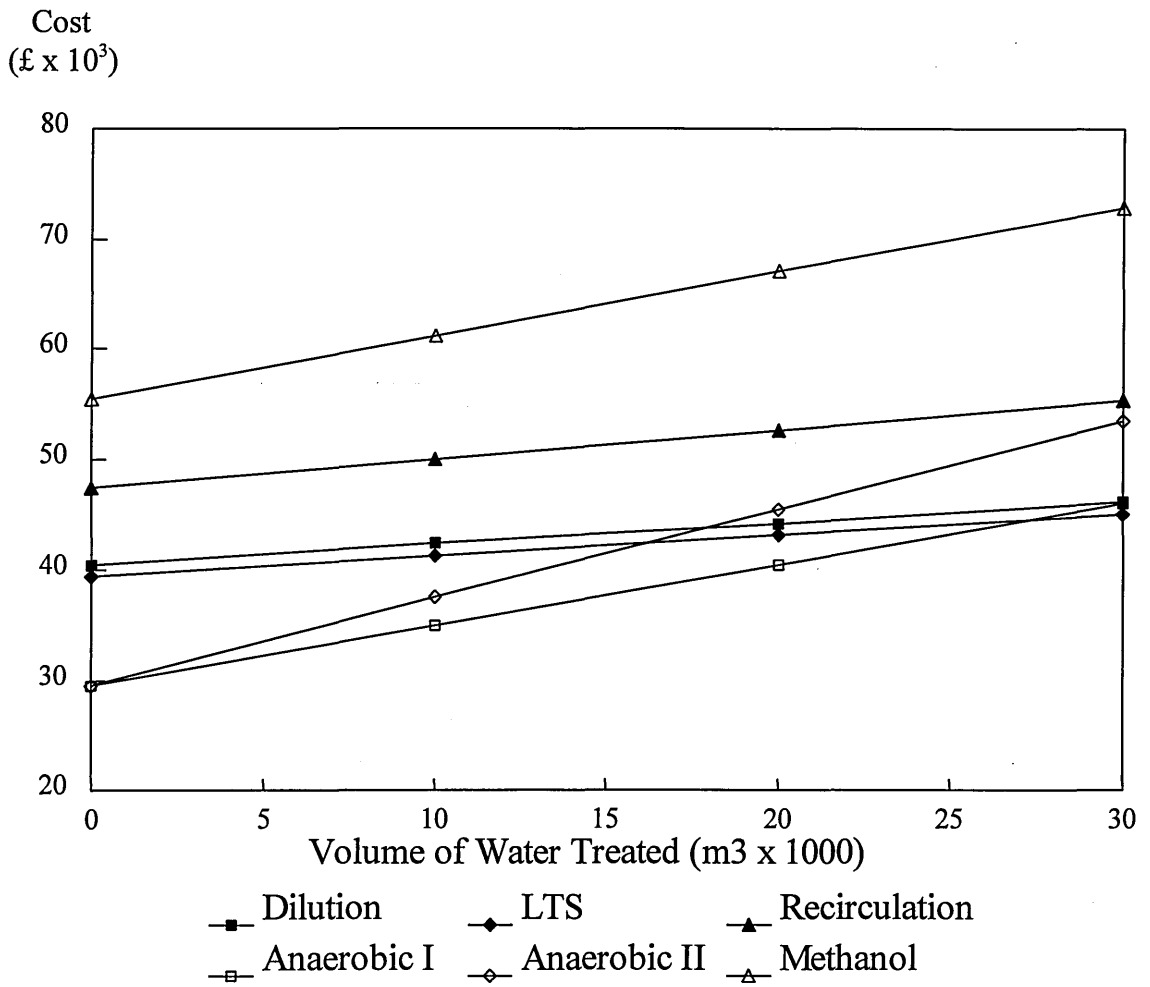
6.7.2 Cost Comparison

The comparative cost of the alternative methods of on-farm nitrate pollution control can now be made and is illustrated in Figure 6.4. The intercept with the y-axis shows the capital component of the costs in each case, and the gradients of the lines give a measure of the operating cost.

It is evident from Figure 6.4 that although on-farm treatment methods offer a capital cost saving because of a reduced design storage capacity, the variable costs, including amortisation of capital, make them less attractive particularly when the additional management and expertise that would be required to operate them is considered.

Overall, the strategy chosen by the farmer for managing high nitrate flows from drained agricultural land will be the one which is both economical and easy to manage, whilst satisfying environmental demands. The most important conclusion from this study is that there are several options available to the farmer, and because all options involve on-farm water storage, a dual benefit of both environmental and water conservation make these options relatively low-cost.

In Chapter 7, as part of a general discussion, the costs of the proposed on-farm methods are compared to the cost of off-farm treatment by the water industry (Section 7.2.3).



where;

Dilution	capital cost = £40500	+	variable cost = 18.7p/m³
LTS (long term storage)	capital cost = £39500	+	variable cost = 18.3p/m³
Recirculation	capital cost = £47500[(1)]	+	variable cost = 25.9p/m³
Anaerobic Strategy I	capital cost = £29500	+	variable cost = 55.0p/m³
Anaerobic Strategy II	capital cost = £29500	+	variable cost = 80.0p/m³
Methanol	capital cost = £55500	+	variable cost = 57.9p/m³

(1) capital cost of recirculation includes £20000 cost for irrigation pipe and equipment

Figure 6.4 Comparative Cost of Alternative Methods of On-Farm Nitrate Pollution Control

6.8 Summary

This chapter has examined possible on-farm treatment strategies for managing unacceptably high nitrate discharges draining from agricultural lands. Those strategies are summarised below.

Originally, dilution and/or blending were proposed as treatment methods; however, because of the over-winter nitrate losses from clay land averaging 22kgN/ha, the average concentration of nitrate in the water makes dilution/blending unfeasible in areas of low excess winter rainfall. Their application in areas of high excess winter rainfall are also limited because additional treatment methods, and therefore additional cost, would still be required.

A simpler strategy is long-term storage, i.e., greater than 6 months. The polluted drainage water is collected and stored on-farm until the nitrate concentration is reduced as a result of natural nitrate reduction at the sediment-water interface (Section 2.3.3). Although not the cheapest option, it is attractive both to the farmer, because it requires minimal management input, and to the water authorities who are keen to promote winter abstraction and storage. Indeed, the Managing Director of Southern Water Services has suggested that more reservoirs could be the only way to prevent water shortages in future (Conservation Matters, 1996). The construction of on-farm ponds would therefore have the dual benefit of both water and environmental conservation.

Recirculation of the water is also an attractive option, especially where irrigation equipment is already to be found in operation, because of the lower water storage costs. Where irrigation equipment needs to be purchased, the cost can be offset against savings made in fertiliser which is also recirculated.

Treatment of the water by anaerobic nitrate reduction was also investigated. It was shown in laboratory experiments that sugar beet could be used as a carbon source. However, an examination of costs has indicated that after the first year's operation, it

would be a more expensive method of treatment than those that have higher capital costs because of an increased storage capacity requirement. Treatment methods could be justified if either the magnitude of water volume stored were unacceptable to water authorities or if reservoir capacities required exceeded limits for on-farm suitability; however, this could be resolved by having a series of smaller ponds.

A future consideration is that the figures given above for average nitrate concentrations are based on mean excess winter rainfall figures published in 1975. Since then the 'Greenhouse Effect' has caused global warming. Temperature rises will effect rainfall patterns to such an extent that arid areas of the UK will become drier and the wet areas wetter. The consequence of this in areas of high rainfall will be to make the option of on-farm dilution/blending more viable. In areas of low rainfall, however, reduced volumes of drainflow would increase average nitrate concentrations whilst allowing reductions in design storage capacity.

Chapter 7

General Discussion and Recommendations to Farmers

7.1 Recent Developments and Future Nitrate Policy

The possibility of a future extension of nitrate controls has been made more probable with the incorporation of the NRA into the new Environment Agency on 1st April 1996. This has seen the amending of Section 161 of the Water Services Act, whereby the Agency will have the legal power to instruct organisations or individuals to carry out work necessary to prevent pollution. The amendment will also allow the agency to require polluters to clean up the pollution they have caused.

With legislation being toughened and its application made more resolute, farmers may soon require additional measures to ensure the protection of watercourses and rivers from pollution. This is especially likely as it is predicted that there will be an increase in agricultural land area being used for sewage sludge disposal (Davis,1996). Provision of an effective pollution prevention strategy would enable continued, and perhaps increased, applications of sewage sludge onto land whilst ensuring freedom from legal action.

Common Agricultural Policy (CAP) price subsidies may also soon be reviewed as European politicians show an increasing concern that environmental aspects of CAP reform should incorporate more EU water legislation, and that good practice should replace good yields (Anyadike, 1996). Indeed, a recent European Union report (EU, 1995) has warned that only partial headway has been made on the implementation of water quality legislation by Member States, concluding that much still needs to be done on the issue of nitrates.

7.2 Feasibility of On-Farm Reduction of Nitrate Pollution

It was the aim of this research study to investigate the feasibility of treating and managing unacceptably high nitrate concentrations in drainage water. By reviewing the nitrogen cycle and its controls it was obvious that anaerobic micro-organisms in the soil reduce nitrate to harmless nitrogen gas under anaerobic conditions. Having identified this as an important process for the reduction of nitrate in agricultural lands, it was recognised as having potential for treating nitrate polluted drainage water. The control parameters for anaerobic nitrate reduction were therefore examined to define the environmental conditions under which nitrate reduction could be optimised. A review of current and proposed methods of nitrate pollution control, and their applicability to the aim of the present study, identified anaerobic treatment as a possible solution, subject to further investigations of specific factors, including cost.

Three possible broad approaches for preventing nitrate pollution from drained lands were identified; these were dilution, long term storage, and anaerobic water treatment. A further option of recirculation is proposed; however, before recommendation there is a need for further investigation.

The main project conclusions and their implications are discussed with specific reference to:

i) On-Farm Treatment
ii) On-Farm Management
iii) Cost.

7.2.1 On-Farm Treatment

It was hypothesised that soil could be used as a medium for supporting anaerobic nitrate reduction, and therefore for treatment of polluting drainage water. However, when the potential for nitrate reduction in soil was investigated under an anaerobic environment,

it was concluded that sustainable nitrate reduction was only achievable if an additional carbon source were supplied.

The problem of nitrate polluted drainage water occurs predominantly in late autumn/early winter when the initial drainflows leach out residual nitrate that has built up in the soil following harvest and autumn cultivations. Hence, if treatment of the water is to be considered as a solution it was necessary for it to work at lower winter temperatures. A temperature of 10°C was chosen for experimentation, which, although higher than ambient over-winter temperatures, would satisfy the aim of this study to assess feasibility.

