CONTINUOUS AEROBIC PROCESSING OF PIGGERY EFFLUENT - A NEW APPROACH TO QUANTIFYING THE FATE OF THE NITROGEN COMPONENT

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SYNOPSIS

The primary objective was the preparation of a complete mass balance around an aerobic treatment system for pig slurry, to quantify the various forms of nitrogen entering and leaving under different conditions. The purpose of this was to assess the effect of such treatment conditions in terms of the amount of polluting forms of nitrogen generated from the slurry. A laboratory scale reactor (designed for this study) was operated under three separate residence times of 2, 4, and 8 days, and aeration level indicated by a redox value in the range of $E_{Ag/AgCl}$ = +100 to +200 mV; the latter two giving nitrifying conditions.

Emissions of di-nitrogen gas are a major component of a nitrogen mass balance, yet one which has been often neglected because of difficulties in distinguishing it from that in the atmosphere. A novel technique was developed in which atmospheric N_2 in the reactor headspace was removed by flushing the system with an 80/20 gas mixture of argon/oxygen. This left microbially derived N_2 available for collection and analysis by mass spectrometry.

Established methods were applied for the measurement of other gaseous nitrogen emissions (NH_3, N_2O, NO) and other forms of nitrogen in the slurry (organic-N, NH_4^+, NO_2^- and NO_3^-). The steam distillation technique for nitrite and nitrate was found to be unreliable, therefore, high performance liquid chromatography was used as an alternative. The existence of the intermediate nitrified N form of hydroxylamine is postulated but was not quantified in this study. The presence of unidentified components in raw slurry was investigated using HPLC, but only chloride and acetate could be recognised with a high degree of confidence.

Mean N_2 concentrations measured were 774 mg l^{-1} in the 4 day treatment and 523 mg l^{-1} in the 8 day treatment. Emissions of the environmentally damaging N_2O gas were quantified as being 514 mg l^{-1} in the 4 day treatment and 219 mg l^{-1} in the 8 day. The lower emissions from the 8 day treatment are attributed to improved contact between oxygen and slurry, reducing the prevalence of zones favourable for denitrification. In the final mass balance study, overall nitrogen leaving the system equalled 86 (±18) % of that entering in the 2 day treatment, 113 (±10) % in the 4 day treatment, and 104 (±21) % in the 8 day treatment. The variation in values was attributed to errors in the liquid phase analysis of slurry nitrogen compounds.

Dedicated to the memory of Albert George Greatorex.

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NOTATION

BOD_5	5 day biological oxygen demand (mg l^{-1})
C	concentration of atmospheric N_2 in headspace at time t (%v/v)
C_0	concentration of atmospheric N_2 in headspace at zero time (%v/v)
COD	chemical oxygen demand (mg l^{-1})
DO	dissolved oxygen
F	titration correction factor
M_N	mg of N_2 emitted per litre of slurry (mg l^{-1})
N	mg of nitrogen recovered per ml of slurry sample (mg ml^{-1})
N_k	Kjeldahl nitrogen (mg l^{-1})
N_{ox}	oxidizable nitrogen
N_{tot}	total nitrogen (mg l^{-1})
Q	flow rate of flushing gas (l min^{-1})
Q_n	rate of N_2 emission by denitrifiers (l min^{-1})
SD	standard deviation
t	time (mins)
T	titre value after subtraction of blank (ml)
TS	total solids (%w/w)
V	headspace volume (l), or sample volume in steam distillation experiments (ml)
VPM	volumes per million
Z	percentage of N_2 detected by mass spectrometer (%v/v)
θ	residence time of gas in headspace (mins)

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PART A

Background to Project Work

1. INTRODUCTION

1.1 Background to aerobic treatment process

Increased production of pig slurry has become a significant environmental problem in areas of intensive pig production. This is due to the problems of air and water pollution caused by different forms of nitrogen associated with the slurry, as well as gaseous emissions. There has also been a rise in complaints by the general public, particularly with regard to odour nuisance (NRA, 1992; MAFF, 1992; MAFF, 1993). These problems have lead to the search for improved treatment and handling techniques. One option which the farmer can consider, is aerobic treatment of the slurry. Although it has disadvantages compared with anaerobic digestion, mainly on the grounds of running cost, it offers a number of advantages including: the ability to transform nitrogen between various forms in controlled ways, faster breakdown of organic matter and simpler process configuration.

Much research has been undertaken, therefore, to identify and study the effects of the various factors which affect aerobic slurry treatment. One particular area of interest is the fate of nitrogen during the treatment process. This interest arises from the dual nature of nitrogen as either a key component of soil fertility, or as a major pollutant of the air or ground water.

The exact configuration of a practical aerobic treatment process will depend on the farmer's specific requirements. For example, if ammonia emissions are to be diminished, a nitrifying treatment would be appropriate to convert ammonia into nitrate. However, if the nitrate produced from such a process also has to be diminished (because of the problem of ground water

pollution after spreading the treated slurry), then a denitrifying treatment would be appropriate to convert nitrate into nitrogen gases. In practice, it is convenient to combine both nitrification and denitrification in a simultaneous process, occurring in the same reaction vessel. Therefore in the context of this project, the study of the nitrogen transformations by nitrification and denitrification, were made in a reactor of just such a configuration.

Caution should be exercised when developing a treatment process since it is possible that a process which removes one type of pollution problem, e.g., nitrate removal, may create another unanticipated problem, e.g., nitrate removal treatments can produce nitrous oxide - a powerful greenhouse gas and ozone depletant (McElroy et al., 1977). Potential problems of this sort can be identified by comparing nitrogen quantities in the entry and exit streams of a slurry reactor (i.e., by drawing up a nitrogen mass balance) and investigating any imbalances which become apparent.

1.2 Hypothesis to be tested

In the light of the above, the aim of the project was to test the following hypothesis: constructing a complete nitrogen mass balance, around a continuous aerobic treatment process for pig slurry, will enable all forms of nitrogen to be accounted for under different sets of treatment conditions. This will allow these conditions to be assessed for the amounts of polluting forms of nitrogen which they induce from the treatment process. Also a complete mass balance will reinforce the assertion that the nitrogen analyses performed were done so accurately.

The novelty of the project is founded on the fact that no mass balance study, in the past, had made a comprehensive attempt to measure all the nitrogen forms arising out of an aerobic slurry

treatment process. The main reasons for this were difficulties in measuring emissions of di-nitrogen gas, a key problem which is addressed in the work presented here, and also a lack of awareness among some workers as to the scale of emissions of gaseous oxides of nitrogen (e.g., in the case of nitrous oxide which, from some treatments, can reach concentrations in the order of thousands of ppm).

In order to construct a complete mass balance, as accurately as possible, it was necessary to quantify all measurable forms of nitrogen believed to occur. A laboratory scale, continuous aerobic slurry reactor was constructed for this purpose.

1.3 Nitrogen analyses used to construct mass balances

Figure 1.1 shows the different forms of nitrogen measured in this work. In the raw and treated slurry phases, techniques are well established to quantify organic nitrogen by the Kjeldahl method and ammoniacal (i.e., $NH_4^+ + NH_3$) nitrogen by steam distillation (Dimmock & Martinez, 1994; APHA, 1985). Techniques for the determination of nitrite (NO_2^-) and nitrate (NO_3^-) from slurry, using steam distillation, were found to be less repeatable, and as yet, no attempt has been made to recover hydroxylamine (not shown in fig. 1.1) - an intermediate in the nitrification pathway which has been found, by some workers, to accumulate under conditions of oxygen deficiency (Yang & Alleman, 1992). High Performance Liquid Chromatography (HPLC) was found to be a more reliable method for accurately determining nitrite and nitrate, whilst offering the possibility of quantitatively recovering hydroxylamine.

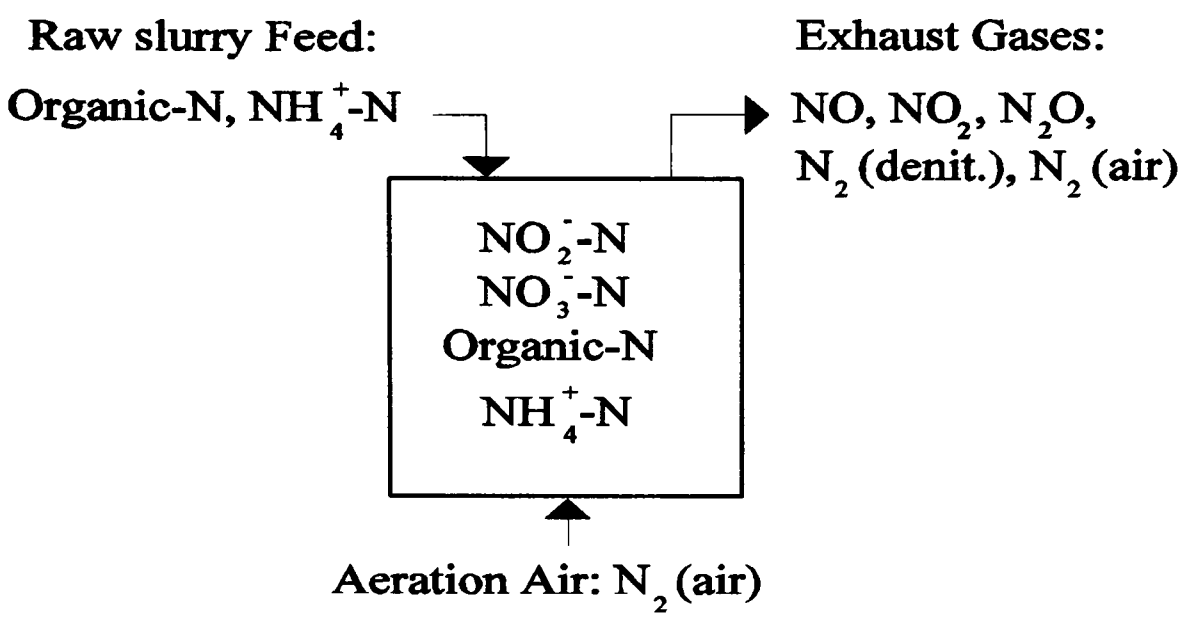


Fig. 1.1, Representation of nitrogen fluxes in-to and out-of an aeration system

N.B., Species inside box are forms of nitrogen detectable in treated slurry. N_2 (air) refers to atmospheric di-nitrogen and N_2 (denit.) refers to di-nitrogen produced by denitrification.

In the gas phase, nitrous oxide (N_2O) was determined by infra red spectroscopy, whilst other gaseous oxides of nitrogen, i.e., nitric oxide (NO) and nitrogen dioxide (NO_2) (only found in traces below 10 ppm in aeration studies), were quantified by Dräger gas analysis indicating tubes (Drägerwerk AG, Lübeck, Federal Republic of Germany). With regard to the omission of N_2 measurements from previous mass balance work, figure 1.1 clearly shows the reason for this, i.e., the difficulties in resolving microbially produced N_2 (N_2 denit.) from atmospheric N_2 (N_2 air), and measuring it, without access to specialised equipment.

A method to quantify only denitrification-derived N_2 had, therefore, to be developed from basics. Originally a technique of stable isotope labelling, using ^{15}N, was considered. This would have

involved adding a ^{15}N labelled ammonium compound to the slurry, and measuring the ratio of $^{29}N_2$ to $^{28}N_2$ in the reactor off-gas. However, this idea was not developed further because of the large cost associated with stable isotope compounds: to have produced a minimum level of 0.1 atom % excess ^{15}N in the reactor off-gas, would have required the dosing of 1.4 g hr^{-1} of labelled ammonium sulphate. The cost of running the reactor at this minimum dosage rate would have been around £100 per hour.

The technique adopted was one of gas flushing, in which air in the reactor headspace was replaced by an 80/20 mixture of argon and oxygen. This reduced interference from atmospheric di-nitrogen, leaving residual, microbially generated di-nitrogen for collection and analysis by mass spectrometry.

1.4 Experimental trials

After the completion of work to assess established methods and develop new techniques as required, full mass balances were constructed for a preliminary series of trials centred on a 4 day residence time, medium aeration level (aerated to a redox potential in the range $E_{Ag/AgCl}$ = +100 to +200 mV) treatment system. These trials involved the measurement of a fairly small number of different slurry parameters, usually on days when samples of headspace gas were collected from the aeration vessel (after flushing with the argon/oxygen gas mixture) for subsequent mass spectrometric determination.

The experience gained from this first series of trials was then used to improve techniques for a second (and final) series of trials, in which the effect of changing residence time on nitrogen

transformations was investigated. These trials were characterised by the measurement of a larger range of slurry parameters on a greatly increased frequency of sampling. Also, access to a data logger enabled the continuous measurement of slurry gases (i.e., O_2, CO_2 and N_2O) and physical parameters such as temperature, pH and redox. For this final piece of work, full mass balances were constructed around medium aeration treatments with 2, 4 and 8 day residence times.

1.5 Project Objectives

A summary of the main project objectives (and thesis chapters where they are dealt with) follows:

(i) Construct a laboratory scale aerobic reactor (based on a continuously fed, stirred tank design), to treat and monitor 10 litre volumes of pig slurry, under conditions able to induce nitrification and denitrification of the slurry nitrogen (chapter 3).

(ii) Develop a technique for the determination of di-nitrogen gas, evolved from the reactor by denitrification activity, based on the findings of a validated mathematical model (chapter 4).

(iii) Evaluate existing analytical techniques for the determination of organic nitrogen, ammonium, nitrite and nitrate (chapter 5), and develop HPLC methods to recover and quantify nitrite, nitrate and hydroxylamine from pig slurry (chapter 6).

(iv) Prepare nitrogen mass balances around the laboratory scale aerobic reactor, according to the trials outlined in section 1.4, and include other analytical data, such as changes in COD and solids content of the slurry, to define the treatment environment (chapter 7).

During the development of methods using the HPLC, it became apparent that the technique was able to recover a number of components present in raw slurry. The identification of some of these components was pursued, as an aside to the main piece of work, since the treatment process was found to remove them from raw slurry. The study of these components and their removal may have potential for future work, particularly if they are identified as having either a detrimental or beneficial effect on agriculture and the environment.

2. THE BIOCHEMISTRY OF NITROGEN OXIDATION AND REDUCTION, AND ITS APPLICATION TO AEROBIC SLURRY TREATMENT

2.1 Introduction

This chapter reviews relevant literature on the subject of nitrogen transformations (i.e., nitrification and denitrification) with the aim of answering the following questions:

I. Why has aerobic treatment gained importance in recent years and what is its future?

II. How does it compare with the alternative processing option of anaerobic treatment?

III. What are nitrification and denitrification, in terms of their microbiology and biochemistry?

IV. What conditions favour nitrification and denitrification, and what inhibits them?

Obtaining answers to these questions enabled:

1. The importance of aerobic treatment, and its choice for use in this project, to be justified.
2. The potential pollution problems arising from processing pig slurry (as well as from the raw effluent) to be appreciated.
3. A clear understanding of the mechanisms of nitrification and denitrification.
4. Information to be gathered on the parameters governing nitrification-denitrification which could be utilized in the design and operation of the laboratory scale reactor.
5. The extent of knowledge on N_2 measurement in waste treatment systems to be gauged.

2.2 Problems associated with piggery waste production

Since the Second World War, general intensification of agricultural practices has lead to an increase in the volume of animal wastes produced in specific areas (Gasser, 1984). MAFF (1988) quote a figure of 8 Mt of waste produced from pigs, in the UK alone, every year. The production and disposal of such huge quantities of waste in these concentrated regions, means that environmental problems are inevitable. The spreading of waste on to land, for instance, leads to the problem of nitrate leaching into water courses (Goulding & Poulton, 1992) and subsequent problems such as eutrophication (Filip & Middlebrooks, 1976), whilst volatile malodorous compounds are released into the atmosphere, causing a nuisance to local residents (Evans, 1982).

During the period 1985 to 1989, the majority (around 67%) of all reported farm pollution incidents, in England and Wales, were due to animal wastes, 10% being due to pigs alone (National Rivers Authority, 1992). Also for the period 1987 to 1990, pigs were the cause of 650 justifiable odour complaints from the public. This figure represents the largest category (47%) of odour complaints pertaining to the agricultural industry (MAFF, 1992). Additionally, livestock production accounts for the majority of ammonia in the atmosphere. This not only represents a loss in the nutrient value of animal waste; deposition of ammonia, either as gas or dissolved in rainfall, damages vegetation and changes plant populations by acidifying soils (MAFF, 1992).

2.3 The role of aerobic treatment

As intensification and animal numbers has increased, so the means of handling the waste produced by animal production has changed. The popularity of slurry handling systems has grown, whilst straw-based waste systems have become less favoured (Burton & Cumby, 1995). This situation

has arisen from the reduced labour requirements involved in handling waste as a liquid. Interest in aerobic treatment, as a means by which the problem of increased slurry production and the consequential rise in pollution, may be addressed, is growing (e.g., Evans et al., 1986; Williams et al., 1989; Beaudet et al. 1990; Burton et al., 1993a), although the technique has been available for a number of years (Owens et al., 1973). Continuous aerobic processing of slurry can control offensive odour for between several days and several months (depending on the residence time employed) as well as reducing COD and BOD_5, whilst the encouragement of a nitrifying and/or denitrifying environment can be used to reduce pollution by ammonia and nitrate (Burton, 1992).

The effect of aerating slurry is to bring about an environment which is favourable (when sufficient free oxygen is available) for aerobic microorganisms to dominate anaerobes. The aerobic digestion process yields a greater amount of energy than its anaerobic counterpart, leading to faster and more extensive breakdown of organic matter. Also the nitrification process is aerobic, hence a treatment system which supplies adequate oxygen is essential.

2.4 Comparison of aerobic and anaerobic processing of animal waste

Anaerobic activity occurs in slurry which has not received sufficient oxygen to support the proliferation of aerobic microorganisms. The characteristic offensive odours are derived from the products of anaerobic processes, such as volatile fatty acids (VFAs), phenols, cresols and indoles, and from the respirative pathways which utilise chemically bound oxygen, e.g., sulphate ions are reduced to the pungent smelling hydrogen sulphide gas (Burton, 1992). The production of bio-gas, which can be used as a source of fuel, is the result of complex organic substrates being progressively broken down, ultimately into very simple gaseous molecules such as carbon dioxide

and the combustible methane. The principal reasons for selecting aerobic processing, rather than anaerobic processing, are as follows:

1. Aerobic treatment offers the option of effective nitrogen control through nitrification and/or denitrification. Williams et al. (1989) achieved a 91 % reduction in ammonia nitrogen levels from a 4 day residence time, continuous aerobic treatment of pig slurry. Anaerobic treatment does not allow this capability, and ammonia levels from a reactor are typically very high due to the breakdown of organic nitrogen without subsequent nitrification. For example, Lo et al. (1985) recorded a doubling of ammonia nitrogen from a 16 day residence time, continuous anaerobic treatment of screened dairy manure. In addition to polluting the environment, ammonia, at high concentrations in an anaerobic treatment, can inhibit breakdown of VFAs. Zeeman et al. (1985) found that 4.5 g l^{-1} of NH_4^+ caused VFA accumulation (as a percentage of acidification) of 60 % in anaerobic digestion of cow slurry.

2. Aerobic treatment allows shorter residence times and faster breakdown of organic matter, hence enabling a larger quantity of waste to be treated in a given reactor volume, compared with anaerobic treatment. Williams et al. (1991) compared an aerobic and an anaerobic treatment process for odour control. Although both processes gave odour reductions between 75 and 86 %, a residence time of 10 days was required for the anaerobic process compared with 2 days for the aerobic process. It is not unknown for high loading rates of pig manure (e.g., 9 kg TS m^{-3} d^{-1}) to require residence times in anaerobic digesters of up to 40 days to remove all odorous compounds (Nielsen, 1985).

3. Capital costs of aerobic processing equipment are significantly lower than those for anaerobic processing, although it is accepted that aeration greatly increases running costs. In the Williams et al. (1991) study, projected capital costs were $27 500 for the aerobic plant and $72 500 for the anaerobic plant. The higher capital cost for the anaerobic plant was presumably due to the extra cost entailed in procuring a suitable air-tight reaction vessel, and the gas collection and storage equipment. However, considering the overall cost; i.e., the cost of aeration and the fact that biogases (such as methane) produced by anaerobic digestion can be used as an energy source to off-set treatment cost; aerobic treatment proved to be more expensive at $4.00 per pig produced, whilst anaerobic treatment yielded a small profit of $0.35 per pig produced (n.b., both figures include estimated annual capital and operational costs. It should also be noted that the figures were based mainly on assumptions rather than measured economic data. Therefore, the actual economic performances may have been somewhat different).

Despite increased running costs, therefore, aerobic treatment has significant advantages over anaerobic digestion in terms of nitrogen control and the rate at which slurry can be treated. There are also benefits to be gained from destruction of pathogens, utilisation of heat output and simpler equipment design (Burton, 1992). Additionally, aerobic processing is less sensitive to sudden changes in environmental factors such as temperature, pH and shock loading of nutrients (Peavy et al., 1985). The high costs, however, are more likely to be successfully borne by larger farm units, where profit margins on animals may justify the additional expenditure. Smaller farms with lower profit returns may not benefit, economically, from installing aerobic treatment equipment (Burton & Cumby, 1995).

2.5 Nitrification

Understanding the transformations which occur through nitrification (and subsequent denitrification) is essential, if those polluting forms of nitrogen found in slurry are to be effectively dealt with by aerobic treatment. In considering the nitrogen transformations, examples drawn from animal waste treatment have been used wherever possible. However, where information has not been available or a concept has been more easily explained, examples taken from waste water/activated sludge treatment have been used. The two processes have many similarities, although their principal differences are in the much higher BOD_5 values of animal slurries over domestic sewage (25,000 mg l^{-1} c.f. 250 mg l^{-1}) and also the higher suspended solid concentrations of animal wastes (some 10 to 20 times greater) which generally contain much coarser particles (Hobson & Robertson, 1977).

2.5.1 Mechanism of nitrification

The process of biological nitrification is an oxidation process in which the ammonium present in animal waste is oxidised to nitrate via several intermediates, including hydroxylamine (NH_2OH) and nitrite (NO_2^-):

$$NH_4^+ \rightarrow NH_2OH \rightarrow \text{other intermediates} \rightarrow NO_2^- \rightarrow NO_3^-$$

Overall, it is often perceived as a two step process, ammonium being oxidised to nitrite by the involvement of bacteria from the genus *Nitrosomonas*, and nitrite being oxidised to nitrate by bacteria from the genus *Nitrobacter*.

In the initial oxidation of ammonium by *Nitrosomonas*, the presence of hydroxylamine has been

conclusively demonstrated by experimental work involving selective inhibition of hydroxylamine oxidation (Sharma & Ahlert, 1977). However, the other intermediates before nitrite are considered too unstable to be present in significant concentrations in slurry. This instability also makes them difficult to study, but many workers (in Focht & Chang, 1975) believe that hydroxylamine passes through an unstable nitroxyl structure during its oxidation to nitrite.

The overall stoichiometry of the pathway is given below (Sharma & Ahlert, 1977):

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2^- + 243\text{-}351 \text{ kJ}$$

Oxidation of nitrite to nitrate by *Nitrobacter* is a simpler process (Sharma & Ahlert, 1977):

$$NO_2 + 0.5O_2 \rightarrow NO_3^- + 64.4\text{-}87.4 \text{ kJ}$$

It is clear from the above that nitrification requires oxygen, and thus, *Nitrobacter* and *Nitrosomonas* are defined as aerobic bacteria.

Nitrifying organisms may be described as autotrophic or heterotrophic depending on the nature of the compounds they utilize to produce energy (Painter, 1977). If the oxidation of ammonium through to nitrate is the sole source of energy, then an organism may be classed as autotrophic (e.g. *Nitrosomonas europea* and *Nitrobacter winogradskyi*). If, however, nitrite and/or nitrate are produced from inorganic or organic compounds by reactions which are not necessarily oxidations, and which are not the sole energy sources, then an organism may be classed as heterotrophic (e.g. *Nocardia* spp. and *Agrobacter* spp.). However, in waste water treatment the amount of oxidised nitrogen formed by heterotrophic processes is low. This has been

demonstrated in activated sludge, where selective inhibitors of autotrophic nitrification are usually found to block nitrification completely (Painter, 1977).

The state of autotrophy is not a definite one. A number of workers (reported in Painter, 1977) have found that certain species of *Nitrosomonas* and *Nitrobacter* are able to exhibit heterotrophic behaviour, suggesting that they might be better termed facultative autotrophs. However, on the information available, one must conclude that autotrophic nitrification is by far the dominant process, and little practical significance is attached to the heterotrophic nitrification process.

2.5.1.1 NO and N_2O production from nitrification

In recent years there has been an expansion of published work (mainly in the area of soil science) looking at the production of trace NO and N_2O gases by nitrification processes (e.g., Schuster & Conrad, 1992; Martikainen et al., 1993). Firestone & Davidson (1989) have reviewed some of the literature on this subject. They report that nitrous oxide is produced by ammonium oxidising bacteria, using nitrite as an electron acceptor, when oxygen is limiting (for pathway see figure 2.1 below). Also, nitrifiers can avoid the accumulation of potentially toxic NO_2^- by reducing it to N_2O. The ratio of N_2O over NO_2^- is reported as being small but significant (usually below 1%).

Although Firestone & Davidson (1989) report that rates of NO production, from nitrification, are higher than those for N_2O production, the process is not well understood. There is evidence that NO can be produced via the oxidation of hydroxylamine and/or the reduction of nitrite. Burton et al. (1993a) claim to have detected NO released from an exclusively oxidising environment. Firestone & Davidson (1989) also report that it is unclear if oxygen partial pressure inhibits NO

production by nitrification, or leaves it unaffected.

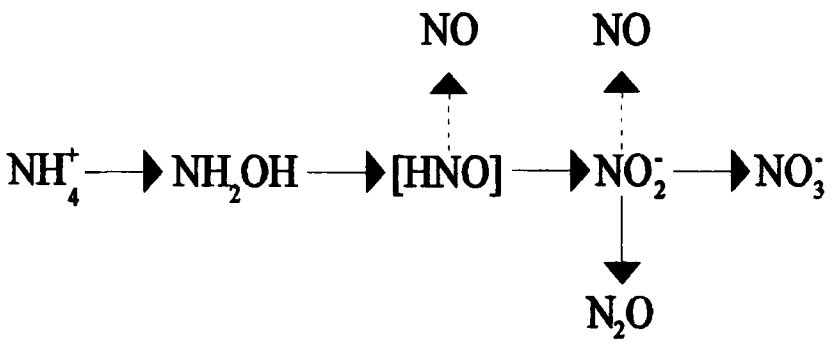


Fig 2.1, Pathway of nitrification showing NO and N_2O production

N.B, Broken lines correspond to unconfirmed pathways,
[HNO] represents the unstable nitroxyl intermediate.

Since the nitrogen transformation processes which occur in soil are analogous to those in slurry aeration, it is not unreasonable to assume that a proportion of any nitric oxide and nitrous oxide produced during waste treatment (albeit small) may be derived from nitrifiers.

2.5.1.2 Inhibition of nitrification

The activities of *Nitrosomonas* and *Nitrobacter* may be inhibited by certain substances (Painter, 1977; Wood et al., 1981). The extent to which this inhibition occurs, however, for each species, may be different. This stems from the fact that the *Nitrosomonas* enzyme system is more complex and hence more susceptible to inhibition than that of *Nitrobacter*. This complexity arises out of the need to oxidise nitrogen from a -3 (NH_4^+) to a +3 (NO_2^-) oxidation state in *Nitrosomonas*, compared with a +3 (NO_2^-) to a +5 (NO_3^-) oxidation state in *Nitrobacter*.

One mode of inhibition of nitrification is by its substrate and the products of its activity. Smith & Evans (1982), report that high concentrations of nitrite and nitrate could exert feedback inhibition of nitrifying activity. In more recent work on pure cultures of *Nitrosomonas*, Groeneweg et al. (1994) observed oscillations in biomass which were attributed to fluctuations between substrate inhibition (influent ammoniacal nitrogen concentration of 392 mg l^{-1}) and product inhibition (NO_2^--N concentration up to 350 mg l^{-1}). This assumption was supported with experimental evidence which showed that oxidation rates were inhibited with ammoniacal nitrogen concentrations above 100 mg l^{-1} and NO_2^- -N concentrations above 80 mg l^{-1}. *Nitrosomonas* cells exposed to NO_2^--N concentrations of 3000 mg l^{-1} showed an oxidation rate 20 % lower than control populations exposed to trace amounts of nitrite.

Other modes of inhibition reported in the literature include:

1. In continuous systems, nitrification fails to become established when residence times of less than 3 days are used (Burton et al., 1993a). This is due to the slow growth rate of nitrifiers (compared with other organisms) which become 'washed-out' of the system before they have developed to a state where they can nitrify ammonium.

2. Mis-conformation of enzymic systems by heavy metals, of which copper is the most notable in animal waste, e.g., Blouin et al. (1988) quote 500 mg l^{-1} of copper in aerated pig waste as being able to inhibit all microbial breakdown of soluble nitrogen compounds.

3. In nitrifying systems deficient in oxygen, hydroxylamine is believed to accumulate causing acute toxicity problems to *Nitrobacter*. Inhibition of this sort may be detected by a build

up of nitrite in the nitrifying system (Yang & Alleman, 1992).

2.5.2 Effect of environmental conditions

Treatment processes rely on the metabolic activity of microorganisms to effect changes in the piggery waste. Therefore, the biodegradation of organic matter (i.e., carbonaceous as well as nitrogenous matter) is a function of various environmental conditions (e.g., aeration level, pH and temperature) under which the microorganisms are maintained, as well as the slurry composition. It should also be realised that environmental factors have chemical (as well as microbiological) effects. This is of particular importance in the NH_4^+/NH_3 equilibrium, where both pH and temperature will determine the relative amounts of each molecular species, and hence the availability of NH_4^+ to participate in nitrification.

2.5.2.1 Level of available oxygen

This is necessary to maintain the aerobic bacterial population in treatment processes which oxidise organic compounds. The quantity of nitrogen oxidised is less than that of carbon. Evans et al. (1983) state that when dissolved oxygen (DO) is maintained above 15% of saturation and the mean residence time is 3 days, the carbonaceous oxygen demand for pig slurry treatment is about 188 g of O_2 per pig place per day (at a treatment temperature of 25 to 45 ^{0}C). On the other hand, the oxygen demand for nitrification is about 82 g of O_2 per pig place per day (25 to 35 ^{0}C), amounting to about 30 % of the total oxygen requirement (Evans et al., 1986).

Although aerobic treatment is an effective method for odour control, large amounts of energy must be expended at high aeration rates, which limits cost effectiveness (Williams et al., 1989).

Therefore much work has been done to assess what level of aeration is necessary to give an acceptable amount of treatment as regards slurry stabilization (in terms of reducing odour and ammonia emission) during subsequent storage.

Stenstrom & Poduska (1980) reviewed the work of a number of researchers, in the late seventies, who were investigating the effect of DO on nitrification in pure and mixed cultures, and cultures found in waste water systems. They reported a wide range of DO values at which the maximum rate of both nitrification reactions occurred, from 0.3 mg l^{-1} to 4.0 mg l^{-1}. Therefore, they were unable to suggest any clearly defined DO concentration for optimal nitrification. This variation is due to a number of factors, including: the amount and nature of microbiological floc, degree of agitation, temperature and the concentration of surface active agents (Cumby, 1987).

Smith & Evans (1982) looked in detail at piggery slurry treatment at various DO concentrations, and found that aeration efficiency was greatest at DO as low as 1% of saturation. As much as 90% of the soluble nitrogen (i.e., convertible nitrogen as opposed to non-convertible nitrogen - roughly equivalent to the organic nitrogen of the slurry solids) was lost at low DO, this being due to the occurrence of simultaneous nitrification and denitrification (see section 2.6.3) in the completely mixed reactor.

The residence time of slurry in continuous treatments can also be used to improve the efficiency of mass transfer at lower DO concentrations. Stenstrom & Song (1991) constructed a simulation (based on nitrification in an activated sludge process) which predicted increased nitrate production, for DO levels below 2 mg l^{-1}, at a residence time of 6 days compared with 3 days.

This suggests that low DO and long residence time could have a similar effect on the amount of nitrate production as high DO and short residence time. Unfortunately they fail to present, or give reference to, the results of earlier experimental work which would support the model with hard experimental evidence.

Measurement of oxidation environment using redox

Where treatment conditions involving low DO (below 10 % of saturation) are required, a number of workers prefer to measure oxygen availability using redox potential, e.g., Williams et al. (1989) and Burton et al. (1993a). The reason for this, as reported by Gray (1992), is that redox gives greater reliability, when compared with DO measurement, at low DO concentrations. This arises from the greater stability of response from redox probes, which are not unduly influenced by the formation of gas bubbles on their surfaces, as is the case with DO probes. An additional advantage of redox is that it measures the whole oxidising environment of a treatment system, rather than just the single parameter of free oxygen availability. This provides information about the state of oxidative processes (such as nitrification and carbonaceous breakdown) which DO does not.

Williams et al. (1989) found that nitrification was inhibited in pig slurry aerated to redox potentials in the range E_{cal} = -195 to -45 mV. Burton et al. (1993a) record similar findings, however for treatment times of 4 days, redox potentials in the range of E_{cal} = -70 to -30 mV were found to be suitable for nitrification and denitrification to occur simultaneously.

2.5.2.2 pH

During nitrification, a decrease in pH is encountered (Owens et al., 1973). This is due to the production of protons as ammonium is oxidised to nitrate (see equation on page 14). As pH falls, nitrification activity is reduced as the formation of free nitrous acid inhibits both *Nitrosomonas* and *Nitrobacter* (Sharma & Ahlert, 1977). Evans et al. (1986) found that under high aeration conditions (DO > 15% of saturation), nitrification stopped when the pH fell to about 5.5 . Using a continuous culture system, this acidity was subsequently diluted and neutralised by fresh, incoming slurry of alkaline pH. The conditions were then suitable for the nitrifying population to resume growth and activity. Evans et al. (1986) claim that the result of this continuous cycling between nitrification and its inhibition was that only a proportion of the total slurry nitrogen was oxidised, the percentages being 40% at 15 ^{0}C, 60% at 25-35 ^{0}C and 40% at 40 ^{0}C. Although pH inhibition may have had some involvement, another likely explanation is that the inhibition was due to substrate limitation by exhaustion of the soluble nitrogen fraction.

Smith & Evans (1982) also found effluent pH values of 5 to 6 at high DO. Under conditions of low DO, they found higher pH values of 6.5 to 7.0, presumably due to hydroxyl ions released during denitrification and the effect of feed slurry ammonium. Similar work by Murray et al. (1975), also found an elevation in slurry pH from 5 to 8 as conditions of high DO were subsequently replaced with conditions of low DO.