This study showed that significant nitrate reduction i.e., 100mg/l (22.6mg/l NO_3-N) was possible at low temperatures, when nitrate reducing anaerobic bacteria had a readily utilisable carbon supply. Optimum nitrate reduction efficiency occurs when the ratio of carbon atoms to nitrogen atoms is such that maximum nitrate reduction is obtained for the minimum amount of carbon supplied to the system. This study confirmed the optimum C : N ratio to be 1.65 to 1, when glucose is used as the carbon source in nitrate reducing water treatment systems. The main conclusion of the study, however, was that attached growth treatment systems using sand and soil as the porous support media would be unsuitable for on-farm use because clogging, as a result of biomass growth, would eventually cause hydraulic failure.

The phenomenon of anaerobic nitrate reduction is a result of anaerobic bacteria utilising nitrate instead of oxygen for respiration. Maintenance of an anaerobic environment is therefore vital to obtain high rates of nitrate reduction. As drainage water contains dissolved oxygen, a higher flow rate of water into the treatment system increases the import of oxygen. The literature review identified the environmental threshold for anaerobic nitrate reduction to be a redox potential of 200mV, a figure confirmed in the low temperature study. Hence, it is possible to maintain an anaerobic environment, i.e., less than 200mV, by controlling the flow rate of water through the treatment system. Treatment performance has therefore to be described relative to the nitrate concentration

reduced, volume of water treated, and the volume of bio-reactor, and is achieved by quoting performance in terms of grams of nitrate-nitrogen reduced per cubic metre of bio-reactor per day ($g/m^3/d$).

It was proposed that organic materials be used to support anaerobic nitrate reduction in the treatment systems by being both the attached growth site, and, upon degradation by the micro-organisms, provide a readily utilisable carbon supply. A biodegradation study was undertaken to assess their potential use in which three organic materials were compared to glucose. This study concluded that glucose could only be a sustainable carbon source providing it was applied continuously to the bio-reactor. Importantly, the study showed batch sugar beet to be a degradable carbon source that could support anaerobic nitrate reduction. As it was a requirement of any proposed solution that a non-continuous input was desirable, it was concluded that water treatment systems utilising very soluble carbon sources were unsuitable for on-farm operation, but that there was a potential for using sugar beet in further studies.

Subsequent continuous flow treatment studies using sugar beet concluded that a sustainable nitrate reduction performance of $23g/m^3/d$ could be achieved. This is higher than the $19g/m^3/d$ recorded in the glucose studies, but this low figure was primarily a result of poor hydraulic performance in the glucose experiments. Indeed, management of biological clogging is a feature of industrial attached growth water treatment systems, which consequently achieve greater performances than those reported in this study.

In both biodegradation and flow studies pH was a major control parameter. It was therefore concluded that optimal nitrate reducing performance required a near neutral pH environment to be maintained. The mixing of crushed limestone with the sugar beet was shown to be an effective method of pH control, and could therefore be recommended for use in a treatment system.

Identification of an optimum performance for a treatment system utilising sugar beet as the carbon source allowed, for specific on-farm pollution incidents, the design of

appropriate treatment systems, including definition of bio-reactor volume and cost. Those figures showed that the scale of the proposed systems were acceptable, especially where land is available e.g. through set-a-side. The cost of the systems, however, were in the long-term high relative to other more simple, easier to manage strategies.

7.2.2 On-Farm Management

The objective of this study was to monitor, identify and handle nitrate polluted drainage water. Investigations into available technology lead to the conclusion that solid-state Ion Specific Electrode's (ISE) were the cheapest and easiest option to monitor and identify nitrate, whilst being robust enough for on-farm use.

Being able to identify the polluting drainage water allows it to be intercepted, stored, and managed effectively. Once stored, the proposed options for managing the drainage water are dilution, recirculation and long-term storage. Detailed examination of each strategy allowed their applicability to the project aim, and cost, to be assessed.

Dilution is only possible by collecting all the initial polluting drainflows and then diluting those with later drainflows that have a lower nitrate concentration. This solution, however, has only a limited application in areas of high excess winter rainfall i.e., greater than 200mm, where average nitrate concentrations in late season drainage water will be lower than the acceptable 50mg/l nitrate limit. If dilution were used, the drainage water would require additional treatment/management options, and therefore cost, to ensure pollution protection.

Long term storage i.e., greater than 6 months, is an attractive option as it is simple to manage and can be applied in almost all cases where the necessary land can be made available. It is also suggested that a 6 months storage time may in fact be conservative for the required nitrate reduction. This is because the figures researched were for nitrate reduction in reservoirs, whereas in an on-farm pond the water-sediment interface i.e.,

where nitrate reduction occurs, is proportionally greater, and so nitrate reduction may occur at a faster rate. The study showed that the ratio of drained land area to pond area required is approximately 30 to 1, assuming a pond depth of 3m. Where there are limits upon the size of water storage structure permitted, a series of ponds/reservoirs would still permit the effective management of the polluting water. Long term storage also offers the additional benefit of on-farm water conservation, a factor that may help justify cost, and which in time could become as important as pollution prevention. Large scale on-farm water storage also opens up the possibility for water transfer schemes, in which water stored during winter is released during the summer. This water could then be abstracted further downstream either by farmers for irrigation or by the water industry to supplement domestic supply.

Drainflow volume comprises preferential flow through macropores between clay aggregates and piston flow through the micropores within the clay aggregates. Following initial drainflows in late autumn, i.e., when preferential flow predominates, there is scope for the clay to adsorb further water until field capacity is reached. Recirculation of drainage water onto clay in late autumn may therefore exploit this phenomenon. A reduction of nitrate losses would result primarily by reducing the volume of drainage water in which the nitrate is carried. Secondary losses through the promotion of anaerobic zones may also occur. Storing the drainage water and recirculating it for irrigation is also an option; however, the main restriction of this solution is that it is site specific. This is primarily because the problem being addressed is associated with drained clay lands, where farmers are less likely to need irrigation water, as significant soil moisture deficits are improbable and the crops grown rarely justify irrigation. Recirculation would as an option, however, allow water storage costs to be reduced, making the strategy attractive to farmers who already irrigate land. Likewise it could be considered by dairy farmer's who own slurry spreading equipment.

7.2.3 Cost

A cost comparison was made in Chapter 6 between the proposed on-farm methods for controlling water pollution. This comparison concluded that treatment methods offered a capital cost saving because of a reduced design storage capacity. Variable costs, including annual amortisation of capital, however, made them less attractive, particularly when the additional management and expertise that would be required to operate them was considered. However, to put the cost of the different options into context it is necessary to consider the cost of treating the water by the water industry within a large capacity, off-farm treatment plant.

For example, the cost of a new activated sludge plant to treat 20000m^3/day of water with levels of nitrate above the 50mg/l limit is £8.85million, with an associated running cost of 4.8p/m^3 of water treated (Cooper at al., 1995). Amortisation[1] of capital over a 21 year period indicates costs of 14.1p/m^3, assuming the plant treats 7.3 million cubic metres of water per year. Hence, the total cost per cubic metre of water treated is 18.9p/m^3.

Using the figure of 18.9p/m^3 for off-farm treatment, the cost of treating 30000m^3/year from a 30ha drained agricultural catchment would be approximately £5700. In Section 6.7.2 the costs per cubic metre for possible on-farm methods were given, and hence the cost of treating 30000m^3/year using dilution, long term storage, recirculation, and anaerobic water treatment (sugar beet and methanol), are approximately £5600 (18.7p/m^3), £5500 (18.3p/m^3), £7800 (25.9p/m^3), and £17000 (56.7p/m^3) respectively. It is therefore apparent that the cost of the on-farm management strategies i.e., long term storage, storage and recirculation, and storage and dilution, would be similar to off-farm treatment, all of which are significantly cheaper than on farm treatment.

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1 Amortisation Cost = £116 per £1000 invested for a 21 year repayment period @ interest rate of 10% (Weatherhead, 1996).

The above costs for on-farm solutions, however, do not take into account the cost of land and production lost as a result of the construction of on-farm water storage facilities. For example, for a 30ha catchment growing cereals, approximately 1ha of land would be lost at a cost of £5000/ha, and a gross margin of £780/ha/year for winter wheat (Nix, 1996). If set-a-side payments were sanctioned to compensate for the production loss at the maximum of £341/ha/year (Nix, 1996), and the capital cost for the land was amortised over 21 years, the cost per cubic metre would be equivalent to 5.4p (see Appendix VII). Hence, the additional cost for on-farm treatment of 30000m^3/year would be approximately £1600. Grant aid from MAFF, the Countryside Commission, and English Nature is available for pond construction, and may help reduce this cost.

Hence, the approximate total annual cost of the proposed management strategies i.e., storage and dilution, long term storage, and storage and recirculation, would be £7200, £7100, and £9400 respectively. It can therefore be concluded that the on-farm management methods are not currently competitive in terms of cost with off-farm treatment. It may be unlikely that subsidies and grants will be given towards the construction of on-farm water storage facilities, however; it is possible that the construction of on-farm water storage facilities would increase land values as they would supplement existing water supplies. Any increase in land value could be offset against the cost of the proposed on-farm methods, so allowing them to be considered as an alternative option for controlling nitrate pollution.

7.3 Recommendations to Farmers

The above discussion leads to the following recommendations to farmers:

i) Farmers in areas of high excess winter rainfall i.e., above 200mm, can use the following strategies: dilution, long term storage, and anaerobic treatment. Long term storage is a simple strategy requiring a low management input; however, greater volumes of drainage water will require larger water storage structures, the construction and land costs of which may prove prohibitive. Anaerobic treatment and dilution are more expensive options, but allow scope for reduced design storage capacities, whilst offering greater flexibility to the farmer for managing the polluted water.

ii) Farmers in areas of low excess winter rainfall i.e., less than 200mm, are limited in the strategies available because the average nitrate concentration of drainage water, if all collected, will be above the 50mg/l acceptable limit. Long term storage and anaerobic treatment are the options available, of which long term storage is more cost effective and requires less management than treatment systems. Treatment systems are more expensive, but allow scope for reduced design storage capacities, whilst offering greater flexibility to the farmer for managing the polluted water.

iii) A further strategy of recirculation is proposed, however, this requires further investigation. It is likely to be an option for farmers in areas of low excess winter rainfall, where high soil moisture deficits will be probable in both late autumn and summer, and where irrigation equipment may also already be available. For farmers in areas of high excess winter rainfall, however, lower soil moisture deficits in late autumn and summer will limit the application of the method, whilst possibly requiring high capital expenditure on irrigation equipment.

Chapter 8

Summary, Conclusions, and Recommendations for Future Work

8.1 Summary

This study has shown that the high peaked discharges of drainflows from agricultural catchments in late autumn present significant management problems. Consequently, all the options outlined in this study require the construction of on-farm water storage facilities to enable the interception and storage of the drainage water. Once stored, the water can be controlled and effectively managed, so preventing pollution of rivers and watercourses.

Investigations into anaerobic nitrate reduction at low temperatures indicate that there is potential for utilising on-farm resources to develop an on-farm method of pollution control. These resources include land for the construction of nitrate reducing anaerobic treatment zones and agricultural crops, in particular sugar beet, for use as carbon sources in those treatment zones. Treatment systems allow a reduction in design storage capacity and offer flexibility to the farmer in managing the polluted water. However, they require a high degree of management expertise and cost more to both construct and operate than alternative water management options.