The optimum pH for nitrification, as found by a number of experimenters, varies quite widely, but Shammas (1986), reporting on the findings of a collection of workers, states that, generally, pH optimum lies between 8 and 9. Also, Barnes & Bliss (1983) state that the optimum pH lies in the

range 7.5 to 8.5, with *Nitrosomonas* optima varying between pH 6 and 9, and *Nitrobacter* optima varying, similarly, between pH 6.3 and 9.4 . The pH values at the top end of the ranges should be regarded with caution, given that the formation of free ammonia at these levels can lead to the inhibition of nitrite oxidisers (Sharma & Ahlert, 1977).

2.5.2.3 Temperature

Temperature directly affects the microbiological activity of a slurry treatment system. The main effect of operating at temperatures below 15 ^{0}C is to reduce nitrification, while at 5 ^{0}C, no nitrification will occur, even with increased slurry concentrations (Owens et al., 1973; Shammas, 1986). Sharma & Ahlert (1977) report that the temperature optimum for nitrifier growth is in the range 28 to 36 ^{0}C. Variation probably arises from differences in the optima of the various species of nitrifiers which were studied.

2.6 Denitrification

2.6.1 Mechanism of denitrification

The metabolism of nitrate by microorganisms, leading to its reduction, may be described in one of two ways (Painter, 1977):

(i) Assimilation in which nitrate is used in the synthesis of cellular constituents. Therefore, nitrate reduction of this sort does not represent an overall loss of nitrogen from a slurry treatment system. Nitrate must, however, first be reduced to ammonium before it can be assimilated.

(ii) Dissimilation is a reductive process in which nitrate-oxygen is used as the terminal hydrogen

acceptor in respirative pathways, rather than molecular oxygen. The nitrogen is not incorporated into cellular material and may be released as nitrite, or the gases: nitric oxide, nitrogen dioxide, nitrous oxide or di-nitrogen, depending on the species of the organism. The name assigned to this process is denitrification, and the production of nitrogen containing gases is significant since they represent a loss of nitrogen from a treatment system.

Dissimilation is the more significant process, since the oxygen demand of cells (for use in respiration) is greater than the nitrogen demand (for use in making organic nitrogen components), hence dissimilatory nitrate reduction occurs at a faster rate than the assimilatory process.

The overall stoichiometry of denitrification is shown below (Mateju et al., 1992). A source of organic carbon is necessary for respiration and growth of the denitrifying bacteria, and here, methanol is included in the equation:

$$6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH$$

There is much concern over the release of gaseous oxides of nitrogen from agricultural sources, and the adverse effects they have on the environment (McElroy et al., 1977). NO_x is a constituent of acid rain and contributes to photochemical smog, whilst N_2O, in particular, is implicated in global warming and the destruction of stratospheric ozone. N_2O has also been detected in supersaturated waters from sewage plant effluents (Cicerone et al., 1978). Concentrations of NO_x gases are rising (Arah, 1992) hence the interest in the denitrification process to see if the emissions may be controlled.

Most of the work on denitrification has been carried out with a limited group of specialized bacteria which has lead to the incorrect view that denitrification can only occur under anaerobic conditions (Mateju et al., 1992). It has been demonstrated by a number of workers, that certain species are quite able to carry out denitrification in the presence of oxygen, e.g., Robertson et al. (1988), Krul & Veeningen (1977), Mateju et al. (1992).

Although a vast range of microorganisms can undertake the assimilatory reduction of nitrate, comparatively few species are able to denitrify (there are still, however, many examples). Some examples of genera known to contain denitrifying species are *Paracoccus, Pseudomonas, Thiosphaera* and *Achromobacter*. In work carried out by Bock et al. (1988) even *Nitrobacter* was found to utilize nitrate as an electron acceptor under anoxic conditions. Most denitrifiers are facultative, i.e., under aerobic conditions they can utilize free oxygen as an electron acceptor, and many are capable of fermentation reactions in the absence of both oxygen and nitrate (Delwiche and Bryan, 1976).

Most sources in the literature represent the reduction of nitrate as proceeding via one or more of the following four steps:

$$NO_3 \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

This over simplifies the real situation in which nitrate can be reduced by a number of pathways, terminating in any one of the products listed above, and involving a single, or a whole variety of species of denitrifying organism at any particular stage or stages. There is also some evidence that NO_2 gas is released during denitrification, possibly as an alternative electron acceptor to NO_2^- in

the initial reduction step of nitrate (Burton et al., 1993b). A more accurate summary of denitrification is presented in figure 2.2.

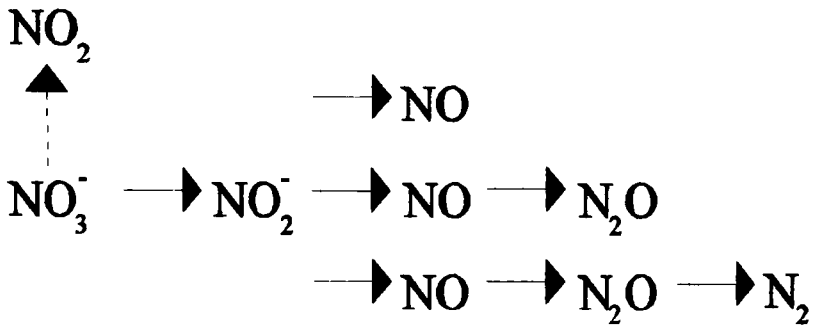


Fig 2.2, The multiple pathways of denitrification

N.B, Broken lines correspond to pathways requiring further investigation.

2.6.1.1 Inhibition

The enzymes involved in denitrification are susceptible to inhibition by a variety of compounds, other than oxygen. The precise modes of action by which the inhibitors take effect are still uncertain. Knowles (1982) lists some of the common inhibitors of denitrification including sulphide, cyanide and acetylene. Acetylene is of interest because it inhibits the reduction of nitrous oxide to di-nitrogen. This feature has been exploited by some workers (e.g., Ryden & Dawson, 1982) for the measurement of di-nitrogen emissions; although other workers have doubted the accuracy of this technique (Burton et al., 1993a).

Nitrite in high concentrations is able to inhibit nitrate reduction (Focht & Chang, 1975). At lower concentrations (below 130 mg l^{-1} of NO_2^--N), Almeida et al. (1995) found the opposite to be true: Nitrate inhibits nitrite reduction because it is able to out-compete nitrite for common biochemical pathways. Earlier workers (reviewed in Focht & Chang, 1975) explained this observation as nitrite and nitrate reductions occurring at different redox potentials. The vagueness of this explanation, together with the advances in biochemical techniques over the last twenty years, would seem to make the Almeida et al. (1995) explanation the more plausible one.

2.6.1.2 Nutritional requirements

In order to reduce nitrate ions, denitrifiers require a substrate to act as a source of electrons. In the case of agricultural wastes, this is the carbonaceous material which is, therefore, oxidised by the denitrifiers in reducing nitrate (Barnes & Bliss, 1983). Most denitrifiers may therefore be termed heterotrophic, and the amount of nitrogen they denitrify is linearly dependent on the concentration of carbon source in the medium (Mateju et al., 1992). Some denitrifiers however, e.g., bacteria of the genera *Paracoccus, Thiobacilus* and *Thiosphaera*, are able to undergo autotrophic denitrification using hydrogen, carbon dioxide or reduced sulphur compounds as energy sources. Their denitrifying activity is therefore independent of organic carbon substrate concentration (Mateju et al., 1992). However, the importance of maintaining an adequate organic substrate for denitrification indicates that, in waste treatment, heterotrophic denitrifiers are more significant than autotrophic ones.

2.6.2 Effect of environmental conditions

2.6.2.1 Dissolved oxygen

The general assumption regarding the effect of oxygen on denitrification, is that it represses nitrogen oxide reductases, and hence denitrification (Knowles 1982). Voβwinkel et al. (1991), for instance, report that the N_2O-reductase enzyme of a *Pseudomonas aeruginosa* strain appeared to be particularly susceptible to low concentrations of oxygen and its formation required strict anaerobiosis. Also, Prakasam & Loehr (1972) found that strict anaerobic conditions were necessary to achieve complete denitrification of oxidized nitrogen in poultry waste, whilst many standard texts on agricultural waste treatment (e.g., Hobson & Robertson (1977)) suggest periods of non-aeration in order to reduce the nitrate concentration.

However, there are instances of denitrification having been reported in aerated systems (Murray et al., 1975). Krul & Veeningen (1977), working with a number of denitrifying bacteria isolated from activated sludge and drinking water, found a range of results whilst testing for the synthesis of nitrate reductase enzyme under aerobic conditions. Whilst the synthesis of this enzyme was completely repressed in some bacteria, in others, there was an almost total non-repression by oxygen. Voets et al. (1975) found that intensive nitrate reduction in highly nitrogenous wastes only occurred under oxygen deficient conditions. However, nitrite reduction took place at a considerable rate under both anaerobic and aerobic conditions.

Although the work of Krul & Veeningen (1977) gives some biochemical evidence that aerobic denitrification is possible, non-repression of nitrate reductase provides no hard, direct evidence, e.g., it fails to distinguish assimilatory from dissimilatory denitrification. The production of

nitrogen containing gases would, however, provide more direct evidence. A number of workers have produced this evidence. For example, Davies et al. (1989), using *Paracoccus denitrificans* and *Pseudomonas aeruginosa*, detected N_2 and N_2O in media containing DO concentrations of 100 μM. Also Burton et al. (1993b), working on pig slurry aeration, reported the evolution of N_2O (640 vpm), NO (70 vpm) and NO_2 (10 vpm) gases for a four day treatment time and aeration to a redox potential of -50 mV E_{cal}. The ability of some denitrifiers to undergo aerobic denitrification has implications for simultaneous nitrification-denitrification (see section 2.6.3).

2.6.2.2 pH

The rate of denitrification is pH sensitive with an optimum around 6.5 to 7.5 (Barnes & Bliss, 1983). This optimum varies with the organism, nitrate concentration and age of culture (Delwiche & Bryan, 1976). The overall effect on pH, of a culture undergoing denitrification, is a decrease in the hydrogen ion concentration of the slurry medium due to neutralisation by the hydroxyl ions formed during nitrate reduction. The process replaces almost 50 % of the alkalinity consumed by the nitrification reaction (Barnes & Bliss, 1983). If one considers the stoichiometry of nitrification and denitrification, by comparing the overall nitrification equation below with the denitrification equation on page 23, the figure of 50 % becomes apparent, since six OH^- ion are formed by denitrification for every twelve H^+ ions formed by nitrification:

$$6NH_4^+ + 12O_2 \rightarrow 12H^+ + 6H_2O + 6NO_3^-$$

The activities of nitrogen oxide reducing enzymes are pH dependent. Voβwinkel et al. (1991) report that *Pseudomonas aeruginosa* produced exclusively N_2O above pH 7.5, whereas NO was the main gas produced between pH 6.5 and 7.5 .

In animal waste treatment, the control of pH to achieve denitrification is less important than that of other parameters, such as aeration. For example, Prakasam & Loehr (1972) found that the complete reduction of nitrate nitrogen to zero concentration in nitrified poultry waste, without pH control, was possible within 4 to 6 days for mixed liquors containing 1 to 6% of concentrated solids (provided pH did not reach extremes of 4 and 11).

2.6.2.3 Temperature

Most studies of the effect of temperature on denitrification generally agree that optimum reaction rates occur in the range 30 to 60 ^{0}C (Barnes & Bliss, 1983; Knowles, 1982; Delwiche & Bryan, 1976). Experiments carried out by Fenlon & Robinson (1977), using a semi-continuous treatment plant, showed that the general denitrification reaction rate, in pig waste, increased with temperature by a factor of between 2.2 and 1.8 for every 10 ^{0}C rise in temperature, in the range 15-37 ^{0}C. This corresponds to results reviewed in Focht & Chang (1975).

2.6.3 Simultaneous nitrification and denitrification

Often in waste treatment, large losses of gaseous nitrogen are encountered, despite the use of apparently aerobic conditions. For example, Murray et al. (1975) found nitrogen loss from an oxidation ditch aerated at a rate of 85 mg of O_2 l^{-1} hr^{-1}. Cases such as these may be due to the emission of ammonia, and/or the occurrence of simultaneous nitrification and denitrification. There appears to be two distinct modes by which simultaneous nitrification-denitrification can proceed. The first relates to the physical environment of denitrifying organisms, whilst the second relates to the physiology of certain heterotrophs.

In the first case, an oxygen gradient is set up so that some cells may be at zero DO. This gradient may be set up in the stratification of an oxidation ditch (Murray et al., 1975) or by the generation of micro-environments, as would be encountered in a floc particle in a slurry treatment reactor. Rittmann & Langeland (1985) clearly describe how anoxic zones can be formed in biological floc. The effect in both these situations is to provide zones of low DO (in which denitrification can occur) that coexist with zones of higher DO suitable for nitrification.

In the case of physiological characteristics, it has been found that certain heterotrophic bacteria, notably *Thiosphaera pantotropha*, are able to carry out nitrification and denitrification simultaneously. For example, Kshirsagar et al. (1995), working with activated sludge inoculated with *T. pantotropha*, found that an initial NO_3^--N concentration of 425 mg l^{-1} could be reduced by between 75 and 85 % at a DO concentration of 2.5 mg l^{-1}. The precise mechanism by which these organisms are able to do this are as yet not understood, but they may be able to achieve it by switching between autotrophic and heterotrophic metabolic pathways (Robertson et al., 1988).

In general terms it appears that simultaneous nitrification-denitrification can occur in a process where oxygen (as opposed to nutrient) availability is limited, e.g., as a consequence of low aeration. The main advantage of combining denitrification with nitrification in waste treatment is that it provides a simple solution to the problem of nitrogen removal. Both processes can be carried out in the same vessel leading to increased capacity of treatment facilities (Rittmann & Langeland, 1985).

2.7 Discussion of key points arising from literature review

In section 2.1, a number of questions were raised concerning aerobic treatment and nitrogen transformation processes. Literature reviewed in this chapter has provided the following answers to these questions:

I. Why has aerobic treatment gained importance in recent years and what is its future?

Over the last 50 years, the production of animal wastes in certain areas, together with the associated pollution problems and complaints from the public, has risen. Therefore, there is a need for an effective treatment system to deal with these problems. Continuous aerobic treatment is identified as such a system.

With the numbers of livestock increasing, and the continued change in waste handling practices (favouring slurry production), the future of aerobic processing in reducing the environmental impact of slurry production, seems assured. However, in the case of smaller producers, the amount of profit returned for each animal may not justify the investment in aerobic treatment plant.

II. How does it compare with the alternative processing option of anaerobic treatment?

Continuous aerobic treatment can eliminate odour, pathogens and polluting forms of nitrogen from animal waste. Anaerobic digestion, on the other hand, does not offer control of nitrogen, pathogen control is more limited, odour reduction takes significantly longer and equipment is more complicated and expensive. The advantages associated with aerobic processing therefore offset the higher running costs compared with anaerobic

processing.

III. What are nitrification and denitrification, in terms of their microbiology and biochemistry?

Nitrification is essentially an aerobic, autotrophic process in which slurry ammonium is converted to nitrate by microbial activity. Denitrification is more complex, and in waste treatment the most significant aspects of the process are the anoxic, heterotrophic reduction of nitrate to di-nitrogen gas and various other gaseous oxides of nitrogen. Under certain conditions (e.g., where free oxygen availability is limited, but nutrient availability is not) nitrification and denitrification can occur simultaneously.

The processes of nitrification and denitrification can be controlled in continuous aerobic slurry treatment by the conditions of aeration level, residence time and temperature. However, some of the products may be damaging to the environment, e.g., nitrous oxide.

IV. What conditions favour nitrification and denitrification, and what inhibits them?

The residence time of slurry and oxygen availability are important considerations in continuous treatments. Residence times of 3 days or more are recommended to avoid nitrifier 'washout'. Increasing residence time can also improve the efficiency of mass transfer at low DO concentrations.

The availability of free oxygen is essential to drive nitrification, while in some systems, the presence of free oxygen represses denitrification. Much evidence has been reported, however, regarding the occurrence of denitrification under aerobic conditions. For a 4

day continuous treatment of pig slurry, the optimum redox potential for simultaneous nitrification-denitrification to occur is in the range E_{cal} = -70 to -30 mV. Above this, denitrification is repressed whilst below it, both nitrification and denitrification are repressed.

Temperature affects the metabolic rate of microorganisms, the optimum range for nitrification being 28 to 36 ^{0}C, whilst that for denitrification is 30 to 60 ^{0}C. Therefore, ignoring any practical considerations, temperature should be controlled in a range of 30 to 36 ^{0}C for optimum nitrifying-denitrifying conditions.

The optimum pH ranges for nitrification and denitrification are 8 to 9, and 6.5 to 7.5, respectively. However, in waste-treatment nitrogen control, pH is of less importance since nitrification and denitrification readily occurs outside this range, provided extremes of pH (e.g., 4 and 11) are not reached.

Nitrite and ammonia are potential inhibitors of nitrification, if present in sufficient concentration (e.g., in pure cultures oxidation rates can be inhibited by > 100 mg l^{-1} of NH_4^+-N and > 80 mg l^{-1} of NO_2^--N). In denitrification, nitrite has been observed to inhibit nitrate reduction, whilst at lower nitrite concentrations (e.g., below 130 mg l^{-1} of NO_2^--N) the reverse has been observed, i.e., nitrate inhibits nitrite reduction.

Regarding the extent of knowledge on N_2 measurement in waste treatment systems: no reliable attempts at measuring N_2 were found in the literature for continuous aerobic treatment of animal

wastes. In the area of soil science however, there is a great deal of information on N_2 measurement by acetylene inhibition and stable isotope techniques (discussed in section 4.1.3). The reliability of the former is in doubt, whilst the latter is expensive and requires access to specialist equipment.