The water management options outlined include long term storage, dilution, and recirculation of the stored water. These options meet all the requirements for on-farm suitability i.e., control of nitrate pollution, they require a minimum of management expertise to operate, operable at low temperatures, integration into current farming systems, and a comparable cost to existing off-farm treatment. By satisfying these

requirements, these strategies can be recommended to farmers as on-farm methods of reducing nitrate pollution in subsurface drainage water.

8.2 Conclusions

1) In soil, significant continuous nitrate reduction can only be achieved under anaerobic conditions when a readily utilisable carbon source is continuously present.

2) At the low temperature of 10°C nitrate concentrations of 100mg/l (22.6mg/l NO_3-N) were reduced in attached growth water treatment systems where glucose was supplied as the carbon source. Anaerobic treatment of nitrate polluted drainage water is therefore possible in winter.

3) In attached growth water treatment systems, glucose can be used as a readily utilisable carbon source, however, it is a non-sustainable carbon source unless continuously supplied.

4) In water treatment systems utilising glucose as the carbon source, the most efficient application ratio is 1.65 glucose-carbon atoms to 1 nitrate-nitrogen atom. This is the ratio at which all the nitrate can be reduced with the minimum amount of glucose (energy) utilised.

5) A redox potential of 200mV is the environmental threshold for anaerobic nitrate reduction in water treatment systems.

6) Biologically degraded sugar beet is a sustainable source of readily utilisable carbon. When used as a carbon source in a small-scale laboratory anaerobic water treatment system, an average optimum performance of 23 grams of nitrate-nitrogen reduced per cubic metre of bio-reactor per day was achieved.

7) In water treatment systems which utilise soluble carbon sources e.g. glucose, clogging of the porous media as a result of biomass growth results in a decline in flow rate and poor performance, and unless remedial action is taken will eventually result in hydraulic failure. Clogging is not a problem when sugar beet is used as both the carbon source and the microbial growth site, and therefore flow rates though the water treatment system are both sustainable and controllable.

8) Low environmental pH reduces nitrate reduction in anaerobic water treatment systems utilising organic materials as the carbon source. Optimum nitrate reducing performance is obtained with a near neutral pH environment, which can be maintained by adding crushed limestone to the bio-reactor.

9) This study has shown that on-farm nitrate reducing treatment systems are feasible, however, construction and maintenance costs, combined with the additional management expertise required to operate them, will make them less attractive to the farmer.

10) Farmers in areas of high excess winter rainfall i.e., greater than 200mm, can use long term storage, dilution, or treatment to reduce nitrate pollution. Farmers in areas of low excess winter rainfall i.e., less than 200mm, however, can only use long term storage or treatment to reduce nitrate pollution. Recirculation is a possible further option in all areas, however, requires further investigation.

11) The proposed pollution protection strategies require the interception and on-farm storage of drainage water, adding considerable cost to those strategies. For long term storage, however, an additional environmental benefit of on-farm water conservation would be provided, at a cost approximately 25% greater than that for off-farm water treatment alone.

8.3 Recommendations for Future Work

The conclusions of this study have identified the following areas for future work:

1) Undertake Field Scale Studies of Recirculation Strategy

An objective of this study would be to examine the practical aspects of the method, including identification of appropriate irrigation equipment. It is suggested that standard irrigation scheduling programmes that incorporate the Penman equation be used to determine timeliness of water applications.

Measurement of both water and nitrogen application and discharge rates would allow a water and nitrogen balance to be made, because once the water has drained through the soil again its volume and nitrogen content can be re-measured, and any benefit quantified.

2) Investigate Nitrate Reduction in On-Farm Ponds

The aim of this study would be to examine nitrate reduction rates in drainage water stored over a 9 month period in ponds and water storage facilities with volumes not greater than 30000m^3. The objective would be to identify the optimal shape of pond for greatest rates of nitrate reduction, whilst providing the maximum storage capacity, with the minimum loss of land required for pond construction.

3) Investigate the Application of Controlled Drainage under UK Conditions

Controlled drainage has been used to reduce nutrient pollution from free draining soils in the USA. Application of the method on drained clay land would have the significant advantage of low cost i.e., because the method would make use of the installed subsurface drainage network. Investigations have already examined the effect of prolonged waterlogging upon yields, however, the objectives of this work would include the examination of nutrient losses and the practical application of the method including waterlevel control needs for increased nitrate reduction and satisfactory tractor fieldwork.

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Appendix I

Analysis of Sandy Loam Soil Used in Soil and Low Temperature Studies

pH			7.32
% Organic Matter -			
	Furnace Method		5.67%
	Titration		3.17%
Particle Size Analysis by pipette method			
	-	Fine	21.48%
Sand	-	Medium	42.69%
	-	Coarse	10.32%
		Total	74.49%
Silt	-		12.86%
Clay	-		12.65%
Dry Bulk Density - Undisturbed			1.44gm/cm^3
Keen Box -	Dry Bulk Density (air dry)		1.31gm/cm^3
	Dry Bulk Density (saturated)		1.28gm/cm^3
	Density of solid constitutents		2.37gm/cm^3
	Porosity (air dry)		0.45gm/cm^3
	Porosity (saturated)		0.46gm/cm^3
	Void Ratio (air dry)		1.14gm/cm^3
	Void Ratio (saturated)		0.85gm/cm^3
	%Volume Expansion		2.41%

Appendix II

Calculation of the Chemical Quantites Required for Formulations Used in Soil and Low Temperature Studies

1) Formulation of the 100mg/l Nitrate Solution :

Molecular weight of KNO_3 = 101.10324

Molecular weight of NO_3 = 62.0049

$$\frac{101.10324}{62.0049} = 1.6305$$

Hence, 163.05mg KNO_3 added to 1 litre of distilled water will give a nitrate concentration of 100mg/l, and is equivalent to 22.6mg/l NO_3-N.

2) Formulation of the Carbon to Nitrogen Ratios (C : N) :

Values given below in Table A2.1 are in grams and are used to make up a 20 litre solution of the influent for the C : N ratio required:

Table A2.1 Quantites of Chemicals Required to Produce Standard Solutions of Carbon to Nitrogen Ratios Used in Experimental Studies

C : N Ratio	Glucose ($C_6H_{12}O_6$) (grams)	Potassium Nitrate (KNO_3) (grams)
3 : 1	3.390	3.261
2 : 1	2.260	3.261
1.65 : 1	1.865	3.261
1 : 1	1.130	3.261
Control	0.000	3.261

Appendix III

Low Temperature Study Data

i) Feasibility of Nitrate Reduction (C : N = 3 : 1)

(Data Summarised in Table 5.3)

Test No.1

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)		Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)
1	0.51	4.34		25	0.4	3.28
2	0.5	3.82		26	0.4	3.2
3	0.5	3.7		27	0.4	3.84
4	0.49	3.43		28	0.4	3.78
5	0.49	4.51		29	0.4	4.18
6	0.48	2.46		30	0.39	3.66
7	0.48	2.75		31	0.39	3.28
8	0.48	4.3		32	0.39	3.9
9	0.48	4.15		33	0.39	3.7
10	0.47	2.79		34	0.39	3.72
11	0.47	2.85		35	0.38	4.05
12	0.46	3.16		36	0.39	3.59
13	0.46	3.28		37	0.37	3.59
14	0.45	2.99		38	0.37	3.68
15	0.45	2.83		39	0.37	3.88
16	0.44	2.87		40	0.37	3.82
17	0.44	2.97		41	0.37	3.41
18	0.43	2.91		42	0.36	3.84
19	0.43	3.2		43	0.36	3.39
20	0.43	2.89		44	0.36	3.61
21	0.43	3.55		45	0.36	4.74
22	0.42	3.43		46	0.36	5.63
23	0.42	3.45		47	0.35	3.72
24	0.41	3.41		48	0.35	3.47

Average Flow Rate = 0.42 l/hr

Average NO_3-N Reduction = 3.56 mg/l

Test No.2

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)		Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)
1	0.46	2.44				
2	0.44	2.12				
3	0.43	2.03				
4	0.41	2.07				
5	0.4	2.46				
6	0.39	2.39				
7	0.38	2.19				
8	0.38	1.62				
9	0.37	1.6				
10	0.37	1.76				
11	0.37	1.96				
12	0.36	1.87				
13	0.35	2.1				
14	0.35	2.26				
15	0.35	1.82				
16	0.34	1.96				
17	0.34	2.28				

Average Flow Rate = 0.38 l/hr
Average NO_3-N Reduction = 2.05 mg/l

Test No.3

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)		Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)
0.5	0.36	10.12		12.5	0.3	6.54
1	0.32	12.38		13	0.3	4.31
1.5	0.32	11.22		13.5	0.3	2.9
2	0.3	12.06		14	0.3	3.54
2.5	0.3	5.78		14.5	0.29	3.77
3	0.29	9.99		15	0.3	2.88
3.5	0.3	11.17		15.5	0.3	2.52
4	0.3	11.39		16	0.3	2.96
4.5	0.29	9.44		16.5	0.29	2.94
5	0.29	5.25		17	0.29	2.4
5.5	0.3	2.4		17.5	0.29	2.54
6	0.32	4.57		18	0.29	3.46
6.5	0.3	3.3		18.5	0.29	2.68
7	0.3	3.66		19	0.29	2.92
7.5	0.3	4.78		19.5	0.29	3.28
8	0.3	7.74		20	0.28	3.1
8.5	0.3	6.44		20.5	0.28	3.46
9	0.3	5.51		21	0.28	3.46
9.5	0.31	9		21.5	0.29	6.7
10	0.31	6.33		22	0.29	4.59
10.5	0.31	2.74		22.5	0.28	4.96
11	0.31	2.96		23	0.28	4.49
11.5	0.3	3.54		23.5	0.27	9.34
12	0.31	2.56		24	0.28	5.8

Average Flow Rate = 0.30 l/hr
Average NO_3-N Reduction = 5.41 mg/l

Test No.4

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)		Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)
1	0.36	5.64		25	0.26	4.66
2	0.36	4.93		26	0.25	3.75
3	0.36	5.86		27	0.25	3.37
4	0.37	4.24		28	0.25	5.27
5	0.36	6.59		29	0.24	5.8
6	0.36	4.31		30	0.23	4.96
7	0.36	7.96		31	0.23	3.71
8	0.35	3.6		32	0.22	1.62
9	0.33	5.06		33	0.22	4.77
10	0.33	4.54		34	0.22	3.52
11	0.33	4.07		35	0.21	4.26
12	0.32	5.31		36	0.2	2.7
13	0.32	5.78		37	0.2	5.7
14	0.31	7.21		38	0.2	3.9
15	0.3	6.07		39	0.19	3.95
16	0.3	6.93		40	0.19	6.79
17	0.29	3.9		41	0.19	3.58
18	0.29	3.63		42	0.18	2.78
19	0.29	6.71		43	0.18	3.71
20	0.28	4.37		44	0.17	3.71
21	0.28	3.39		45	0.17	5.27
22	0.27	3.32		46	0.16	3.8
23	0.27	1.55		47	0.17	5.16
24	0.26	2.48		48	0.16	3.54