3. DESIGN AND CONSTRUCTION OF THE LABORATORY SCALE AERATOR

3.1 Introduction

In order to test the hypothesis in section 1.2, equipment was assembled to contain and control a continuous aerobic process for pig slurry. The configuration selected was one which encouraged simultaneous nitrification-denitrification. This lead to the production of the various forms of nitrogen which would be expected from a nitrogen removal process. Thus, the effect of changing control parameters on the production of these nitrogen forms could be conveniently studied in a single reactor vessel. A prime consideration, therefore, was to design the equipment in such a way that it would facilitate the measurement of the various forms of nitrogen to be included in the mass balance determinations. The following features were considered to be important in designing the apparatus:

1. The equipment should be reliable and straight forward.
2. The volume of treated slurry in the reactor should be sufficient to allow sampling for laboratory analysis.
3. Sufficient headspace above the liquid should be allowed, within the reactor, to enable control of foaming and collection of gases.
4. The vessel should be completely air tight to avoid any ingress of atmospheric air which would interfere with the analysis of microbially produced N_2.
5. Conditions which affect nitrifying and denitrifying activity, such as aeration level, residence time and temperature, should be controllable so as to maintain an environment which is suitable for simultaneous nitrification-denitrification.

3.2 Choice of configuration and scale

The choice of process configuration was based on a single stage, Continuously Stirred Tank Reactor (CSTR). This involves the regular addition and removal of slurry from the aeration vessel, which is maintained at an approximate steady state. This type of process is easily controlled and provides a consistent level of treatment of slurry, compared with batch processes (Burton, 1992), thus allowing the maintenance of a nitrifying-denitrifying environment.

Simplicity and cost dictated that experiments be carried out at the laboratory scale. Collection and analysis of samples of N_2 and NO_x gases were greatly simplified and considerably cheaper at the laboratory scale, the working volume of slurry selected being a nominal 10 litres. However, the size still contained a sufficient volume of slurry, from which suitable volumes of liquid samples could be withdrawn for laboratory analysis. The overall reactor vessel volume was 20 litres. This left 10 litres of headspace available for foam control.

3.3 Control parameters affecting the treatment environment

3.3.1 Residence time

To encourage nitrification a residence time of 4 days was chosen, thus avoiding the problem of nitrifier 'washout' - as encountered with treatments below 3 days. For a 10 litre working volume, a 4 day residence time was achieved with a mean slurry flow rate of 100 ml per hour.

3.3.2 Aeration requirements

3.3.2.1 Aeration level

Aeration level is an important consideration when designing the treatment process since this

provides the oxygen input that brings about aerobic breakdown of organic matter, and oxidation of ammonium nitrogen to nitrate. Controlling aeration level is also important if conditions of simultaneous nitrification-denitrification are required, since too high an input of O_2 may inhibit denitrifiers. A minimum rate of aeration is required however, and the amount of air which needs to be supplied in order to achieve this can be calculated from empirical observations of O_2 utilization by COD and nitrogen oxidation.

3.3.2.2 Air supply

Typical values of COD breakdown and raw slurry total solids (TS) used in the aerator are:

$$\Delta COD = 13 \text{ g } l^{-1} \text{ (Burton, 1992)}$$

$$TS = 30 \text{ g } l^{-1}$$

Total nitrogen content of slurry is approximately 7% of TS (Burton, 1992), therefore,

$$N_{tot} = 0.07 \text{ x } TS$$

$$= 0.07 \text{ x } 30$$

$$= 2.1 \text{ g } l^{-1}$$

of this, the actual oxidisable nitrogen content makes up around 50%. Therefore the oxidisable nitrogen concentration of a typical raw slurry is, $N_{ox} = 1.0 \text{ g } l^{-1}$. The stoichiometry of the nitrification process can be represented as,

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$$

Therefore, the mass ratio of O_2 required to oxidise 1 mole of ammonium nitrogen is 64/14, i.e.,

$$4.6 \text{ g of } O_2 \text{ per } 1 \text{ g of } NH_4^+\text{- N}$$

Therefore, the mass of O_2 per litre of slurry required to oxidise 1.0 g of N_{ox} is,

$$4.6 \times 1.0 = 4.6 \text{ g } l^{-1}$$

For a COD requirement of 13 g l^{-1}, the overall O_2 requirement is 17.6 g l^{-1}. At standard temperature and pressure, the number of moles of gas contained in 1 litre of air is 0.0416 moles, 21% of which are O_2. Therefore, 1 litre of air contains 0.28 g of O_2.

The volume of air required to oxidise 1 litre of slurry is therefore 17.6/0.28 = 63 litres. For a 4 day residence time, 1 litre of slurry is fed over a 10 hour period. Therefore, the minimum flow rate of air should be 6.3 l hr^{-1}, i.e., 0.10 l min^{-1}, assuming that all the O_2 entering the aerator is utilized.

3.3.3 Temperature

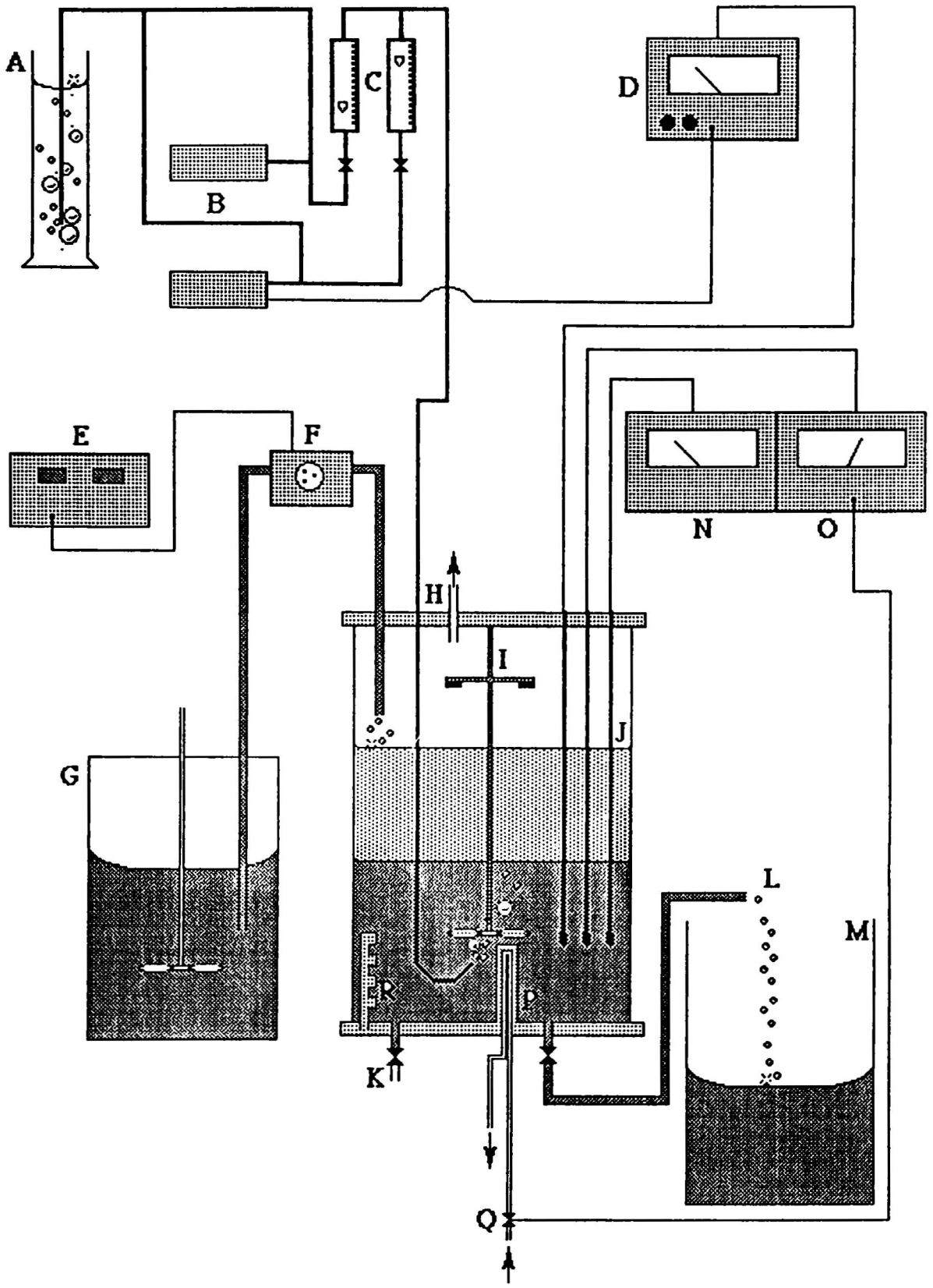
The temperature of the slurry was maintained in the mesophilic range, at 25 ± 2 ^{0}C. This was a compromise between the expected optimum temperature of simultaneous nitrification-denitrification (30 to 36 ^{0}C) and the influence of ambient temperature in the laboratory which prevented the temperature inside the reactor from rising above 30 ^{0}C. Operating the reactor at this temperature did not, however, appear to greatly retard the activity of nitrifiers and denitrifiers in the slurry.

3.4 Construction of aeration system

3.4.1 General description

Plate 1 shows the reactor and ancillary equipment, which is detailed in figure 3.1. The vessel consisted of a 500 mm length of glass QVF tubing, internal diameter 225 mm and nominal volume 20 litres. To the flanges around the top and bottom of the QVF, were bolted a stainless steel lid and base. These were sealed by rubber gaskets and silicone sealant. It was important to make the vessel gas tight, so as to avoid any ingress of atmospheric air during N_2 flushing experiments (described later). Projecting from the base of the vessel, a stainless steel cooling finger **[P]** sat within the vessel contents. Cooling water entered and exited this via two external ports. Also located in the base was a ball valve sampling port **[K]**, and a port connected to an 'S' shaped 0.5" diameter HDPE tube of height 550 mm **[L]**. This tube was an overflow for the treated slurry, and maintained the vessel contents at constant weight without the need for any drain pump. Ports in the lid accommodated probes **[J]**, feed pipes for raw slurry and aeration air, and an outlet tube for the sampling of headspace gases **[H]**.

In the centre of the lid sat a gas tight bearing housing for the impeller shaft. The impeller shaft consisted of a 455 mm long, stainless steel round bar, of diameter 0.5". Located 120 mm from the top of the shaft was a mechanical foam breaker **[I]**. This was made from a 116 mm diameter ABS plastic disc with four stainless steel blades fixed beneath it (plate 2 and Appendix IV for diagram). Bubbles in the foam were broken by high shear forces generated by the foam breaker, and impaction of the foam, at high velocity, against the walls of the reactor, where it trickled back down into the bulk liquid slurry.



A	Water column vent for surplus air
B	Air pumps for high and low aeration rates
C	Air flow meters and valves
D	Redox meter and controller for high flow air pump
E	Time controller for slurry feed pump
F	Slurry feed pump
G	Raw slurry tank
H	Off gas sample port
I	Impeller shaft, incorporating mechanical foam breaker
J	pH, redox and temperature probes
K	Slurry sample port
L	Outlet pipe for treated slurry
M	Treated slurry tank
N	pH meter
O	Temperature meter and controller for cooling water
P	Cooling finger
Q	Water supply and solenoid valve for cooling finger
R	Baffle

Fig. 3.1, Diagram of aerator components

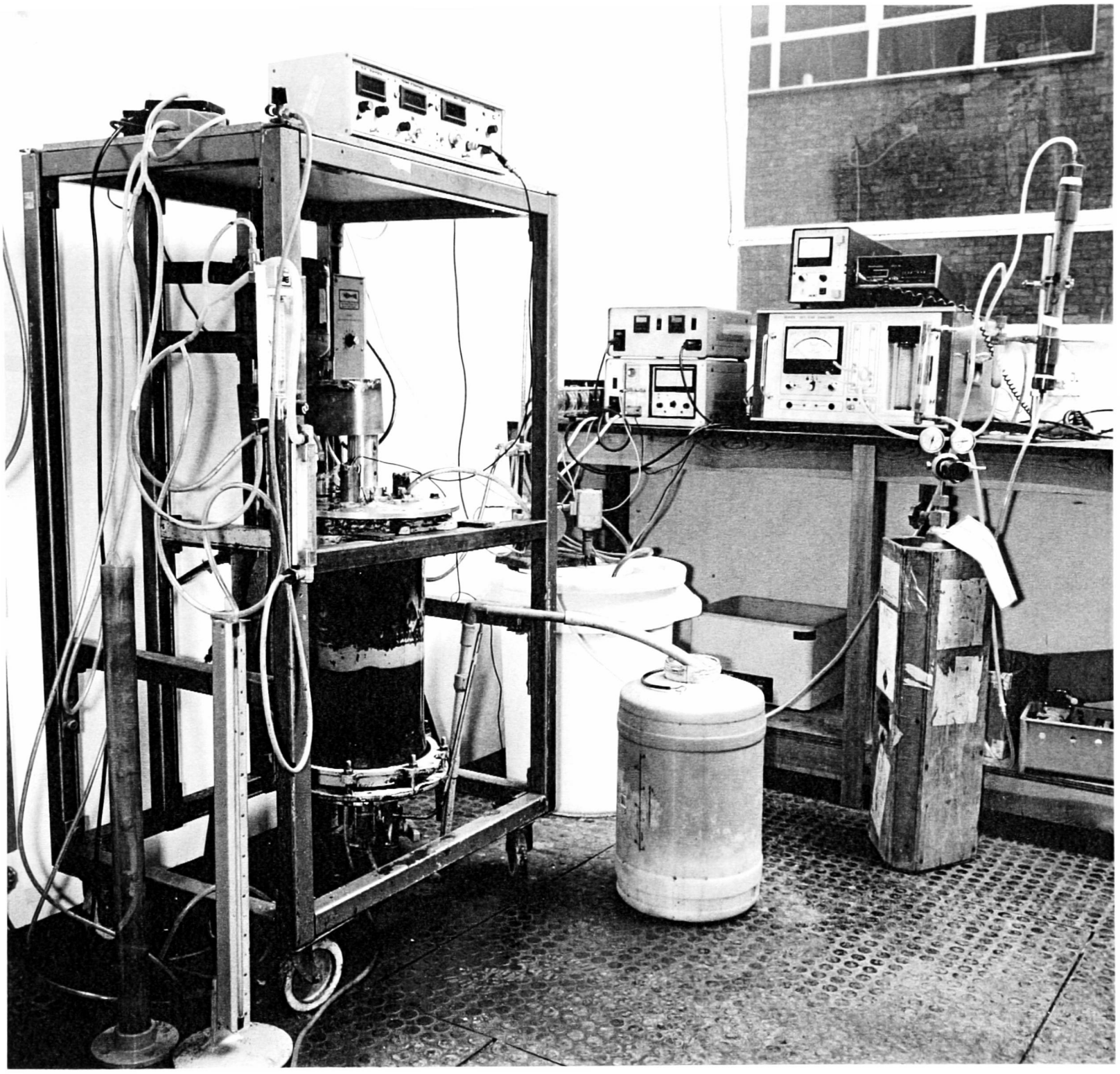


Plate 1, Laboratory scale aerator and ancillary equipment

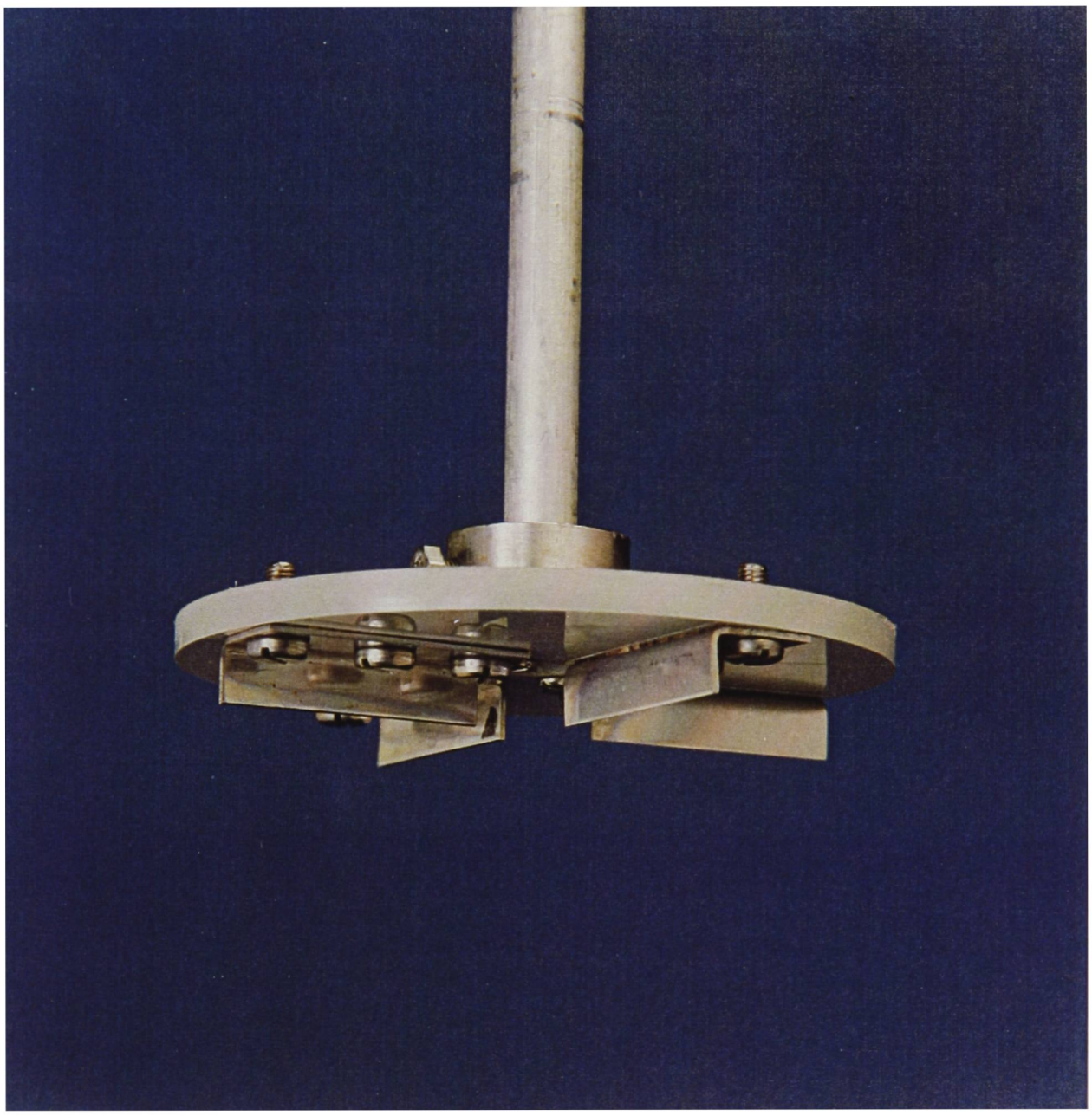


Plate 2, Mechanical foam breaker

The whole arrangement, with a motor to drive the impeller (at a speed of 800 rpm), was located in a steel frame. The motor (NECO variable speed, single phase direct current, 187 watts) was offset 180 mm between the bearing housing and the back of the frame. This afforded easier access to the probes from the vessel lid, easier removal of the lid itself and also avoided the problems of direct couplings. The motor drove the impeller by a simple pulley and belt drive.