Average Flow Rate = 0.26 l/hr
Average NO_3-N Reduction = 4.54 mg/l

Test No.5

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)		Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)
1	0.22	5.73		25	0.15	4.76
2	0.21	4.03		26	0.15	4.9
3	0.21	2.81		27	0.15	4.79
4	0.21	5.07		28	0.15	5.87
5	0.2	2.98		29	0.15	5.07
6	0.21	3.28		30	0.14	4.92
7	0.2	3.11		31	0.14	5
8	0.2	3.71		32	0.14	5.05
9	0.19	3.71		33	0.14	5.69
10	0.19	3.04		34	0.13	3.99
11	0.19	4.26		35	0.13	5.98
12	0.19	3.28		36	0.13	5.09
13	0.18	3.43		37	0.13	5.29
14	0.18	3.02		38	0.12	5.16
15	0.18	3.68		39	0.13	6
16	0.17	3.98		40	0.12	4.65
17	0.17	3.58		41	0.12	5.03
18	0.17	3.79		42	0.12	6.09
19	0.17	3.88		43	0.12	5.93
20	0.17	4.01		44	0.12	6.16
21	0.16	4.66		45	0.11	6.02
22	0.16	3.94		46	0.11	5.93
23	0.16	4.5		47	0.11	6.09
24	0.16	4.03		48	0.11	6.4

Average Flow Rate = 0.16 l/hr
Average NO_3-N Reduction = 4.61 mg/l

Test No.6

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)		Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)
1	0.24	1.54		25	0.13	4.97
2	0.2	1.23		26	0.13	3.17
3	0.18	2.49		27	0.13	5.72
4	0.17	1.35		28	0.12	3.06
5	0.16	1.79		29	0.12	2.59
6	0.16	2.07		30	0.12	3.9
7	0.16	2.63		31	0.13	4.4
8	0.15	3.09		32	0.12	4.49
9	0.15	2.03		33	0.12	4.86
10	0.14	2.68		34	0.12	4.03
11	0.13	1.28		35	0.11	4.08
12	0.13	2.1		36	0.12	4.97
13	0.13	3.39		37	0.12	3.66
14	0.13	3.5		38	0.12	3.75
15	0.12	3.8		39	0.12	4.82
16	0.12	2.91		40	0.11	4.9
17	0.12	3.24		41	0.11	5.23
18	0.13	4.38		42	0.11	5.27
19	0.13	4.47		43	0.11	5.16
20	0.12	4.77		44	0.11	5.91
21	0.12	5.14		45	0.11	6.91
22	0.12	5.42		46	0.11	6.25
23	0.12	5.66		47	0.1	6.21
24	0.13	4.86		48	0.11	7.72

Average Flow Rate = 0.13 l/hr
Average NO_3-N Reduction = 3.92 mg/l

Test No.7

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)		Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)
1	0.12	9.07		25	0.08	8.56
2	0.11	10.76		26	0.08	9.42
3	0.11	9.52		27	0.08	9.52
4	0.11	7.88		28	0.08	9.57
5	0.11	7.25		29	0.08	9.42
6	0.11	7.61		30	0.08	9.57
7	0.1	8.48		31	0.08	10.02
8	0.1	6.85		32	0.08	10.22
9	0.1	7.36		33	0.08	10.52
10	0.1	8.16		34	0.08	10.61
11	0.1	7.51		35	0.08	10.86
12	0.1	7.55		36	0.08	11.02
13	0.1	6.94		37	0.08	11.28
14	0.09	7.63		38	0.08	11.28
15	0.09	8.14		39	0.08	11.72
16	0.09	7.44		40	0.08	11.72
17	0.09	8.18		41	0.07	12.03
18	0.09	8.48		42	0.07	12.13
19	0.09	8.65		43	0.07	12.47
20	0.09	8.69		44	0.07	12.75
21	0.09	8.27		45	0.07	12.55
22	0.09	9.12		46	0.07	10.61
23	0.09	8.58		47	0.07	13.33
24	0.09	9.23		48	0.07	13.9

Average Flow Rate = 0.09 l/hr
Average NO_3-N Reduction = 9.63 mg/l

Test No.8

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)		Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)
1	0.06	5.75		25	0.04	12.4
2	0.05	7.48		26	0.04	13.29
3	0.05	9.03		27	0.04	13.77
4	0.05	10.4		28	0.04	15.12
5	0.06	6.47		29	0.03	14.71
6	0.05	8.06		30	0.04	14.73
7	0.05	7.7		31	0.03	15.75
8	0.05	9.95		32	0.03	15.09
9	0.06	5.26		33	0.03	16.13
10	0.06	6.21		34	0.03	16
11	0.05	7.23		35	0.03	16.36
12	0.05	8.88		36	0.03	16.31
13	0.06	6.62		37	0.03	15.29
14	0.06	6.65		38	0.03	15.14
15	0.05	7.65		39	0.03	16.34
16	0.05	8.57		40	0.03	16.52
17	0.05	11.21		41	0.03	14.74
18	0.05	11.49		42	0.03	16.24
19	0.04	11.79		43	0.03	16.52
20	0.04	12.13		44	0.03	17.03
21	0.04	11.92		45	0.03	15.15
22	0.04	11		46	0.02	14.29
23	0.04	13		47	0.03	15.97
24	0.04	13.57		48	0.03	16.88

Average Flow Rate = 0.04 l/hr
Average NO_3-N Reduction = 12.25 mg/l

Test No.9

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)		Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)
4	0.04	13.31		100	0.01	20.81
8	0.04	13.22		104	0.01	20.94
12	0.03	15.34		108	0.01	20.83
16	0.03	16.08		112	0.01	20.79
20	0.03	17.87		116	0.01	20.83
24	0.03	18.8		120	0.01	20.83
28	0.03	19.68		124	0.01	20.83
32	0.03	20.33		128	0.01	20.89
36	0.02	20.69		132	0.01	19.95
40	0.02	20.88		136	0.01	20.38
44	0.02	20.99		140	0.01	20.51
48	0.02	21.01		144	0.01	20.55
52	0.02	20.96		148	0.01	20.53
56	0.02	20.88		152	0.01	20.58
60	0.02	20.99		156	0.01	20.48
64	0.02	20.89		160	0.01	20.5
68	0.02	20.68		164	0.01	20.1
72	0.02	20.88		168	0.01	20.79
76	0.02	20.53		172	0.01	20.89
80	0.02	20.88		176	0.01	20.86
84	0.02	20.81		180	0.01	20.6
88	0.02	20.84		184	0.01	20.06
92	0.02	20.78		188	0.01	20.76
96	0.01	20.88		192	0.01	20.83

Average Flow Rate = 0.017 l/hr
Average NO_3-N Reduction = 20.07 mg/l

ii) Efficiency of Nitrate Reduction - Nitrate-Nitrogen Reduction Data (mg/l) for Three C : N Ratios
(illustrated in Figure 5.3)

Day	Carbon to Nitrogen Application Ratio			
	1 : 1	1.65 : 1	2 : 1	Control
1	5.39	5.34	5.67	2.55
2	3.65	5.21	5.52	3.35
3	3.46	3.72	6.07	1.28
4	7.57	12.97	8.27	4.04
5	3.28	16.83	10.25	1.32
6	4.15	19.21	8.56	4.06
7	4.51	21.51	13.51	0.09
8	6.54	19.99	14.79	2.8
9	18.82	22.35	19.4	2.29
10	10.61	23.02	20.89	2.1
11	15.63	22.27	22	-0.22
12	16.25	23.14	22.48	0.92
13	16.96	23.07	22.6	2.26
14	16.6	23.23	22.7	0.34
15	16.57	23.1	22.92	2.93
16	16.84	23.29	22.87	2.22
17	16.2	23.15	23	0.92
18	16.3	22.5	22.94	4.93
19	16.23	23.04	22.95	3
20	16.08	23.23	23.1	2.11
21	15.93	23.35	23.19	0.75
22	16.15	23.26	23.2	0.6
23	16.38	23.26	22.41	1.93
24	16.27	23.19	22.38	-0.78
25	16.75	23.06	22.51	-2.43
26	16.72	23.1	22.53	-0.61
27	16.86	22.98	22.71	-2.72
28	17	22.87	23.04	-2.03
29	17.08	23.64	23.04	-1.39
30	18.19	23.39	23.18	-1.13

ii) Efficiency of Nitrate Reduction - Redox Data for Three C : N Ratios
(illustrated in Figure 5.4)

Days	Carbon to Nitrogen Application Ratio			
	1 : 1	1.65 : 1	2 : 1	Control
1	437	114	138	334
2	339	200	262	258
3	262	149	138	244
4	150	133	-10	229
5	120	-68	-52	222
6	171	-123	-13	152
7	141	-166	-12	196
8	3	-182	-25	145
9	10	-179	-37	183
10	28	-173	-30	178
11	-10	-184	-59	161
12	4	-182	-123	148
13	3	-178	-128	167
14	4	-180	-121	155
15	2	-64	-118	154
16	0	-180	-99	151
17	-23	-171	-145	160
18	-25	-173	-102	150
19	-28	-178	-60	160
20	-24	-178	-54	150
21	-29	-187	-68	166
22	-24	-181	-78	140
23	-20	-186	-86	152
24	-15	-184	-100	156
25	-12	-166	-129	164
26	-35	-144	-115	163
27	-46	-195	-90	167
28	-151	-174	-231	163
29	-102	-186	-228	177
30	-147	-184	-234	175

C : N - 1 : 1 **Replicate I**

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.41	5.14	20.9	4.07	28.13
2	0.38	4.93	20.39	4.14	24.94
3	0.34	2.4	21.48	8.95	10.82
4	0.29	8.15	19.75	2.42	31.19
5	0.29	4.51	21.98	4.87	17.2
6	0.26	4.18	21.62	5.17	14.28
7	0.16	5.52	21.84	3.96	11.95
8	0.09	9.21	21.69	2.36	10.73
9	0.04	18.75	20.54	1.1	10.01
10	0.04	16.56	21.4	1.29	8.83
11	0.03	18.18	21.19	1.17	7.93
12	0.03	17.44	20.97	1.2	7.37
13	0.03	17.64	21.55	1.22	7.03
14	0.03	17.59	21.62	1.23	6.59
15	0.03	17.26	21.55	1.25	6.12
16	0.02	17.83	21.55	1.21	5.7
17	0.02	17.94	21.4	1.19	5.58
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	0.02	16.82	21.26	1.26	4.59
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	0.02	17.51	20.9	1.19	3.75
26	-	-	-	-	-
27	0.01	17.66	20.68	1.17	3.43
28	0.02	18.17	20.18	1.11	4.7
29	0.02	18.47	20.03	1.08	3.81
30	0.01	19.16	20.18	1.05	3.35