3.4.2 Provisions for aeration

Aeration was provided by two, 240 V ac diaphragm pumps **[B]** with a maximum individual flow rate of 3 l min^{-1}. One of the pumps could be switched on or off, according to the redox level of the slurry (see section 3.5) to provide control, whilst the other was on continuously. The flow rate of air from each pump could be controlled manually using a gap meter with a needle valve (Platon Flowbits Ltd., Basingstoke, UK) **[C]**. Excess air, generated when the needle valves were partially or fully closed, was vented through a water column **[A]** (head depth 700 mm) to generate a back pressure. Air was fed, via the reactor lid, through a rigid pipe to a depth of 470 mm (just below the impeller). This arrangement was easier to dismantle and clean than a floor mounted sparger.

3.4.3 Slurry feed

To maintain a 4 day residence time, necessary for nitrifying conditions, an accurate and reliable feed system was required to deliver the raw slurry into the reactor. The system employed was a peristaltic pump (Model 5025, Watson and Marlow Ltd.) **[F]** controlled by a digital, dual time switch **[E]**. This allowed the accurate control (in minutes and seconds) of the pumping duration,

and the interval between pump activations. The pumping duration was calibrated so as to deliver 100 ml of raw slurry every hour for residence times of 4 days, 50 ml per hour for 8 day residence times, and 100 ml every half an hour for 2 day residence times.

3.4.4 Mixing

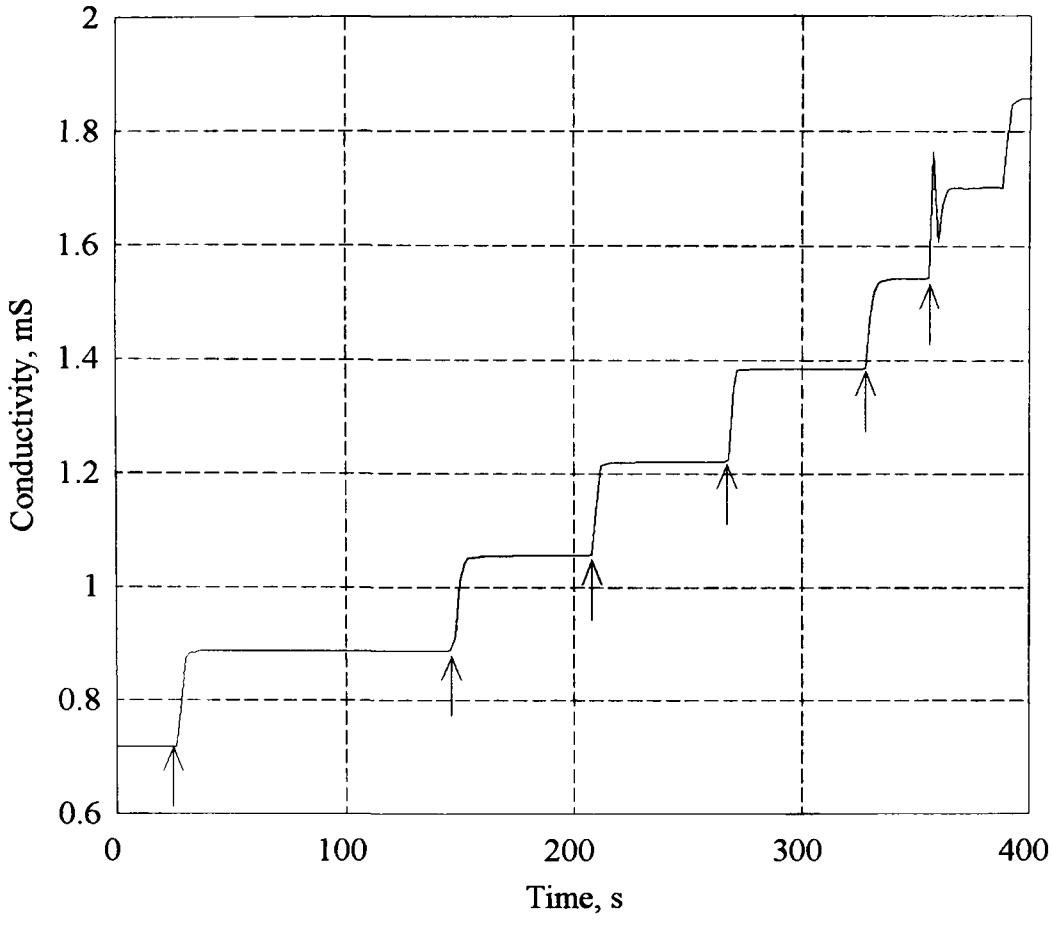
Mixing was achieved with a stainless steel Convex Bladed Mixed Flow (CBMF) impeller (see plate 3), mounted on the end of the impeller shaft. The CBMF impeller was chosen because it combined the gas dispersing power of a Rushton turbine, with the solids mixing ability of a marine type propeller (i.e., it suspended and uniformly distributed insoluble solids, whilst homogenising the liquid fraction). The design was based on that of Niranjan et al., (1990).

3.4.4.1 Mixing experiment

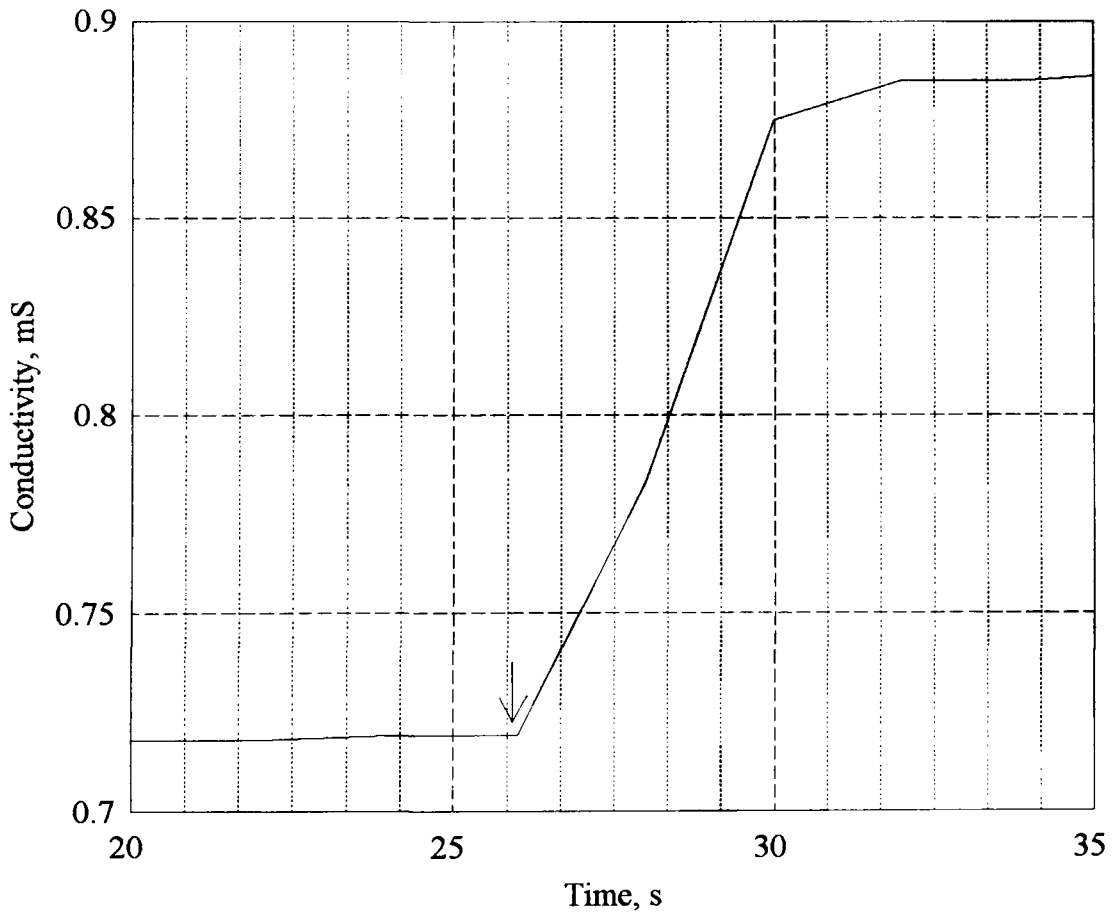
Although the efficiency, in terms of power consumption and running cost, were not of great importance to the project, the need for a well mixed system was essential. This was tested by determining the mixing time of a salt tracer in the reactor. The reactor was filled with 10 litres of water and the impeller started at a speed of 400 rpm. A conductivity probe was positioned in the water at half the depth and one third of the radius as measured from the circumference. 5 ml aliquots of a sodium chloride solution (200 g l^{-1}) were pipetted into the reactor at intervals, and the resulting changes in conductivity recorded (see graphs 3.1 and 3.2).

The arrows on graphs 3.1 and 3.2 indicate additions of sodium chloride solution. Mixing time was taken as the time for conductivity to increase from one plateau to the next. The steepness of the slope between plateaus, in graph 3.2, indicates that the mixing time is 6 seconds.

Plate 3, Convex bladed mixed flow impeller



Graph 3.1, Effect of mixing on a salt tracer



Graph 3.2, Enlargement of first plateau

The mixing time is sufficiently short (relative to the feed cycle time of 15 minutes and the residence time of 4 days) to indicate a well mixed system. When the impeller was used in slurry, the speed was doubled to 800 rpm. The purpose of this was, primarily, to increase the velocity of the foam breaker, as 400 rpm did not lead to sufficient foam destruction. However, a secondary effect of this was to further improve mixing. Additionally, a baffle was included in the reactor design to aid mixing by encouraging turbulent flow of the slurry.

3.5 Control and monitoring of environmental parameters

3.5.1 Redox potential

The parameter of redox potential was chosen for monitoring and controlling aeration. Redox offers greater reliability, compared with dissolved oxygen, in measuring the effect of the low levels of aeration necessary for simultaneous nitrification-denitrification. The equipment used to monitor and control redox potential was centred upon an indicator/control unit, Model 40/144R (supplied by PHOX Systems Ltd., Shefford, Bedfordshire, UK) **[D]**. This could measure a range of -700 to +700 mV, with a discrimination of 5 mV. Output could be monitored from an analogue display or from a millivolt output socket. The redox electrode used was a Ag/AgCl DIP electrode, model 9126 (PHOX Systems Ltd.). The calibration of the electrode was made using Zobell's solution, this consisting of the following components made up to 1 litre with deionised water:

Potassium Chloride (KCl)	7.4560 g
Potassium Ferricyanide ($K_3Fe(CN)_6$)	1.0975 g
Potassium Ferrocyanide ($K_4Fe(CN)_6.3H_2O$)	1.4080 g

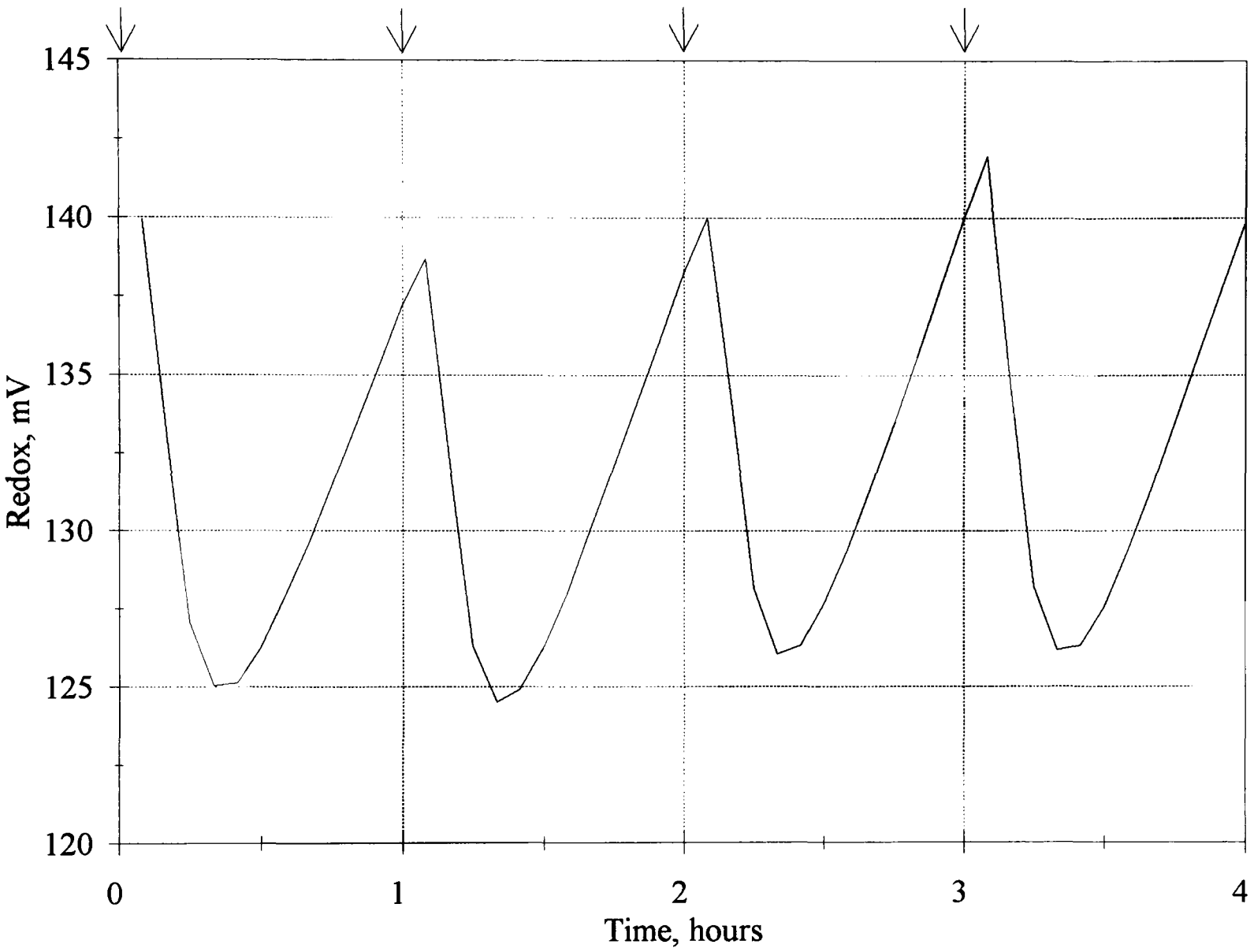
The redox potential of this solution is +235 mV for a Ag/AgCl electrode (Jacob, 1970).

3.5.1.1 Control of aeration

The set point controller could be used to energise or de-energise a relay controlling a 240 V ac supply to one of two diaphragm air pumps **[B]**. Control was achieved by allowing one of the diaphragm pumps to pump air continually at a low flow rate, whilst the other was activated by the redox controller when the redox fell below a certain point (+50 mV was found to be appropriate for conditions favourable for simultaneous nitrification-denitrification).

The intermittent pump, controlled by redox, was set between 1.0 and 2.0 l min^{-1}. Initially, the continuous pump was set to give a flow rate of 0.10 l min^{-1} (as calculated in section 3.3.2.2). In practice, this air flow rate was found to be insufficient to bring about the required conditions. Increased aeration demands result from a variety of factors including the amount and nature of microbiological floc, degree of agitation and temperature. A flow rate of around 0.4 to 0.6 l min^{-1} was found to yield suitable conditions for simultaneous nitrification-denitrification, corresponding to a utilization of between 15 and 25 %.

This arrangement of air pumps allowed a constant stream of air to meet the main part of the aeration demand. When the system lacked aeration (indicated by a fall in redox below set point) then the control pump was automatically switched on to boost the aeration and return redox back above set point. Redox was seen to cycle (within acceptable limits) in the reactor, an example of which, for a 4 day residence time, is shown in Graph 3.3 (raw slurry was added to the reactor at the start of each hour). From this graph the redox can be seen to fall some 15 mV after the addition of slurry, before it begins to rise again. This can be explained by the time taken for the aeration system to respond to the extra load of raw slurry.