C : N - 1 : 1 **Replicate II**

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.29	6.47	20.83	3.22	25.2
2	0.24	1.99	18.88	9.49	6.39
3	0.24	4.08	21.69	5.32	12.82
4	0.21	7.2	21.76	3.02	20.19
5	0.21	3.31	12.25	3.7	9.38
6	0.19	4.08	21.69	5.32	10.49
7	0.16	4.75	12.9	2.72	10.15
8	0.14	5.18	21.69	4.19	9.83
9	0.1	18.5	21.55	1.16	23.36
10	0.09	8.78	21.62	2.46	10.17
11	0.05	14.1	21.33	1.51	9.36
12	0.04	16.08	21.48	1.34	8.78
13	0.03	17.5	11.53	0.66	7.46
14	0.03	16.1	21.48	1.33	6.63
15	0.03	17	21.62	1.27	5.88
16	0.03	16.94	21.48	1.27	6.25
17	0.03	15.11	21.4	1.42	6.42
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	0.02	14.87	21.4	1.44	4.83
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	0.02	16.15	20.97	1.3	4.18
26	-	-	-	-	-
27	0.02	16.3	20.83	1.28	3.58
28	0.02	16.52	20.39	1.23	4.35
29	0.02	17.58	20.47	1.16	3.69
30	0.01	18.73	20.39	1.09	3.65

C : N - 1 : 1 **Replicate III**

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.42	4.55	21.33	4.69	25.42
2	0.36	4.04	20.83	5.16	19.49
3	0.31	3.9	21.62	5.54	15.93
4	0.29	7.36	21.91	2.98	28.67
5	0.28	2.01	21.98	10.94	7.37
6	0.26	4.2	21.84	5.2	14.35
7	0.23	3.27	15.35	4.69	9.82
8	0.17	5.24	12.97	2.48	11.93
9	0.11	19.21	21.19	1.1	28.5
10	0.09	6.49	21.69	3.34	7.5
11	0.05	14.6	15.28	1.05	10.04
12	0.04	15.23	13.76	0.9	8.71
13	0.04	15.74	21.55	1.37	7.95
14	0.03	16.12	11.82	0.73	7.48
15	0.03	15.45	21.69	1.4	7.09
16	0.03	15.74	21.69	1.38	6.49
17	0.03	15.56	21.4	1.38	5.87
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	0.02	16.11	21.55	1.34	4.87
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	0.02	16.59	21.48	1.29	3.75
26	-	-	-	-	-
27	0.02	16.62	21.33	1.28	3.49
28	0.02	16.32	21.33	1.31	5.06
29	0.02	15.19	21.33	1.4	3.82
30	0.02	16.68	21.4	1.28	3.66

C : N - 1.65 : 1 Replicate I

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.26	6.05	37.11	6.13	20.58
2	0.15	7.65	37.19	4.86	15.07
3	0.05	6.09	36.9	6.06	4.13
4	0.02	18.02	37.19	2.06	4.98
5	0.02	22.52	35.82	1.59	6.61
6	0.02	25.03	36.32	1.45	6.98
7	0.02	26.26	36.39	1.39	6.01
8	0.01	26.09	36.18	1.39	5.12
9	0.01	25.95	36.18	1.39	4.58
10	0.01	25.82	36.18	1.4	4.15
11	0.01	25.57	35.82	1.4	3.9
12	0.01	25.63	36.97	1.44	3.63
13	0.01	25.37	37.19	1.47	3.31
14	0.01	25.96	37.33	1.44	2.93
15	0.01	25.57	36.39	1.42	2.78
16	0.01	26.28	35.53	1.35	2.49
17	0.01	25.74	35.24	1.37	2.29
18	0.01	24.62	35.31	1.43	2.11
19	0.01	25.46	35.67	1.4	2.1
20	0.01	25.82	35.1	1.36	1.95
21	0.01	26.13	35.24	1.35	1.93
22	0.01	25.93	35.1	1.35	1.79
23	0.01	25.7	34.23	1.33	2.45
24	0.01	25.78	34.59	1.34	1.97
25	0.01	25.78	34.23	1.33	1.99
26	0.01	25.76	34.3	1.33	3.15
27	0.01	25.55	33.8	1.32	2.17
28	0.01	25.39	33.08	1.3	2.38
29	0.01	26.97	33.73	1.25	2.4

C : N - 1.65 : 1 Replicate II

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.17	4.86	37.26	7.67	11.04
2	0.1	4.45	34.73	7.81	5.68
3	0.06	1.13	34.66	30.68	0.88
4	0.03	10.15	34.45	3.39	4.05
5	0.04	11.08	35.38	3.19	6.56
6	0.04	16.86	36.18	2.15	8.26
7	0.03	19.73	36.54	1.85	8.81
8	0.03	22.56	36.54	1.62	10.26
9	0.01	26.04	36.03	1.38	4.95
10	0.01	26.43	35.89	1.36	3
11	0.01	26.24	36.75	1.4	2.55
12	0.01	26.26	36.39	1.39	0.98
13	0.01	26.48	36.97	1.4	0.72
14	0.01	26.54	36.61	1.38	1.08
15	0.01	26.32	36.46	1.39	0.5
16	0.01	26.24	35.31	1.35	0.08
17	0.01	26.35	35.31	1.34	0.08
18	0.01	26.3	35.46	1.35	1.78
19	0.01	26.35	35.02	1.33	1.3
20	0.01	26.45	34.95	1.32	0.44
21	0.01	26.48	34.88	1.32	0.15
22	0.01	26.41	34.59	1.31	0.15
23	0.01	26.6	34.23	1.29	0.11
24	0.01	26.32	34.01	1.29	0.13
25	0.01	26.48	34.95	1.32	0.08
26	0.01	26.54	33.51	1.26	0.11
27	-	-	-	-	-
28	-	-	-	-	-
29	0.01	26.45	33.51	1.27	0.02

C : N - 1.65 : 1 Replicate III

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.17	5.1	36.54	7.16	11.62
2	0.12	3.52	36.82	10.46	5.75
3	0.07	3.93	37.4	9.52	3.55
4	0.04	10.74	36.75	3.42	4.99
5	0.03	16.88	34.95	2.07	6.06
6	0.03	18.74	35.82	1.91	7.58
7	0.03	21.53	35.6	1.65	8.9
8	0.03	20.33	34.81	1.71	8.92
9	0.02	24.07	35.1	1.46	7.46
10	0.02	25.82	35.89	1.39	7.53
11	0.02	24.01	36.75	1.53	5.17
12	0.01	26.54	36.54	1.38	4.64
13	0.01	26.37	36.18	1.37	2.93
14	0.01	26.19	36.54	1.4	2.64
15	0.01	26.41	36.18	1.37	2.37
16	0.01	26.34	35.31	1.34	2.07
17	0.01	26.35	34.95	1.33	1.82
18	0.01	25.59	34.88	1.36	1.61
19	0.01	26.32	34.81	1.32	1.69
20	0.01	26.43	34.73	1.31	1.45
21	0.01	26.45	34.52	1.31	1.14
22	0.01	26.45	34.01	1.29	0.61
23	0.01	26.47	33.65	1.27	0.29
24	0.01	26.48	34.09	1.29	0.06
25	0.01	25.91	34.52	1.33	0.02
26	-	-	-	-	-
27	-	-	-	-	-
28	-	-	-	-	-
29	0.01	26.5	34.23	1.29	0.02

C : N - 2 : 1 **Replicate I**

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.25	11.54	33.22	2.88	38.45
2	0.19	4.85	44.68	9.21	11.92
3	0.19	6.27	44.9	7.16	15.91
4	0.19	8.58	45.04	5.25	21.93
5	0.19	8.72	45.11	5.17	21.8
6	0.17	8.96	44.82	5	20.38
7	0.16	10.02	44.82	4.47	20.81
8	0.13	11.84	38.19	3.23	20.54
9	0.08	17.53	44.9	2.56	19.23
10	0.06	20.1	44.82	2.23	17.01
11	0.05	21.78	36.1	1.66	14.5
12	0.03	23.53	44.46	1.89	10.58
13	0.04	23.85	44.9	1.88	13.36
14	0.03	23.83	36.25	1.52	11.01
15	0.04	23.84	44.9	1.88	11.36
16	0.03	23.71	36.46	1.54	8.41
17	0.02	23.91	44.32	1.85	7.7
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	0.03	24.18	44.25	1.83	9.52
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	0.02	24.55	43.6	1.78	5.57
26	-	-	-	-	-
27	0.02	24.93	43.02	1.73	5.51
28	0.02	25.32	42.09	1.66	7.37
29	0.02	25.23	42.59	1.69	7.13
30	0.01	25.32	35.24	1.39	4.9

C : N - 2 : 1 **Replicate II**

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.44	6.33	44.1	6.97	37.26
2	0.39	6.05	44.25	7.31	31.44
3	0.27	5.7	45.04	7.9	20.45
4	0.26	7.18	45.04	6.27	24.66
5	0.27	7.75	44.97	5.8	27.64
6	0.23	8.92	44.75	5.02	27.22
7	0.18	10.52	44.61	4.24	25.4
8	0.13	10.4	35.53	3.42	17.47
9	0.07	17.39	44.68	2.57	16.85
10	0.05	22.74	44.25	1.95	15
11	0.04	23.91	44.25	1.85	12.86
12	0.04	23.93	44.25	1.85	12.11
13	0.03	23.89	44.39	1.86	10.42
14	0.03	23.97	44.18	1.84	9.69
15	0.03	23.96	44.25	1.85	9.13
16	0.03	23.96	44.18	1.84	8.54
17	0.03	24.02	43.67	1.82	8.05
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	0.02	24.15	43.17	1.79	6.59
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	0.02	24.49	43.31	1.77	5.55
26	-	-	-	-	-
27	0.02	25	42.45	1.7	5.41
28	0.02	25.32	42.09	1.66	7.11
29	0.02	25.44	42.23	1.66	7.19
30	0.02	25.6	42.09	1.64	5.13

C : N - 2 : 1 **Replicate III**

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.23	15	43.81	2.92	46.4
2	0.2	0.65	44.25	6.81	1.71
3	0.1	6.25	44.32	7.09	8.2
4	0.06	9.06	44.54	4.92	7.8
5	0.06	14.27	35.67	2.5	10.93
6	0.04	7.79	30.99	3.98	3.68
7	0.04	19.98	44.46	2.23	10.06
8	0.03	22.13	44.18	2	9.84
9	0.03	23.28	44.39	1.91	9.54
10	0.03	22.84	44.32	1.94	9.89
11	0.03	23.3	44.25	1.9	9.47
12	0.03	22.99	38.05	1.66	9.38
13	0.03	23.06	44.39	1.93	9.26
14	0.03	23.3	44.68	1.92	8.55
15	0.03	23.97	44.18	1.84	7.97
16	0.02	23.95	44.39	1.85	7.15
17	0.02	24.06	44.39	1.85	5.88
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	0.02	24.24	42.3	1.75	6.31
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	0.01	26.9	34.3	1.28	1.01
26	-	-	-	-	-
27	0.01	24.19	41.36	1.71	1.31
28	0.01	24.49	41.36	1.69	1.73
29	0.01	24.44	41.22	1.69	1.87
30	0.01	24.63	40.86	1.66	1.61