Graph 3.3, Redox cycle

Note: Arrows indicate additions of raw slurry

3.5.2 Temperature

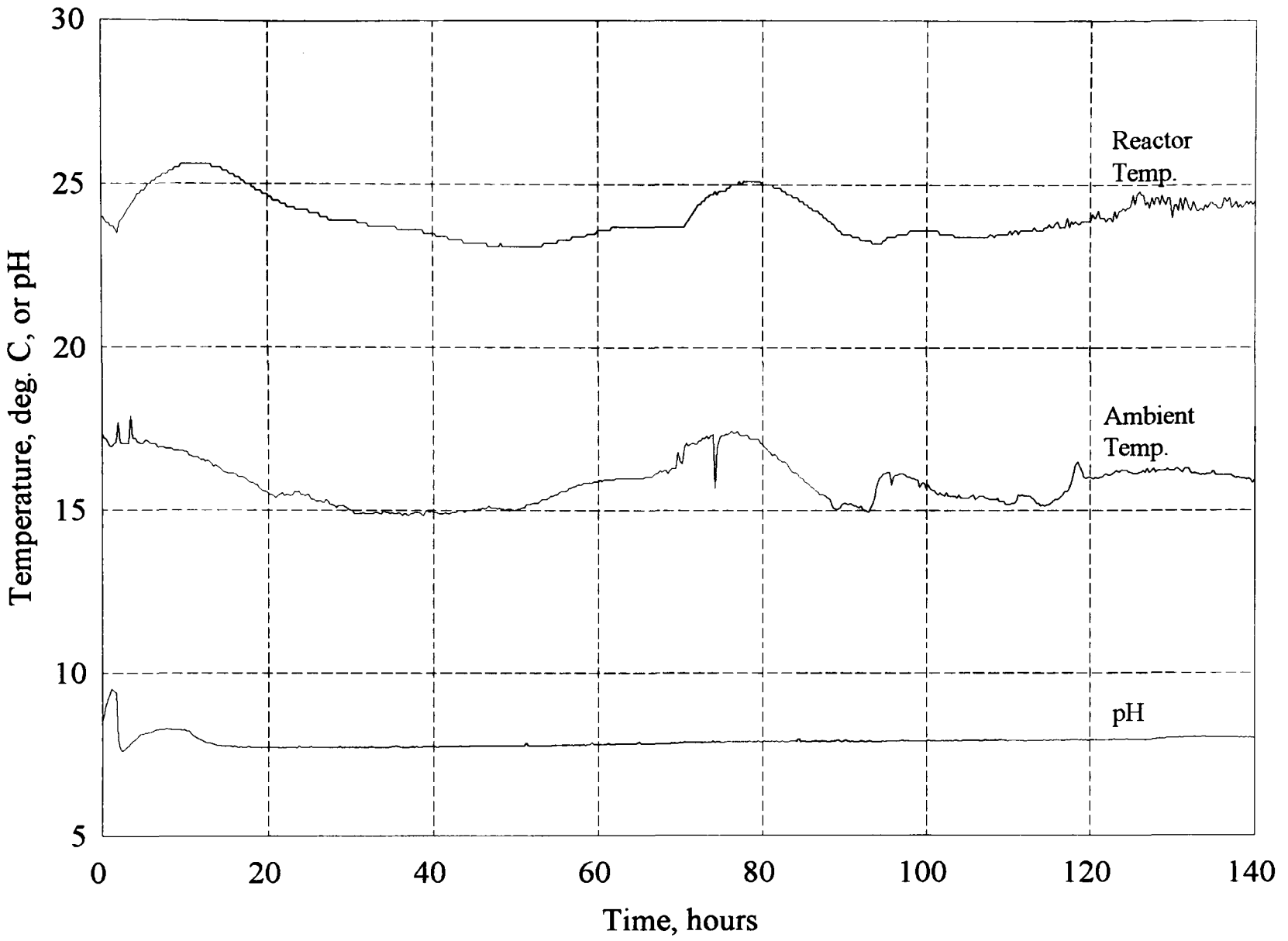
Temperature was monitored in the reactor with a simple probe made from a thermistor sealed into a 10 mm (outside diameter) x 460 mm length of stainless steel tubing. The probe was connected to a temperature meter/set point controller **[O]**. The output from the set point controller activated a 240 V ac solenoid valve (Series 410, KV Automation, Milton Keynes, UK) **[Q]**. This controlled the supply of cold water from a constant head tank to the reactor cooling finger **[P]**. A constant head tank supplied cooling water, thus avoiding the problem of high pressure associated with water taken directly from the mains.

The aerated slurry temperature was generally maintained within ± 2 ^{0}C of the 25 ^{0}C set point. However, the temperature control (cooling finger) was less precise during hot weather leading to a wider band of ± 4 ^{0}C. This was the consequence of a slow response time arising from inadequate capacity of the cooling finger. The poor performance of the cooling finger, as a device for temperature control, is demonstrated in graph 3.4 where ambient temperature clearly exerts an influence on the reactor temperature.

3.5.3 Other monitoring equipment

3.5.3.1 Gases: nitrous oxide, carbon dioxide and oxygen

Gases were monitored, via the outlet tube **[H]**, for CO_2 and N_2O using infra red analyzers (ADC, Hoddesdon, UK), and O_2 using a Makareth type electrode (Neotronics Ltd.).



Graph 3.4, Reactor temperature and pH

Note: Data presented was obtained from a medium aeration, 4 day treatment.

3.5.3.2 pH

pH was monitored with a double junction gel filled electrode (Whatman International Ltd., Maidstone, Kent, UK). The electrode was epoxy bodied and of sturdy construction for robustness and maintenance free operation.

pH was not controlled, but was still a useful parameter to monitor. A fall in pH to values in the range 6.0 to 7.5, indicates that nitrification and denitrification activity is occurring, because of the increased proton concentration (arising from ammonium oxidation) in the slurry environment. Also, stability of pH is a more direct and reliable indicator of steady state nitrification-denitrification conditions than redox. Graph 3.4 shows a typical pH response from the reactor. Except for an early peak (due to a blocked air pipe) the pH remained at a constant 6, over the four day period, confirming satisfactory nitrifying-denitrifying conditions.

It should be stressed that pH measurement is, at best, simply a guide to the state of nitrifying-denitrifying activity. The most reliable information can only be obtained by taking actual measurements of the different forms of nitrogen encountered during aerobic treatment, and this forms the basis of part B of the thesis.

PART B

Experimental Work

4. MEASUREMENT OF HEADSPACE DI-NITROGEN GAS

4.1 Possible methods for measuring N_2

4.1.1 Introduction

In order that a complete nitrogen mass balance can be made around the laboratory scale reactor, it is essential that measurements are taken of the N_2 produced by denitrification activity. In the past, there have been few attempts in animal waste research to quantify N_2. This is due to the large amount of N_2 present in the atmosphere, which poses a problem in trying to obtain an accurate measurement of N_2 produced by denitrification. In the sphere of soil research, however, there has been much effort to try and quantify denitrification derived N_2. The techniques developed were not considered appropriate to the work of this project, and the reasons for this are now discussed.

4.1.2 Use of acetylene inhibition

This involves partial inhibition of denitrification by the addition of 0.5% acetylene to aeration air. Any N_2 which would have been produced by microbial activity, is instead released as the more easily measured N_2O. Even allowing for the safety hazards associated with acetylene/air mixes, concerns have been expressed about the value of the acetylene inhibition technique. Burton et al. (1993a), used a method based upon the work of Ryden et al. (1982), but were unable to produce conclusive results beyond confirming that N_2 is released during denitrification. The technique is also prone to error: Yeomans and Beauchamp (1982) found acetylene enhanced denitrification rates, possibly due to its utilisation as a carbon substrate. Porter (1992) found that 2.68% (v/v) acetylene could inhibit NH_4^+

oxidation by 90 % in soil. However, Walter et al., (1979) found that as little as 0.1% (v/v) was sufficient to completely inhibit nitrification in soils. Porter (1992) failed to explain the lower sensitivity of inhibition in the more recent study: It seems that the soil used had been dry stored for two years prior to use. This may have encouraged mechanisms of acetylene decomposition that were absent in the earlier study.

4.1.3 Use of stable isotopes

The possibility of using stable isotopes was also considered, in the light of work by Pruden et al. (1985) on ^{15}N measurement in soil. This would have involved dosing raw slurry with an ^{15}N labelled ammonium compound, and attempting to measure ^{15}N by mass spectrometry in the N_2 collected from the reactor headspace. The technique was considered impractical because of the excessive dilution of labelled N_2 by aeration air, and the high cost of ^{15}N labelled compounds.

4.1.4 Use of nitrogen free air

In the light of the problems discussed in 4.1.2 and 4.1.3, a new technique had to be developed. The method proposed was one of gas flushing to eliminate atmospheric N_2 from the reactor headspace. It did not suffer any of the above limitations, mainly because manipulation of biochemical pathways were not involved, as is the case with acetylene inhibition, or the use of isotopes. Microbially derived N_2 was collected by, first flushing atmospheric N_2 from the headspace of the aerator with a mixture of inert gas and oxygen. An 80:20 (v/v) argon:oxygen gas mixture was used: The argon effectively replaced headspace di-nitrogen, whilst oxygen maintained the aerobic environment in the slurry.

Secondly, the headspace gas (from which atmospheric N_2 had been flushed) was collected and analysed by mass spectrometry to determine the concentration of denitrification derived N_2.

Before samples of headspace gas could be collected an accurately analysed for N_2, two questions needed to be answered: Firstly, approximately how much nitrogen free air must be flushed through to reduce atmospheric N_2 in the reactor to a level where microbially derived N_2 can be accurately measured? Secondly, which is the most reliable form of sample gas containment in terms of avoiding contamination through ingress of atmospheric N_2? The first question was answered by the development and validation of a mathematical model to describe the nitrogen flushing process (section 4.2), whilst the second question was answered by collecting gas samples in different containers and analysing the contents by mass spectrometry (section 4.3)

4.2 Development of flushing technique by use of a modelling approach

4.2.1 Model derivation

In considering the loss of di-nitrogen from the reactor during flushing, there are two main factors of importance (refer to figure 4.1). These are firstly, the rate of emission of N_2 into the headspace from microbial activity (Q_n, l min^{-1}), and secondly, the amount of atmospheric N_2 (of concentration C, % v/v) removed from the headspace. C is dependent on the flow rate of the flushing gas through the aerator (Q, l min^{-1}).

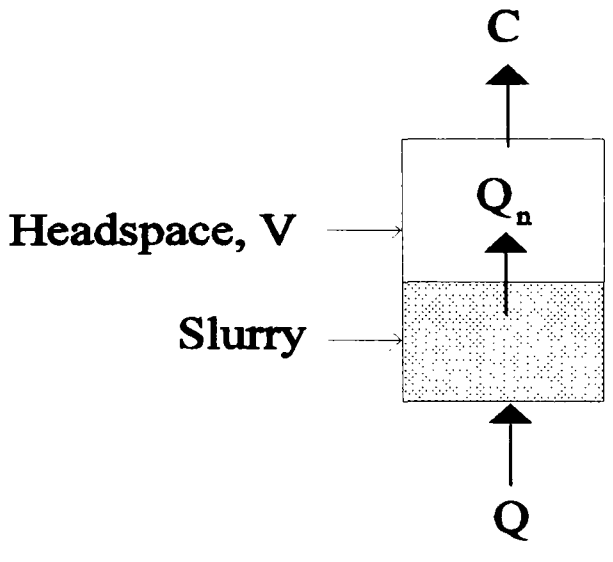


Fig. 4.1, Summary of factors to be included in model

The decrease in concentration of N_2 at time, t, for an aerator with headspace volume, V, can be expressed as:

$$\delta C = \left(\frac{Q_n - QC(t)}{V} \right) \delta t$$

Assuming that Q_n is constant, and that at time $t = 0$, $C = C_o$ (initial N_2 concentration in air),

$$\int_{C_o}^{C} \frac{dc}{(Q_n - QC)} = \int_{o}^{t} \frac{dt}{V}$$

$$\left[-\frac{1}{Q} \log_e (Q_n - QC) \right]_{C_o}^{C} = \frac{1}{V} \left[t \right]_o^t$$

$$-\left[\log_e(Q_n - QC) - \log_e(Q_n - QC_o)\right] = \frac{Qt}{V}$$

$$-\log_e \frac{(Q_n - QC)}{(Q_n - QC_o)} = \frac{t}{\theta}$$

Where $\theta = \frac{V}{Q}$ = residence time of gas in headspace (mins)

$$\frac{Q_n - QC}{Q_n - QC_o} = e^{-\frac{t}{\theta}}$$

$$C = \frac{1}{Q}\left\{Q_n - (Q_n - QC_o)\, e^{-\frac{t}{\theta}}\right\}$$

$$C = \frac{Q_n}{Q}\left(1 - e^{-\frac{t}{\theta}}\right) + C_o e^{-\frac{t}{\theta}} \qquad (4.1)$$

$\frac{t}{\theta}$ is equal to the number of headspace volumes to be flushed. The first term (on the RHS of the equation) refers to the N_2 emitted from the slurry by denitrification, and the second term refers to the residual N_2 from the air.

Equation (4.1) enables the total N_2 concentration present in the headspace, C, to be calculated after

a given number of headspace volumes of gas have been passed. In practice, to calculate the volume of flushing gas required to reduce the atmospheric N_2 in the reactor from 79 % by 6 orders of magnitude (i.e., to 0.8 ppm), it is not necessary to know the value of Q_n. This is because the second term alone, in equation (4.1), refers to the residual N_2 from the atmosphere. Therefore, to reduce the N_2 from 79 % to 0.8 ppm would theoretically need 13.8 headspace volumes of flushing gas. At this point, any N_2 released from the slurry could be easily distinguished.

The model assumes that the system is well mixed. This is certainly the case in the headspace and slurry. However, it is unlikely to be the case in the foam layer. This would not be disadvantageous though, as gas flow through the foam layer will be a plug flow, hence the time taken to flush atmospheric N_2 from this zone of the aerator may well be faster than that of the headspace. The model also assumes no ingress of atmospheric N_2 from outside the reactor. This is an important point to note, since the possibility of atmospheric N_2 ingress will increase as the residual N_2 concentration inside the reactor falls. It is essential therefore, that the reactor is made sufficiently gas tight to allow for this.

4.2.2 Model validation

4.2.2.1 Introduction

In order to test the model with the equipment available, an alternative to N_2 flushing had to be sought, given the absence of mass spectrometry equipment at Silsoe Research Institute. The approach adopted was to introduce N_2O gas into the reactor and measure the decay in N_2O concentration, with

an Infra Red analyzer, as the headspace was flushed with atmospheric air. From the data produced, the number of headspace volumes, $\frac{t}{\theta}$, required to reduce the N_2O concentration by 10% (one order of magnitude) could be determined and compared with the theoretical value.

4.2.2.2 Experimental method

The reactor was set up with approximately 10 litres of slurry, which had been aerated for a period of 1 week. N_2O produced by denitrification was not measured in the headspace. N_2O gas, of concentration 1000 ppm, was flushed into the reactor (headspace volume approximately 10 litres), through the air input lines, until the concentration in the effluent gas reached a value between 500 and 1000 ppm (as measured by the infrared analyzer (ADC, Hoddesdon, UK)). This was taken as the value of C_0. N_2O was then replaced with atmospheric air at a flow rate of 2 l min^{-1} and the decay in N_2O concentration monitored from the IR analyzer. The experiment was carried out in duplicate.

4.2.2.3 Results

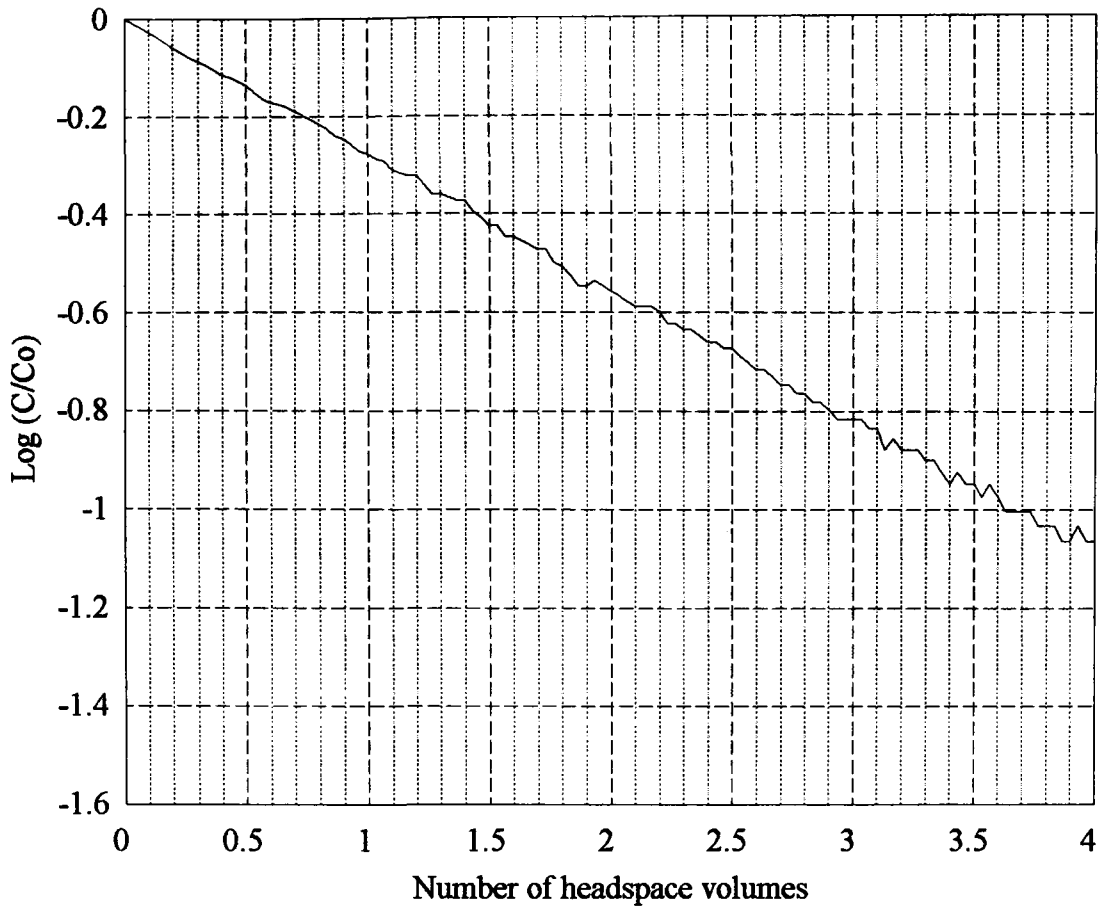
The N_2O concentration data were plotted as $\log\frac{C}{C_0}$ versus the number of headspace volumes, $\frac{t}{\theta}$ (see graphs 4.1 and 4.2). From these plots, the number of headspace volumes of air which caused a 10-fold reduction of the N_2O concentration could be simply read, since a fall to 10 % of the original concentration corresponded to an interval of -1 on the log scale. The results are presented for comparison in table 4.1.