CONTROL **Replicate I**

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.27	4.62	0	0	16.43
2	0.15	4.06	0	0	8.27
3	0.1	4.25	0	0	5.61
4	0.09	4.53	0	0	5.44
5	0.11	3.93	0	0	5.47
6	0.09	6.87	0	0	8.53
7	0.09	-0.42	0	0	-0.48
8	0.09	3.5	0	0	3.95
9	0.08	1.94	0	0	1.94
10	0.07	1.96	0	0	1.76
11	0.07	0.49	0	0	0.42
12	0.06	-0.98	0	0	-0.82
13	0.06	0	0	0	0
14	0.06	-2.29	0	0	-1.97
15	0.1	1.55	0	0	2.15
16	-	-	-	-	-
17	0.09	3.58	0	0	4.07
18	-	-	-	-	-
19	0.06	2.83	0	0	2.23
20	0.03	4.6	0	0	2.12
21	0.03	3.13	0	0	1.15
22	0.03	2.55	0	0	0.89
23	0.03	3.3	0	0	1.1
24	0.03	3.02	0	0	1.08
25	0.03	0.75	0	0	0.25
26	0.03	0.06	0	0	0.02
27	0.02	-1.75	0	0	-0.57
28	0.03	-2.29	0	0	-0.77
29	0.02	0.71	0	0	0.21

CONTROL **Replicate II**

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.35	2.4	0	0	11.19
2	0.35	2.83	0	0	13.28
3	0.24	-1.78	0	0	-5.76
4	0.24	3.28	0	0	10.54
5	0.24	-0.33	0	0	-1.04
6	0.23	2.66	0	0	8.04
7	0.21	0.23	0	0	0.64
8	0.23	1.59	0	0	4.86
9	0.22	2.87	0	0	8.49
10	0.22	2.66	0	0	7.81
11	0.21	0.28	0	0	0.79
12	0.21	2.16	0	0	6.15
13	0.22	5.18	0	0	14.88
14	0.21	3.19	0	0	8.93
15	0.21	4.43	0	0	12.09
16	-	-	-	-	-
17	0.21	-0.39	0	0	-1.06
18	0.1	5.24	0	0	6.84
19	0.09	4.66	0	0	5.67
20	-	-	-	-	-
21	0.09	-2.12	0	0	-2.53
22	0.09	2.39	0	0	2.76
23	0.09	0.8	0	0	0.93
24	0.09	-0.46	0	0	-0.53
25	0.08	-4	0	0	-4.11
26	0.08	1.88	0	0	1.97
27	0.08	-2.66	0	0	-2.73
28	0.08	-2.45	0	0	-2.53
29	0.08	-2.53	0	0	-2.68

CONTROL **Replicate III**

Day	Flow (l/hr)	Nitrate Reduced (mg/l)	Carbon Utilised (mg/l)	C : N	Performance ($g/m^3/d$)
1	0.41	0.64	0	0	3.45
2	0.36	3.15	0	0	15.06
3	0.36	1.36	0	0	6.51
4	0.35	4.3	0	0	20.08
5	0.36	0.36	0	0	1.7
6	0.33	2.66	0	0	11.58
7	0.32	0.47	0	0	2.01
8	0.33	3.32	0	0	14.42
9	0.32	2.07	0	0	8.78
10	0.32	1.68	0	0	7.11
11	0.31	-1.43	0	0	-5.9
12	0.31	1.57	0	0	6.36
13	0.31	1.59	0	0	6.45
14	0.32	0.12	0	0	0.51
15	0.31	2.81	0	0	11.65
16	-	-	-	-	-
17	0.34	-0.42	0	0	-1.89
18	0.24	5.29	0	0	16.75
19	0.23	1.51	0	0	4.61
20	0.23	1.73	0	0	5.16
21	0.21	1.25	0	0	3.54
22	0.21	-3.14	0	0	-8.67
23	0.21	1.68	0	0	4.6
24	0.19	-4.91	0	0	-12.58
25	0.19	-4.05	0	0	-10.09
26	0.18	-3.76	0	0	-8.76
27	0.17	-3.76	0	0	-8.56
28	0.17	-1.34	0	0	-2.95

Calculation of Average Treatment Performance ($g/m^3/d$)

Data for flow rates above 0.1 l/hr.
All data from C : N experiments (Control not included).

Flow Rate (l/hr)	$g/m^3/d$		Flow Rate (l/hr)	$g/m^3/d$
0.44	37.26		0.21	9.38
0.42	25.42		0.21	20.19
0.41	28.13		0.2	1.71
0.39	31.44		0.19	10.49
0.38	24.94		0.19	21.93
0.36	19.49		0.19	15.91
0.34	10.82		0.19	21.8
0.31	15.93		0.19	11.92
0.29	28.67		0.18	25.4
0.29	25.2		0.17	11.62
0.29	31.19		0.17	11.93
0.29	17.2		0.17	20.38
0.28	7.37		0.17	11.04
0.27	20.45		0.16	11.95
0.27	27.64		0.16	10.15
0.26	24.66		0.16	20.81
0.26	14.35		0.15	15.07
0.26	14.28		0.14	9.83
0.26	20.58		0.13	20.54
0.25	38.45		0.13	17.47
0.24	6.39		0.12	5.75
0.24	12.82		0.11	28.5
0.23	46.4		0.1	8.2
0.23	27.22		0.1	5.68
0.23	9.82		0.1	23.36

Average performance = 19 $g/m^3/d$

Appendix IV

Biodegradation of Organic Materials Study Data

(Data illustrated in Figures 5.7, 5.9, & 5.10)

Glucose - G1		Glucose - G2		Glucose - G3	
Time (hours)	Cumulative Carbon Dioxide (mg)	Time (hours)	Cumulative Carbon Dioxide (mg)	Time (hours)	Cumulative Carbon Dioxide (mg)
0	0	0	0	0	0
23.25	48.13	23.25	38.5	3.5	27.5
75.5	122.38	75.5	110	75.5	49.5
172.25	354.75	122.5	140.25	122.5	126.5
188.5	629.75	172.25	442.75	144	599.5
189.75	863.5	176.75	1005.13	154.25	847
192	1028.5	188.5	1310.38	167.25	1078
202	1199	189.5	1607.38	176.75	1333.75
213.5	1625.25	192	1848	189.5	1508.38
218.25	1848	196.75	2026.75	240.25	1618.38
219.75	2002	202.25	2211	289	1728.38
223	2172.5	212	2398	339	1832.88
224.5	2277	223	2530	380.25	1887.88
233.75	2541	236.5	2675.75	505.25	2025.38
234.5	2722.5	246	2818.75	555	2245.38
236.5	2824.25	258.25	3036	599	2712.88
240.25	2970	263.25	3214.75	624	3271.13
246	3195.5	265.25	3322	645	3609.38
258.25	3470.5	272.5	3514.5	671	3997.13
258.5	3707	283.5	3789.5	723	4241.88
265.25	3869.25	284.25	4023.25	773	4420.63
339	4015	286.5	4237.75	849	4558.13
380.25	4226.75	292.25	4397.25	976	4668.13
431	4353.25	316.75	4581.5	1178	4838.63
505.25	4496.25	339	4710.75	-	-
555	4666.75	380.25	4837.25	-	-
887	4930.75	431	4963.75	-	-
1178	5313	505.25	5142.5	-	-
-	-	555	5302	-	-
-	-	839.25	5568.75	-	-
-	-	935	5827.25	-	-
-	-	1178	6305.75	-	-

(Data illustrated in Figures 5.7, 5.9, & 5.10)

Sugar Beet -SB1		Sugar Beet -SB2		Sugar Beet -SB3	
Time (hours)	Cumulative Carbon Dioxide (mg)	Time (hours)	Cumulative Carbon Dioxide (mg)	Time (hours)	Cumulative Carbon Dioxide (mg)
0	0	0	0	0	0
68.5	17.05	68.5	60.5	68.5	30.8
90.5	44.55	75.5	102.3	115	63.8
96.75	71.5	80.5	155.1	126	132.55
101.5	132.55	90	278.85	139	228.8
103	160.6	92	295.35	145.25	308.55
112	353.1	95	335.5	151	397.65
113	382.25	96.75	354.2	161.75	562.1
114.75	444.4	101.5	399.85	165	632.5
119	506.55	103	414.15	170.5	716.1
126	623.7	112	508.2	175.25	817.3
128	667.7	113	524.7	185.5	958.65
136.75	826.1	115	548.9	195.5	1032.9
139	856.35	117	617.1	209.5	1081.3
145.25	941.6	126	680.9	232.5	1131.9
151	954.8	139	757.9	244.25	1169.3
161.75	1009.8	151	830.5	258.75	1197.35
165	1053.25	165	909.7	380	1268.85
175.25	1089	170.5	984.5	433.5	1296.35
185.5	1112.65	175.25	1064.8	482	1323.85
209.5	1133.55	185.5	1158.3	598	1372.53
232.5	1152.25	195.5	1236.4	741.75	1448.43
258.75	1179.75	209.5	1311.2	886	1496.28
380	1215.5	232.5	1342	1056.5	1564.48
817	1295.25	258.75	1356.3	-	-
1056.5	1368.4	380	1375	-	-
-	-	1056.5	1415.15	-	-

Control									
Time (hours)	0	23.5	75.5	123.5	236.5	334	380.25	501.25	1178
Cumulative Carbon Dioxide (mg)	0	66.83	104.23	123.2	158.4	195.25	209.55	256.3	288.2

(Data illustrated in Figure 5.8)

Time (hours)	pH						
	G1	G2	G3	SB1	SB2	SB3	Control
0	6.45	6.76	6.51	6.48	6.42	6.61	6.48
300	7.66	7.54	5.52	5.69	5.64	8.72	7.85
380.25	8.93	8.85	6.52	-	-	-	-
742.5	-	-	-	6.22	5.36	9.31	-
864	9.21	9.13	8.37	-	-	-	-
906	8.93	8.85	8.63	-	-	-	-
928.5	-	-	-	6.29	5.17	8.88	-
1050	8.8	8.8	8.59	-	-	-	-
1056.5	-	-	-	5.43	5.09	8.93	-
1178	9.12	8.97	8.44	-	-	-	7.75

Appendix V

Combined Low Temperature and Organic Material Study Data

Flow Study : Without pH Control - Combined Data

(Sample of Data presented in Table 5.13)

Flow Rate (l/hr)	NO_3-N Reduction (mg/l)	NH_3-N (mg/l)	Redox (mV)	pH
0.54	-0.27	5.14	351	5.1
0.54	0.12	5.41	363	4.9
0.54	0.87	5.19	318	4.8
0.54	0.01	6.3	334	4.8
0.54	-0.46	6.06	318	4.7
0.53	2.94	5.25	266	4.7
0.48	3.03	5.22	284	4.6
0.46	0.26	5.19	214	4.7
0.45	0.92	5.57	295	4.8
0.44	0.73	5.46	206	4.8
0.44	0.26	5.84	263	4.8
0.39	0.94	5.65	90	4.8
0.36	0.5	5.65	211	4.8
0.36	-0.33	5.25	284	4.7
0.32	0.81	5.61	82	4.6
0.29	0.71	5.98	51	4.7
0.28	0.81	5.54	44	4.6
0.25	0.2	5.34	20	4.6
0.2	1.72	6.18	62	4.5
0.19	0.01	5.81	256	4.5
0.19	0.34	5.62	285	4.5
0.19	0.36	5.94	283	4.5
0.19	1.15	5.67	288	4.5
0.16	1.09	5.84	177	4.5
0.15	0.97	5.32	169	4.5
0.1	2.5	7.4	248	4.6

0.09	2.89	7.14	248	4.4
0.08	1.57	6.23	132	4.4
0.08	0.36	5.72	100	4.5
0.07	-0.56	5.67	149	4.5
0.07	-0.31	5.7	92	4.4
0.07	1.57	8.4	136	4.4
0.07	0.79	7.26	84	4.3
0.07	2.21	7.95	227	4.2
0.07	0.52	5.09	183	4.4
0.07	-0.38	6.68	36	4.4
0.06	3.87	6.26	26	4.3
0.06	0.48	5.41	132	4.3
0.06	3.61	6.3	167	4.3
0.05	1.57	5.82	116	4.4
0.05	2.83	6.06	59	4.4
0.05	1.9	6.08	60	4.3
0.05	2.58	6.33	260	4.3
0.04	1.68	6.2	154	4.2
0.03	1.56	6.46	130	4.3
0.01	1.73	7.25	105	4.3
0.01	1.18	7.3	234	4.2
0.01	3.32	7.8	202	4.2

Flow Study : With pH Control - Replicate I

(Data illustrated in Figure 5.13)

Experiment Time (hours)	Flow Rate (l/hr)	Nitrate-Nitrogen Reduction (mg/l)		Experiment Time (hours)	Flow Rate (l/hr)	Nitrate-Nitrogen Reduction (mg/l)
0	-	-		560	0.22	13.98
112	0.11	18.48		609	0.22	11.45
136.5	0.08	21.72		629.5	0.22	11.12
161	0.08	20.98		654.5	0.22	9.41
185	0.08	19.06		676	0.22	5.05
263	0.07	20.43		700	0.21	7.99
282.5	0.41	8.18		727.5	0.21	7.86
285.5	0.05	6.16		749.5	0.2	7.97
288.5	0.3	6.87		778.5	0.21	7.99
292.5	0.3	6.54		797.5	0.2	7.84
304.5	0.01	22.13		805.5	0.36	3.97
323	0.23	9.14		820	0.35	3.95
328	0.33	5.29		842.5	0.35	4.25
337	0.62	18.4		870	0.35	3.37
339	0.28	15.03		893.5	0.35	4.94
343	0.76	2.89		917.5	0.35	4.95
348	0.62	5.02		939.5	0.35	5.62
351.5	0.64	6.92		947.5	0.34	5.94
366.5	0.62	2.99		959	0.05	20.58
370.5	0.62	3.37		963	0.05	20.64
374	0.25	5.04		978	0.04	20.68
386	0.25	9.77		988.5	0.04	20.64
410	0.25	10.9		1005.5	0.04	20.7
434	0.25	11.54		1081	0.04	20.68
460	0.22	12.26		1171	0.04	20.7
535	0.22	16.61				

Flow Study : With pH Control - Replicate II

(Data illustrated in Figure 5.14)

Experiment Time (hours)	Flow Rate (l/hr)	Nitrate-Nitrogen Reduction (mg/l)		Experiment Time (hours)	Flow Rate (l/hr)	Nitrate-Nitrogen Reduction (mg/l)
0	-	-		560	0.18	7.76
112	0.07	16.99		609	0.18	9.27
136.5	0.03	19.81		629.5	0.18	8.96
161	0.04	16.95		654.5	0.18	9.41
185	0.03	8.88		676	0.18	6.07
263	0.05	16.4		700	0.18	10.8
282.5	0.38	6.56		727.5	0.18	15.82
285.5	0.4	5.01		749.5	0.18	13.23
288.5	0.39	2.89		778.5	0.18	12.66
292.5	0.4	3.06		797.5	0.19	12.01
304.5	0.4	3.16		805.5	0.29	7.7
323	0.83	1.68		820	0.29	7.39
328	0.86	1.34		842.5	0.29	7.29
337	0.22	4.77		870	0.29	7.5
339	0.26	5.84		893.5	0.3	7.46
343	0.06	11.28		917.5	0.3	7.13
348	0.6	2.25		939.5	0.3	7.37
351.5	0.6	3.01		947.5	0.3	7.29
366.5	0.6	3.33		959	0.05	20.56
370.5	0.6	1.9		963	0.05	20.62
374	0.26	3.01		978	0.05	20.64
386	0.26	3.97		988.5	0.05	20.62
410	0.26	4.21		1005.5	0.05	20.66
434	0.26	4.11		1081	0.05	20.5
460	0.26	4.03		1171	0.05	20.5
535	0.18	9.29				

Flow Study : With pH Control - Replicate III

(Data illustrated in Figure 5.15)

Experiment Time (hours)	Flow Rate (l/hr)	Nitrate-Nitrogen Reduction (mg/l)		Experiment Time (hours)	Flow Rate (l/hr)	Nitrate-Nitrogen Reduction (mg/l)
0	-	-		560	0.22	9.1
112	0.06	20.56		609	0.22	9.25
136.5	0.04	22.31		629.5	0.22	8.65
161	0.08	17.29		654.5	0.22	7.27
185	0.09	13.02		676	0.22	4.62
263	0.08	10.07		700	0.22	7.11
282.5	0.24	5.78		727.5	0.22	9.31
285.5	0.2	5.74		749.5	0.22	9.15
288.5	0.14	3.24		778.5	0.22	7.35
292.5	0.15	6.81		797.5	0.22	7.11
304.5	0.05	10.94		805.5	0.36	4.25
323	1.51	0.19		820	0.36	3.72
328	0.46	1.5		842.5	0.36	3.72
337	0.73	2.65		870	0.36	4.31
339	0.74	0.58		893.5	0.36	4.17
343	0.74	0.82		917.5	0.36	4.5
348	0.74	3.99		939.5	0.36	4.7
351.5	0.74	3.95		947.5	0.36	4.92
366.5	0.76	1.28		959	0.03	20.68
370.5	0.75	2.35		963	0.02	20.39
374	0.46	1.64		978	0.02	20.35
386	0.46	1.12		988.5	0.02	20.66
410	0.46	0.88		1005.5	0.03	20.54
434	0.43	2.73		1081	0.03	20.45
460	0.22	5.62		1171	0.02	20.56
535	0.22	10.98				

Flow Study : With pH Control - Data from Replicate I

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)	Eh (mV)	pH	NH_3-N (mg/l)	Performance ($gN/m^3/d$)
0	-	-	-	-	-	-
112	0.11	18.48	-122	6.1	13.3	24.2
136.5	0.08	21.72	-48	7.46	1.4	19.93
161	0.08	20.98	-93	7.12	0.68	19.77
185	0.08	19.06	-31	7.03	6.24	18.89
263	0.07	20.43	29	6.36	17.2	16.75
282.5	0.41	8.18	-65	7.11	5.65	41.27
285.5	0.05	6.16	14	6.69	3.6	3.39
288.5	0.3	6.87	70	6.56	5.28	25.24
292.5	0.3	6.54	13	6.56	4.1	24
304.5	0.01	22.13	23	6	12.65	3.25
323	0.23	9.14	15	6.1	3.35	25.5
328	0.33	5.29	20	5.89	2.4	21.35
337	0.62	18.4	60	5.98	10.4	140.55
339	0.28	15.03	80	6.23	7.9	51.14
343	0.76	2.89	50	6.01	1.32	26.7
348	0.62	5.02	42	5.86	1.41	38.31
351.5	0.64	6.92	76	5.91	1.51	53.9
366.5	0.62	2.99	79	6.86	1.7	22.82
370.5	0.62	3.37	42	6.6	2.33	25.48
374	0.25	5.04	44	6.38	5.98	15.67
386	0.25	9.77	19	6.79	6.7	30.15
410	0.25	10.9	-119	5.81	5.95	32.82
434	0.25	11.54	-126	5.98	6.88	34.75
460	0.22	12.26	-52	6.08	6.38	33.33
535	0.22	16.61	-54	6.36	3.4	43.91
560	0.22	13.98	-119	6.47	3.9	36.96
588.5	0.22	12.25	-104	6.34	4.4	32.4
609	0.22	11.45	-25	6.28	4.4	30.27
629.5	0.22	11.12	-73	6.33	4.75	29.39
654.5	0.22	9.41	-95	6.19	3.95	24.88

676	0.22	5.05	-84	6.36	5.3	13.36
700	0.21	7.99	-36	6.6	3.35	20.84
727.5	0.21	7.86	-50	6.07	4.05	20
749.5	0.2	7.97	-45	6.43	3.4	19.91
778.5	0.21	7.99	-53	6.42	3	20.35
797.5	0.2	7.84	-25	6.54	2.8	19.57
805.5	0.36	3.97	56	6.51	0.95	17.51
820	0.35	3.95	130	6.36	1.6	17.14
842.5	0.35	4.25	144	6.28	1.05	18.41
870	0.35	3.37	127	6.3	0.6	14.51
893.5	0.35	4.94	195	6.27	1.15	21.2
917.5	0.35	4.95	189	6.28	0.9	21.23
939.5	0.35	5.62	210	6.31	1.2	23.95
947.5	0.34	5.94	207	6.66	0.7	24.56
959	0.05	20.58	33	6.65	0.75	11.34
963	0.05	20.64	-22	6.54	0.8	11.37
978	0.04	20.68	-30	6.35	0.9	11.14
988.5	0.04	20.64	-18	6.53	0.6	11.12
1005.5	0.04	20.7	-20	6.88	0.16	10.89
1081	0.04	20.68	-120	6.88	0.54	10.88
1171	0.04	20.7	-76	7.02	0.2	10.64