	Experiment 1	Experiment 2	Model prediction
C_0, ppm	750	540	-
$\frac{t}{\theta}$	3.65	1.65	2.30

Table 4.1, Number of headspace volumes of gas causing a 10-fold reduction of headspace N_2O concentration (experimental values compared with theoretical value)

4.2.2.4 Discussion

The two experimental values are comparable with the model value (mean = 2.65 litres), which suggests that the model can provide a reliable estimate of the amount of flushing gas required to lower atmospheric N_2 levels in the reactor. Therefore, to reduce the atmospheric N_2 concentration by 6 orders of magnitude would require: 6 x 2.3 = 13.8 headspace volumes of flushing gas, equivalent to 138 litres. At a gas flow rate of 2.0 l min^{-1}, this would take 69 minutes to achieve, whilst at 0.6 l min^{-1} it would take 230 minutes. The variation between the predicted and experimental values may be due to differences in the volume of slurry in the reactor giving rise to variable headspace volumes. The cause of this variation was traced to poor design of the overflow pipe which maintained the quantity of slurry in the reactor. Improvements to the design of the overflow pipe (particularly regarding elimination of blockages and recalibration to ensure, more exactly, that 10 litres of slurry were held in the reactor) effectively removed the problem of variable slurry volume.



Graph 4.1, Model validation (experiment 1)

Graph 4.2, Model validation (experiment 2)

4.3 Sample containment

4.3.1 Introduction

It was necessary to take samples of headspace effluent gas to a separate site (the School of Chemical Engineering, University of Birmingham) for analysis by mass spectrometry. Reliable sample gas containment was required, which would avoid contamination through ingress of atmospheric N_2. Indeed, gas sample containment proved to be a key factor in the work on N_2 determination.

Containers were tested by filling 3 replicates, of each type, with nitrogen free air (containing < 3 ppm of N_2, N.B., this nitrogen free air was the same gas used to flush atmospheric air from the reactor) and a further 3 replicates with headspace gas collected from the reactor (4 day treatment, medium aeration) after flushing to reduce atmospheric N_2 concentration by 6 orders of magnitude - this enabled the performance of the containers to be tested under typical experimental conditions. After collection, sample gas was analysed the same day for N_2 concentration by mass spectrometry (MM8-80 model, VG Gas Analysis Systems Ltd., Middlewich, Cheshire, UK), yielding results as percentages by volume.

In order to have a target N_2 concentration, which the performance of the containers could be compared against, an approximate N_2 concentration for a 4 day, medium aeration treatment was inferred from mass balance data in the work of Burton et al. (1993b). This N_2 value was 0.16 % (by volume).

4.3.2 Use of gas bags

The first approach was to use bags, as they were simple to fill and empty with sample gas. 40 litre bags, made out of Tedlar, were experimented with first of all. These were each filled with 15 litres of gas, via a simple fitting consisting of a ¼" plastic tube with flange and securing nut (see Plate 4). The fitting was sealed with a screw cap which engaged with an olive and nut. The N_2 concentrations recovered were higher than the 0.16 % expected (see table 4.2), implying N_2 ingress in all the bags. Ingress was due to pin holing arising from the brittleness of the Tedlar material. Leaky fittings may also have been at fault.

Sample and replicate number	N_2 concentration, %
Headspace 1	3.540
Headspace 3	4.328
Headspace 3	6.615
N_2 free air 1	6.043
N_2 free air 2	5.810
N_2 free air 3	2.200

Table 4.2, Concentration of N_2 recovered from Tedlar gas sample bags

The next step was to test a bag made from a more resilient material. A number of 5 litre, five layer bags (Alltech, Carnforth, Lancs., UK) were obtained with screw type on/off valves (see Plate 5). The bag material was made by vacuum deposition of aluminium onto layers of polymer film. 3 litre volumes of gas were collected in these bags, and the test results are presented in table 4.3.

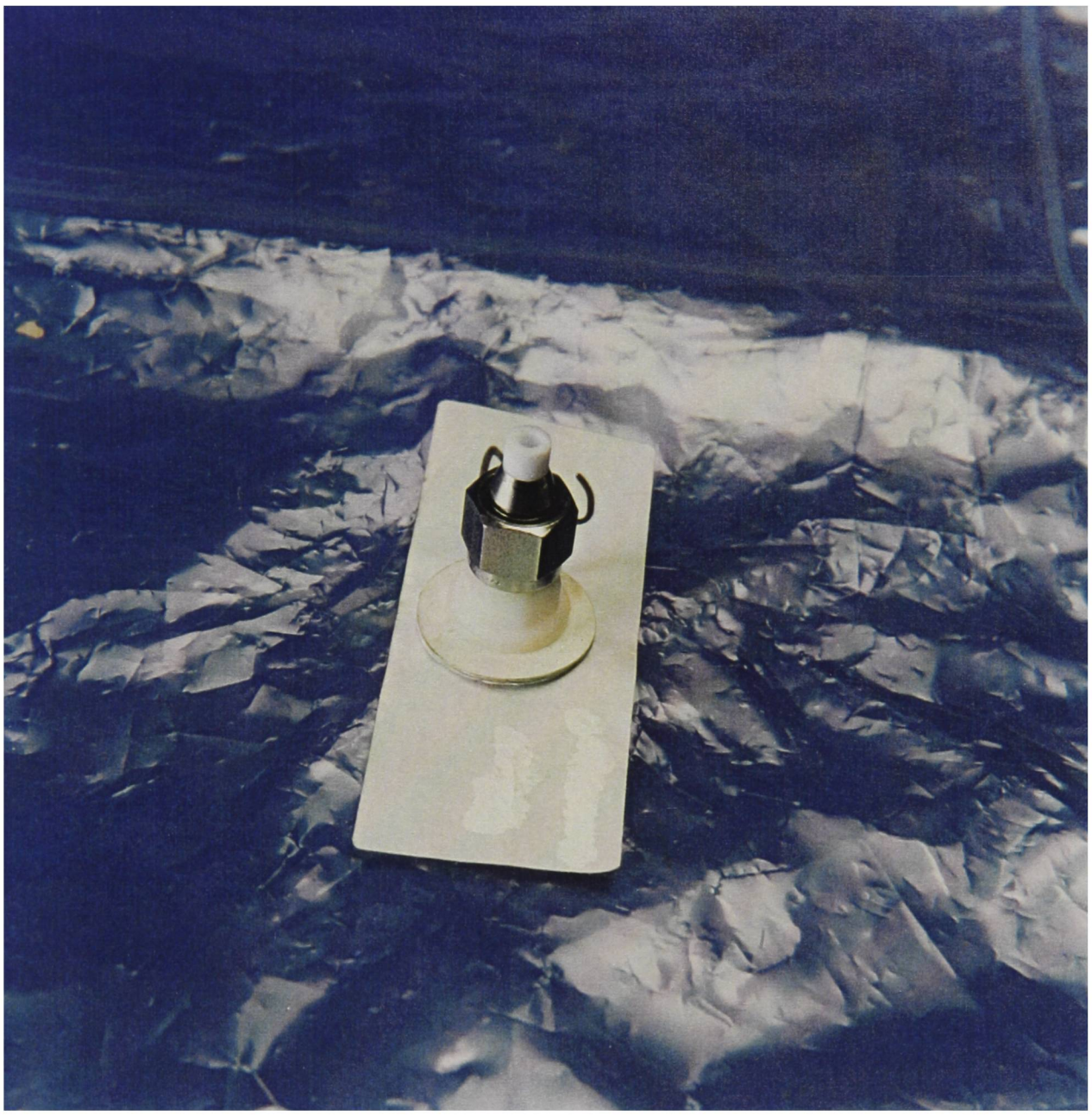


Plate 4, Tedlar gas bag with 0.25" fitting

Plate 5, Five layer bag with screw type on/off valve

Sample and replicate number	N_2 concentration, %
Headspace 1	0.418
Headspace 2	0.824
Headspace 3	0.467
N_2 free air 1	0.379
N_2 free air 2	0.169
N_2 free air 3	0.172

Table 4.3, Concentration of N_2 recovered from 5 layer gas sample bags

It was found that squeezing some of the bags, during analysis by the mass spectrometer, produced lower N_2 concentration readings than for unsqueezed bags, this accounts for the lower values in replicates 1 and 2 of the N_2 free air samples. The cause of this was traced to the valve design. Although an effective seal was maintained during closure, the open valve allowed leakage around the seat during suction emptying by the mass spectrometer, and hence ingress of air into the bag contents. Headspace samples were higher than the 0.16 % expected, whilst the N_2 free air samples were considerably higher than the expected value of 3 ppm. The unreliable performance of the 5 layer bags, in terms of valve design and the risk of puncturing, lead to their rejection as a means of sample collection. It is important to note that, for a bag containing a gas sample with only a small concentration of N_2, even a very modest ingress of atmospheric N_2 will spoil the sample. The use of this type of bag was also disadvantaged by the excessive cost (over £40 per bag).

4.3.3 Use of gas jars

The alternative approach to bags was the use of gas tight, "kilner" type glass jars of three litre volume (see Plate 6). The advantage of jars over bags was the complete elimination of leaks from fittings and punctured bag material. Jar lids were held firmly in place by a wire clamp, whilst the interface between the lid and the jar was effectively sealed by a rubber gasket coated with vacuum grease. The cost of the jars was also low, at £5 each.

The main problem was filling and emptying the jars of sample gas. Filling was achieved by displacing water from the inverted jar in a tap water bath. The lid was clamped shut under water to prevent any air ingress. After transporting the jars to the mass spectrometer, the gas was sampled from each jar by firstly inverting the jar in a water bath, removing the glass kilner lid and replacing with a specially made High Density Poly-Ethylene (HDPE) lid. This lid contained a 1.5" hole filled with a rubber bung through which had been inserted 2 lengths of glass tube, each ending in a syringe tap (Plate 6). One tube was connected to the sampling line of the mass spectrometer, whist the other was connected to a 3 litre beaker of vacuum degassed water (water was degassed to reduce contamination from air bubbles containing atmospheric N_2). Both taps were opened simultaneously, and sample gas drawn into the mass spectrometer, through the first tube, by a diaphragm pump. Gas drawn out of the sample jar was replaced by degassed water, through the second tube, to prevent a build up of vacuum in the jar.

Plate 6, Gas jars (right-hand jar fitted with HDPE sampling lid)

Sample and replicate number	N_2 concentration, %
Headspace 1	0.752
Headspace 3	0.723
Headspace 3	0.706
N_2 free air 1	0.725
N_2 free air 2	0.545
N_2 free air 3	0.574

Table 4.4, Concentration of N_2 recovered from gas jars

From table 4.4, it can be seen that the N_2 concentration values obtained are still higher than expected, but more consistent than the values obtained for the bags. The consistency of the N_2 concentrations measured from the jars, was also observed throughout later work on N_2 determination (examples in tables A18 to A23 of Appendix III). The improved consistency can be explained by the absence of N_2 ingressing into the containers. The high N_2 value may be due to the movement of dissolved N_2 from water, or from air dispersed in the water, into the flushing gas as it is collected in the jars. Although a lower value for the N_2 free air samples would be desirable, the consistency of the results (using the jars as containers) means that the concentration of microbially produced N_2 in the headspace can be determined by subtracting the N_2 free air samples from the total headspace N_2 values. That is:

$$\textit{Headspace } [N_2] - N_2 \textit{ free air } [N_2] = \textit{Microbially produced } [N_2]$$

If this formula is applied to the means of headspace and N_2 free air di-nitrogen concentrations, in the data of table 4.4 (discounting the first replicate for N_2 free air), then a concentration for microbially

produced N_2 of 0.17 % is obtained. This compares favourably with the expected value of 0.16 %. The N_2 free air (not forgetting that this is the same as the flushing gas) samples can therefore be used as blanks to correct for water borne N_2 contaminating the headspace samples as they are collected in the jars.

4.4 Conclusions

1. The amount of nitrogen free air, necessary to reduce atmospheric N_2 in the reactor to a level where it is no longer a significant interference to measuring microbially produced N_2 (i.e., a reduction of 6 orders of magnitude), is estimated as 138 litres. Given that the air flow rate generally employed during the mass balance experiments (chapter 7) was 0.6 l min^{-1}, nitrogen free air will have to be flushed through the reactor for a duration of not less than 230 minutes.

2. The most reliable form of sample containment (and that subsequently used to collect samples of headspace gas for N_2 analysis) was found to be gas jars with sealable lids. However, collecting the gas samples over water introduces the problem of contamination by dissolved atmospheric N_2. Since the degree of this contamination generally remains constant, it can be effectively corrected for by subtracting blank sample values, obtained from collecting samples of flushing gas (i.e., nitrogen free air) separately, from the headspace samples.

5. SLURRY ANALYSIS USING STEAM DISTILLATION TECHNIQUES

5.1 Introduction

The main forms of nitrogen present in slurry include ammoniacal and organic nitrogen, and additionally in treated slurry, nitrite and nitrate. There are also, possibly, other forms of nitrogen such as hydroxylamine (NH_2OH) which have not yet been fully investigated. Therefore, in order that a full nitrogen mass balance can be assembled, all these species must be quantified. This can be achieved using various techniques, for example, colorimetric, titrimetric and chromatographic. A commonly used technique is that of steam distillation and its various modifications (Bremner, 1965).

In the first series of trials to construct full nitrogen mass balances (chapter 7), steam distillation was used to measure all the above forms of nitrogen. However, as the work presented in this chapter will show, steam distillation methods have limitations and this lead to the search for improved techniques for incorporation in the second series of trials; notably High Performance Liquid Chromatography (HPLC) for the determination of NO_2^- and NO_3^-.

Of some interest was the ability of the technique to recover hydroxylamine. No information was found in the literature concerning this aspect, and since there is the possibility that NH_2OH may accumulate in slurries under certain conditions (Yang & Alleman, 1992), it was considered that the use of steam distillation to determine this species should be investigated.

Steam distillation involves the estimation of ammoniacal nitrogen in a sample by co-evaporation

of NH_3, through exposure of the sample to steam in an alkaline environment. The NH_3 is collected as NH_4^+ in weak acid solution (e.g., boric acid), and determined by back titration against standard acid (e.g., H_2SO_4). Boric acid donates a proton to NH_3 thus:

$$NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^-$$

This basic technique is modified, so that different forms of nitrogen can be determined by using some form of pretreatment that converts them into NH_4^+.

5.1.1 Distillation apparatus and conditions

In the work reported here, all analyses were carried out using a Gerhardt Vapodest 4S unit (C. Gerhardt UK Ltd., Brackley, Northants.). This was able to distil and titrate samples automatically to predetermined pH values, using an inbuilt pH probe and controller. Although precise analysis conditions were altered for the different species being studied, all NH_3 released by distillation was collected in 45 ml of 4 % (w/w) boric acid solution. All titrations were performed as back titrations against standardised 0.05 M sulphuric acid.

5.2 Determination of slurry ammonia/ammonium by steam distillation

5.2.1 Introduction

This experiment was designed to assess the NH_4^+ nitrogen content of raw and aerobically treated slurries, using an APHA (1985) based steam distillation method. Results were compared with some literature values.

5.2.2 Method

5 ml of sample were placed in a digestion tube and inserted into the Gerhardt distillation apparatus. The machine was programmed to add 50 ml of 40 % NaOH (low nitrogen) prior to distillation for a time of 4 minutes. The procedure was repeated using 2 blanks of ultra high quality water, 4 raw and 4 treated slurry samples. Results were calculated as mg of N per ml of sample using:

$$N = \frac{1.4\ T\ F}{V} \qquad (5.1)$$

where, N = mg of N per ml of sample,

T = titre value in ml (after subtracting blank),

F = correction factor for standard H_2SO_4, used in the titration (1.03 in this case),

V = volume of sample (ml).

The numerical value 1.4 is the number of mg of N that are equivalent to T ml of 0.05 M standard H_2SO_4 (calculation in Appendix IV).

5.2.3 Results and discussion

Referring to table 5.1, the technique gave highly reproducible results with low standard deviations for both raw and treated slurries. The fall in concentration of 2.46 mg ml^{-1}, between raw and treated samples, indicates that NH_4^+ had been lost through ammonia emission as well as through nitrification (and possible subsequent denitrification). The mean value obtained for raw slurry is comparable with that of Williams et al. (1989) who report a value for NH_3-N of 9 % of TS.