Flow Study : With pH Control - Data from Replicate II

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)	Eh (mV)	pH	NH_3-N (mg/l)	Performance ($gN/m^3/d$)
0	-	-	-	-	-	-
112	0.07	16.99	-128	6.92	9.85	13.52
136.5	0.03	19.81	-110	5.23	3.5	6.06
161	0.04	16.95	-113	6.38	0.9	7.89
185	0.03	8.88	-47	5.96	4.75	3.69
263	0.05	16.4	-8	5.89	14.5	10.84
282.5	0.38	6.56	79	6.08	3.55	30.82
285.5	0.4	5.01	142	6.09	4.66	24.28
288.5	0.39	2.89	179	6.3	2.66	13.7
292.5	0.4	3.06	174	6.08	2.34	14.93
304.5	0.4	3.16	167	6.12	2.3	15.37
323	0.83	1.68	141	5.85	0.52	16.98
328	0.86	1.34	197	5.86	0.64	14.09
337	0.22	4.77	200	6.09	2.45	12.61
339	0.26	5.84	181	6.16	4.5	18.31
343	0.06	11.28	188	6.46	7.45	8.28
348	0.6	2.25	135	6.69	0.04	16.5
351.5	0.6	3.01	135	6.7	0.76	22.1
366.5	0.6	3.33	120	6.14	0.6	24.46
370.5	0.6	1.9	81	5.63	0.49	13.99
374	0.26	3.01	228	5.86	2.78	9.72
386	0.26	3.97	212	5.78	2.74	12.84
410	0.26	4.21	195	6.07	4.3	13.62
434	0.26	4.11	177	5.87	2.25	13.09
460	0.26	4.03	212	5.91	1.9	13.03
535	0.18	9.29	196	6.24	2.15	20.48
560	0.18	7.76	212	6.69	2.75	17.11
588.5	0.18	8.61	196	6.54	2.85	18.96
609	0.18	9.27	180	6.44	3.35	20.43
629.5	0.18	8.96	185	6.67	3.55	19.74
654.5	0.18	9.41	168	6.44	2.55	20.74

676	0.18	6.07	154	6.32	3.15	13.38
700	0.18	10.8	154	6.33	2.95	24.19
727.5	0.18	15.82	173	6.35	2.95	35.62
749.5	0.18	13.23	131	6.35	2.7	29.79
778.5	0.18	12.66	137	6.37	0.85	28.51
797.5	0.19	12.01	121	6.44	1.95	27.35
805.5	0.29	7.7	93	6.42	0.65	27.14
820	0.29	7.39	76	6.25	1.2	26.04
842.5	0.29	7.29	42	6.12	1.4	26.23
870	0.29	7.5	40	6.23	0.65	27
893.5	0.3	7.46	17	6.2	1	27.41
917.5	0.3	7.13	16	6.26	0.75	26.19
939.5	0.3	7.37	9	6.29	1.4	27.05
947.5	0.3	7.29	16	6.72	0.5	27.12
959	0.05	20.56	-55	6.3	0.59	13.59
963	0.05	20.62	-35	6.16	0.54	13.63
978	0.05	20.64	-40	6.64	0.62	13.39
988.5	0.05	20.62	-33	6.7	0.51	13.63
1005.5	0.05	20.66	-26	6.68	0.25	13.15
1081	0.05	20.5	-19	6.65	0.41	12.8
1171	0.05	20.5	-49	6.57	0.4	12.3

Flow Study : With pH Control - Data from Replicate III

Time (hours)	Flow Rate (l/hr)	NO_3-N Reduction (mg/l)	Eh (mV)	pH	NH_3-N (mg/l)	Performance ($gN/m^3/d$)
0	-	-	-	-	-	-
112	0.06	20.56	-27	6.57	9.9	16.11
136.5	0.04	22.31	-36	7.49	3.45	11.74
161	0.08	17.29	-98	7.13	4.6	16.3
185	0.09	13.02	-118	6.9	7.55	13.71
263	0.08	10.07	-215	6.52	8.45	10.23
282.5	0.24	5.78	-51	6.62	5.45	16.99
285.5	0.2	5.74	40	6.41	6.72	14.34
288.5	0.14	3.24	55	6.33	4.16	5.56
292.5	0.15	6.81	14	6.01	5.55	12.51
304.5	0.05	10.94	86	5.67	10.6	7.23
323	1.51	0.19	54	5.56	0.44	3.47
328	0.46	1.5	58	5.44	1.32	8.43
337	0.73	2.65	112	5.35	2.95	23.72
339	0.74	0.58	118	5.61	0.95	5.31
343	0.74	0.82	34	5.37	0.76	7.49
348	0.74	3.99	48	5.54	0.63	36.37
351.5	0.74	3.95	17	5.84	0.78	36
366.5	0.76	1.28	72	5.47	0.6	11.87
370.5	0.75	2.35	54	5.69	0.81	21.63
374	0.46	1.64	66	5.57	2.18	9.18
386	0.46	1.12	60	5.44	1.49	6.26
410	0.46	0.88	54	5.52	0.9	4.98
434	0.43	2.73	35	5.61	2.64	14.43
460	0.22	5.62	-17	5.63	3.85	14.86
535	0.22	10.98	-90	6.5	4.4	29.03
560	0.22	9.1	-51	6.61	6.25	24.05
588.5	0.22	10.37	-83	6.47	5.25	27.42
609	0.22	9.25	-27	6.45	4.25	24.47
629.5	0.22	8.65	-65	6.55	3.95	22.86
654.5	0.22	7.27	-16	6.41	4.3	19.23

676	0.22	4.62	-13	6.31	4.25	12.22
700	0.22	7.11	-102	6.26	4.95	18.8
727.5	0.22	9.31	-18	6.22	4.3	24.61
749.5	0.22	9.15	-3	6.29	3.8	24.19
778.5	0.22	7.35	46	6.18	4.2	19.42
797.5	0.22	7.11	50	6.2	4.2	18.8
805.5	0.36	4.25	76	6.25	1.5	18.72
820	0.36	3.72	134	6.13	1.3	16.39
842.5	0.36	3.72	148	6.03	2.15	16.39
870	0.36	4.31	183	6.19	1.25	18.98
893.5	0.36	4.17	119	6.2	1.85	18.38
917.5	0.36	4.5	128	6.19	2.65	19.85
939.5	0.36	4.7	122	6.22	2.45	20.71
947.5	0.36	4.92	108	6.69	0.55	21.42
959	0.03	20.68	-171	6.41	0.4	6.83
963	0.02	20.39	-220	5.77	0.34	5.74
978	0.02	20.35	-263	5.94	0.7	4.48
988.5	0.02	20.66	-278	5.9	1.1	4.55
1005.5	0.03	20.54	-256	6.25	1.45	7.04
1081	0.03	20.45	-239	5.91	1.63	6.76
1171	0.02	20.56	-183	5.88	1.25	5.54

Calculation of Average Treatment Performance - Flow Study : With pH Control

Performance values taken following 350 hours, flow rate range 0.30-0.18 l/hr.

Flow Rate (l/hr)	Performance ($g/m^3/d$)		Flow Rate (l/hr)	Performance ($g/m^3/d$)
0.25	16		0.18	13
0.25	30		0.18	24
0.25	33		0.18	26
0.25	35		0.18	30
0.22	33		0.18	29
0.22	44		0.19	27
0.22	37		0.29	27
0.22	32		0.29	26
0.22	30		0.29	26
0.22	29		0.29	27
0.22	25		0.3	27
0.22	13		0.3	26
0.21	21		0.3	27
0.21	20		0.3	27
0.2	20		0.22	15
0.21	20		0.22	29
0.2	20		0.22	24
0.26	10		0.22	27
0.26	13		0.22	25
0.26	14		0.22	23
0.26	13		0.22	19
0.26	13		0.22	12
0.18	21		0.22	19
0.18	17		0.22	25
0.18	19		0.22	24
0.18	20		0.22	19
0.18	20		0.22	19
0.18	21			

Average Treatment Performance = 23 $g/m^3/d$

Appendix VI

Calculation of Nitrate Leaching Losses Using Empirical Formulae

For the purposes of this exercise a 30ha catchment, part of Silsoe College Farm, Bedfordshire, was chosen as representative of catchments likely to produce nitrate polluted waters i.e., where nitrate concentrations are likely to exceed the EU nitrate drinking water limit of 11.3mg/l NO_3-N.

The catchment consists of Evesham and Lawford Series clay soils, and hence the nitrate loss regression equation developed by Goss et al.(1993) can be said to be applicable. Climatological data used in the exercise was recorded at Silsoe College's weather station from October-December 1993.

A figure for soil moisture deficit is taken from Climate and Drainage (Smith and Trafford, 1976), and represents the statistically lowest soil moisture deficit for the end of September, i.e., the condition under which the highest drainage volume will occur. The figure for net rainfall is calculated from climate data.

Soil moisture deficit - End of September	=	34mm
Net rainfall from October 1st - October 9th	=	34mm

The calculation is presented in Table A6.1.

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Table A6.1 Calculation of Nitrate-Nitrogen Mass Leached From 30ha Clay Catchment Using Empirical Formulae

Date of Rainfall Event	Rainfall (1) (mm)	Evaporation (mm)	Net Rainfall (mm)	Drainage Volume for 30ha (m^3)	Mass of Nitrate Leached for 30ha (2) (kg NO_3-N)
10-12/10	35.7	5.5	30.2	9060	222.78
9-13/11	20.3	0.6	19.7	5910	134.58
12-13/12	8.1	0.1	8	2400	36.3
17-19/12	9.4	2.7	6.7	2010	25.38
22-23/12	5	0.4	4.6	1380	7.74
28-31/12	9.2	0	9.2	2760	46.38
Totals	87.7	9.3	78.4	23520	473.16 (3)

(1) Rainfall figure is net rainfall as Goss et al. (1993) found that 3mm rainfall required before leaching loss began.

(2) Calculated using following equation:

kg NO_3-N leached/ha = (0.28 x net rainfall) - 1.03 (Goss et al., 1993)

(3) Nitrate Leached per hectare = (473.16/30) = 15.77 kg NO_3-N/ha.

Goss et al. (1993) predicted an annual loss of 22 kg NO_3-N/ha, when no nitrate fertiliser applied in autumn.

Appendix VII

Costs Associated with the Loss of Productive Land for the Proposed On-Farm Methods

The cost for the loss of productive land associated with the construction of on-farm water storage facilities, quoted in Chapter 7, is calculated below:

For a 30ha catchment growing cereals, approximately 1ha of land would be lost for the water storage facility, at a cost of £5000/ha. Amortisation[1] of this cost over a 21 year period will give a variable cost per cubic metre of water treated:
Hence, variable cost for land is: ((£5000/1000) x 116))/30000m^3 = 1.9p/m^3.

With a production gross margin of £780/ha/year for winter wheat (Nix, 1996), and assuming set-a-side payments were sanctioned to compensate for the production loss at the maximum of £341/ha/year (Nix, 1996), then the variable (amortisation) cost calculated for a 21 year period will be:

$$\frac{(((£780-£341) \times 21)/1000) \times 116)}{30000m^3} = 3.5p/m^3$$

Hence, total cost = 5.4p/m^3 of water managed and therefore the variable cost associated with the loss of productive land for 30000m^3/year would be £1620, in addition to the costs quoted in Section 7.2.3.

[1] Amortisation Cost = £116 per £1000 invested for a 21 year repayment period @ interest rate of 10% (Weatherhead, 1996). N.B. Cost is slightly less than by Discounting because risk is not taken into account.