Sample	Ammoniacal nitrogen, mg ml^{-1}	
	Raw slurry	Treated slurry
1	2.78	0.27
2	2.68	0.28
3	2.74	0.29
4	2.75	0.28
mean	2.74	0.28
SD	0.04	0.01
mean as a % of TS	9	1

Table 5.1, Ammoniacal nitrogen content of raw and treated slurries

(More detailed results in Appendix II).

5.3 Determination of Kjeldahl nitrogen

5.3.1 Introduction

The Kjeldahl method determines nitrogen in the trinegative state. It fails to take account of azide, azine, azo, hydrazone, nitrite, nitrate, nitrile, nitro, nitroso, oxime and semi-carbazone (APHA, 1985). The principle is as follows: In the presence of H_2SO_4, potassium sulphate (K_2SO_4) and a catalyst (either selenium or mercuric sulphate), the amino nitrogen of many organic materials is converted to ammonium sulphate ($(NH_4)_2SO_4$), during a digestion period. NH_3 is then liberated from NH_4^+ during subsequent steam distillation with strong alkali. The aim of this exercise was to assess the reproducibility of a modified Kjeldahl method, and to compare results with those of other workers.

5.3.2 Method

The Dimmock & Martinez modification of the Kjeldahl method was used (Dimmock & Martinez, 1994): To four 5 ml aliquots of raw slurry and two 5 ml blanks of ultra high quality water, were added two catalyst tablets (each containing 3.5 g of K_2SO_4 and 3.5 mg of selenium), 10 ml of 98 % H_2SO_4 (low nitrogen grade) and 10 ml of H_2O_2. Samples were then placed in a heating block at 400 ^{0}C, until all colour had been lost, and then left to cool prior to steam distillation using 50 ml of 40 % NaOH (low nitrogen grade). The procedure was repeated using treated slurry in place of raw slurry.

5.3.3 Results and discussion

Results were again calculated using equation (5.1).

Sample	Kjeldahl nitrogen, mg ml^{-1}	
	Raw slurry	Treated slurry
1	3.01	1.20
2	2.84	1.29
3	3.06	1.29
4	2.95	1.27
mean	2.97	1.26
SD	0.08	0.04
mean as a % of TS	10	5

Table 5.2, Kjeldahl nitrogen (N_k) Content of raw and treated slurries

The results (table 5.2) clearly show a large breakdown of N_k by the treatment process, accounting for some 5 % of the total solids content of the slurry. The low standard deviations observed,

again indicate an acceptable level of reproducibility of the method. Comparing the results for raw slurry with literature values, there appears to be close agreement, although some variance would be expected due to differences in pig diet. Williams et al. (1989) report N_k as 12% of TS, whilst Dimmock & Martinez (1994) report N_k as 8% of TS.

5.4 Determination of inorganic nitrogen

5.4.1 Introduction

Inorganic nitrogen, in this context, is taken as meaning the total ammoniacal, nitrite and nitrate nitrogen content of slurry. There are several methods available, but the principle is the same. The NO_2^- and NO_3^- nitrogen is reduced to NH_4^+ during distillation, and the total NH_4^+ nitrogen content is subsequently determined from steam distillation in an alkaline environment (see section 5.2).

There are two common reducing agents which may be employed: One is Devarda's alloy (45 % aluminium, 50 % copper and 5 % zinc), and the other is iron powder reduced by hydrogen (as supplied by the manufacturers). The latter was not used as it had been found to give poor recoveries under the conditions employed in this experiment (Dimmock & Martinez, 1994).

The reduction reaction may be represented by the following equations, where M refers to the metal reducing agent:

$$4H^+ + 2NO_3^- + M \rightarrow 2NO_2^- + M^{2+} + 2H_2O$$

$$8H^+ + 3M + NO_2 \rightarrow NH_4^+ + 3M^{2+} + 2H_2O$$

In this experiment, not only was the reproducibility of the technique assessed, but also the recovery of known quantities of NO_2^- and NO_3^- standard solutions added to slurry samples.

5.4.2 Method

To assess the NO_2^- and NO_3^- nitrogen recovery of the technique, two standard solutions of KNO_2 and KNO_3 were made up, each containing 1 mg of N per ml. 5 g of Devarda's alloy and 5 g of magnesium oxide (MgO) powder were added to each of the following samples (sample volume = 5 ml) immediately before carrying out steam distillation for 4 minutes:

(i) ultra high quality water blank,

(ii) raw slurry,

(iii) raw slurry with nitrite standard,

(iv) raw slurry with nitrate standard,

(v) standards alone.

The samples were carried out in duplicate. The 5 g of MgO powder added were used to create the mild alkaline conditions necessary for NH_3 liberation. MgO was used in preference to NaOH as this was found to produce excessive foaming in the distillation apparatus. After steam distillation and titration against standardised 0.05M H_2SO_4 (correction factor, F = 1.07), the inorganic nitrogen recovery was calculated using equation (5.1).

5.4.3 Results and discussion

Samples	N conc., mg ml^{-1}	Std. recovery, %
NO_2^- std.	0.92	92
NO_3^- std.	0.91	91
raw slurry	4.19	-
NO_2^- std. + slurry	4.40	20
NO_3^- std. + slurry	4.27	9

Table 5.3, Recoveries of NO_2^- and NO_3^- standards from raw slurry

The final column of table 5.3 shows the percentage recovery of each standard from either standard on its own, or from standard added to slurry. The percentage recovery of both standards from the standard/slurry mixtures was greatly reduced compared with the recovery of the standards alone. The poor recoveries could not be explained by possible loss of the standard during sample preparation (e.g., by denitrification due to bacteria in the slurry). Addition of the standards was always the last step prior to steam distillation. A series of experiments was undertaken to try and identify the possible cause of the low recoveries.

5.5 Further experiments using the Devarda's alloy method

5.5.1 Introduction

Various reaction conditions were altered in an attempt to identify the cause of the low standard recoveries in the previous section, 5.4 . Several possibilities were considered:

(i) Increasing the pH from 8 to 10 to enhance NH_3 liberation,

(ii) increasing the distillation time and,

(iii) reducing the sample volume of slurry to increase the relative amount of Devarda's alloy used, and hence increase the reducing power.

It was also postulated that there may have been inhibition of the reduction reaction by an unknown factor in the slurry. Therefore, a number of different slurries were tested, including fresh, stored, low total solids, and slurry supplied from an alternative source.

To simplify experimental procedures, only nitrate standard was used in the tests, since a slurry producing a poor NO_3^- recovery would be expected to have a similarly poor NO_2^- recovery. As in the previous experiment, recoveries were made from: (i) standard and, (ii) standard added to slurry. The two results were then compared.

5.5.2 Methods

(a) Increased distillation time

All conditions were maintained, as for 5.4, but distillation time was increased from 4 minutes to 5 minutes.

(b) Increased pH and reduced sample volume

All conditions maintained as before, including 5 minute distillation time, but pH was increased from 8 to 10 by substituting 5 ml of 0.5 % NaOH in place of MgO, and sample volume was decreased from 5 ml to 1 ml.

(c) Comparison of NO_3^- recoveries from different slurries

Four different slurries were used: A fresh and a stored slurry from the same source (maiden gilts housed at Silsoe College Farm, Silsoe, Beds.); the stored slurry had been kept for 6 months in a large, open top, continuously stirred tank. A low total solids slurry from a weaner house, and a fresh sample collected from the Scottish Agricultural College (Auchincruive, Ayr).

Conditions were maintained as for 5.4, but a sample volume of 2 ml was used for all samples except those from Auchincruive, where excessive foaming, during distillation, necessitated a sample volume of 1 ml. Total solids measurements were also taken of each slurry by drying to constant weight.

5.5.3 Results and discussion

Reaction conditions	Recovery of NO_3^- std., %
distillation time 4 mins	9 (from table 5.3)
distillation time 5 mins	2
sample volume 5 ml, pH 8	9 (from table 5.3)
sample volume 1 ml, pH 10	6

Table 5.4, Effect of different reaction conditions on standard recoveries

From table 5.4 it can be seen that increasing alkalinity, the distillation time and the relative proportion of Devarda's alloy (by reducing sample volume), lead to no improvement. The increase in alkalinity would be expected to enhance the liberation of NH_3 after reduction of NO_3^-, whilst increasing the relative amount of Devarda's alloy (by reducing sample volume) would have

been expected to enhance the reduction of NO_3^-. Since neither of these gave an improvement, and since the NO_3^- reduction is a more complex process then that of NH_3 evaporation, it is possible that the reduction of NO_3^-, by Devarda's alloy, is being interfered with in some way. This was confirmed by testing the samples, after distillation and titration were complete, with Merckoquant nitrate indicator sticks. The sticks confirmed that NO_3^--N, in excess of 500 mg l^{-1} (i.e., > 50% of the standard added), was still present in the slurry/standard mixtures.

Slurry Sample	Total Solids, %	Mean Recovery, %
Silsoe fresh	3.5	34
Silsoe stored	1.5	113
weaner house	0.5	100
Auchincruive	3.5	0.2

Table 5.5, Total solids and standard NO_3^- recovery for a range of slurries from different sources

It was not possible to ascertain the cause of the interference described above, although it is possible to speculate that some chemical agent, e.g., a disinfectant or cleaning agent, used in the piggery houses was responsible. However, it is interesting to note from table 5.5, that recoveries from the stored and low total solids slurries, were much greater than those of the two fresh slurries which had higher solid contents. It is possible that the inhibitory factor may be associated, in some way, with the solids content of slurry. Also, the fact that the same inhibition was encountered in slurries from two separate sources (i.e., Auchincruive and Silsoe) indicates that the phenomenon may be universal to fresh piggery slurries.

Because of the unreliability of measuring NO_2^- and NO_3^- by the Devarda's technique, an HPLC method was developed and used instead (described in chapter 6).

5.6 The Olsen based method for total nitrogen

5.6.1 Introduction

The Olsen (1929) modification of the Kjeldahl method allows the combined determination of Kjeldahl as well as NO_2^- and NO_3^- nitrogen, hence the use of the term total nitrogen (although it is accepted that the method does not include other forms of nitrogen that the Kjeldahl method cannot recover). It is considered here because of the need to acquire total nitrogen values for treated slurry in the series 1 trials, described in chapter 7. At the time these trials were in progress, the HPLC method for NO_2^- and NO_3^- was under development and hence not available for use.

The Olsen modification makes use of a predigestion, in which NO_2^- is oxidised to NO_3^- by H_2SO_4 and potassium permanganate ($KMnO_4$), as in the following equation:

$$5NO_2^- + 2MnO_4 + 6H^+ \rightarrow 5NO_3^- + 2Mn^{2+} + 3H_2O$$

NO_3^- is reduced to NH_4^+ by the hydrogen formed from the reaction of iron with the H_2SO_4 used in the permanganate oxidation.

$$10H^+ + 4Fe + NO_3^- \rightarrow NH_4^+ + 4Fe^{2+} + 4H_2O$$

The whole reaction environment is much more strongly reducing than that for the Devarda's technique, therefore recovery of NO_2^- and NO_3^- should be more successful. Although the

technique was originally developed to give quantitative recoveries of NO_2^- and NO_3^- in total nitrogen studies of soils, Dimmock & Martinez (1994) have successfully used an Olsen based technique on pig slurry.

5.6.2 Method

2 ml of sample and 8 ml of deionised water were placed in a digestion tube together with 10 ml of $KMnO_4$ (50 g l^{-1}) and 20 ml of 25 % (v/v) H_2SO_4. The mixture was allowed to stand for 5 minutes. 5 g of iron powder (reduced by hydrogen) were added and the sample allowed to stand for approximately 15 minutes while the effervescence subsided. The samples were then heated to a temperature of 100 ^{0}C for 45 minutes and cooled before undertaking a normal Kjeldahl digestion, as per the method described in section 5.2.3.2, followed by steam distillation and titration. The method was repeated on a total of 3 samples of raw slurry (with blanks) and 3 samples of treated slurry (with blanks).

5.6.3 Results and discussion

In table 5.6, the reproducibility of each sample is seen to be to be good, with low standard deviations for both treated and raw slurries. Dimmock & Martinez (1994) report a value for raw slurry, using this technique, of 5.30 mg ml^{-1} (SD=0.54). This corresponds to 8 % of TS. They also report a 97.5 % recovery of NO_2^- and 99.1% recovery of NO_3^- standards from raw slurry using the same technique. Interestingly, if these results are compared with the Kjeldahl results, the raw slurry nitrogen values are similar, but for treated slurry the Olsen based method gives a recovery of 0.34 mg ml^{-1} more than the Kjeldahl method. It seems, therefore, that the stronger reducing environment is much more effective at recovering NO_2^- and NO_3. This also adds further

weight to the theory that interference of NO_3^- reduction, or rather an insufficiently reducing environment, was responsible for the inefficacy of the Devarda's method.

Sample	Total nitrogen, mg ml^{-1}	
	Raw slurry	Treated slurry
1	3.20	1.63
2	3.30	1.59
3	3.14	1.58
mean	3.21	1.60
SD	0.07	0.02
mean as a % of TS	11	6

Table 5.6, Total nitrogen (N_{tot}) content of raw and treated slurries

5.7 Recovery of Hydroxylamine by Steam Distillation

5.7.1 Introduction

The aim of this experiment was to investigate the possibility of recovering hydroxylamine (NH_2OH) by the Devarda's alloy method.). If NH_2OH is present in significant quantities, then its quantitative recovery in total nitrogen analysis will be essential. This experiment investigated the recovery of NH_2OH from a standard solution alone, and from standard added to slurry (as in previous experiments).

5.7.2 Method

A standard solution of NH_2OH was made up from hydroxylamine sulphate ($(NH_2OH)_2.H_2SO_4$) and deionised water, containing 1 mg of NH_2OH nitrogen per ml. 5 ml of standard were taken

and analyzed by the Devarda's alloy method. The procedure was repeated using 5 ml of slurry and a mixture of 5 ml of standard plus 5 ml of slurry.

5.7.3 Results and discussion

Samples	N conc., mg ml^{-1}	Std. recovery, %
NH_2OH standard	0.95	95
raw slurry	3.90	-
NH_2OH std. + slurry	4.23	33

Table 5.7, Recovery of hydroxylamine standard by Devarda's alloy method

Table 5.7 shows that NH_2OH standard can be effectively recovered by the Devarda's alloy method. In this respect, NH_2OH behaves like NO_2^- and NO_3^-. It also exhibits similar behaviour in another respect: its recovery from slurry by the Devarda's alloy method was greatly reduced, probably due to poor reduction to ammonia. The experiment suggests that NH_2OH behaves so similarly to NO_2^- and NO_3^-, that any method, which can reliably recover the other two oxides of nitrogen, should also recover NH_2OH. It would be interesting however, to distinguish NH_2OH from the other inorganic forms of nitrogen, to determine its significance as a component of slurry nitrogen. Steam distillation is unable to do this, but high performance liquid chromatography may, in future work, provide a solution.

6. SLURRY ANALYSIS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

6.1 Introduction

High performance liquid chromatography (HPLC) was initially sought as an alternative method to the Devarda's alloy steam distillation method for NO_2^- and NO_3^- recovery from slurry. The technique was found to be more accurate, and versatile in that NO_2^- and NO_3^- could be determined separately. Sample preparation was simple and quick and did not require any reduction step prior to analysis. There was also the possibility of investigating other slurry components, e.g., NH_2OH, phosphate and chloride. (N.B., further information about the principles of HPLC and equipment data are located in Appendix I, part 2).

6.2 Analysis of oxides of nitrogen in slurry.

The aim of this work was to investigate the use of HPLC as an alternative method to steam distillation, for the determination of NO_2^- and NO_3^-. The possibility of identifying NH_2OH in slurry, using HPLC, was also explored.

Sample preparation was important because of the need to remove all suspended material from the slurry samples and avoid damaging the column. This was achieved through prefiltration (to remove coarse particles) and a second, micro filtration, which not only removed all remaining suspended matter, but also removed all slurry bacteria, therefore preventing any post sampling nitrification/denitrification activity.

6.2.1 Determination of nitrite and nitrate nitrogen

6.2.1.1 Introduction

The accuracy of the method was assessed (as with the work on the Devarda's alloy method, section 5.4) by measuring the recovery of standards added to slurry. However, this time recoveries were calculated in terms of their peak areas, obtained from the chromatograms; i.e., recoveries were calculated by subtracting any raw slurry NO_2^-/NO_3^- peak areas from the peak areas for the slurry/standard mixture, and expressing as a percentage of standard NO_2^-/NO_3^- peak areas.

The stationary phase used was Techsphere 5 SAX (strong anion-exchange with particle size 5 µm) supplied in a 150 mm by 4.6 mm stainless steel column (HPLC Technology, Macclesfield, Cheshire, UK). The mobile phase was a 10/90 (v/v) mixture of methanol and 0.05 M phosphate buffer (6.80 g l^{-1} KH_2PO_4 and 2.5 ml concentrated H_3PO_4). The addition of methanol reduced the polarity of the mobile phase, thereby enhancing retention of components on the stationary phase without significantly decreasing analysis time, whilst the buffer maintained pH within the optimum range of 2 to 7 for the stationary phase.

Mobile phase flow rate was set at 1.5 ml min^{-1} and injection volume at 20 µl. Samples were analysed at 214 nm, this being the optimum wavelength in the ultraviolet/visible (UV/VIS) spectrum for absorption of NO_3^-, whilst still giving significant absorption for NO_2^-.

6.2.1.2 Method

A mixed standard was made up, containing 100 mg l^{-1} of NO_2^- nitrogen and 100 mg l^{-1} of NO_3^- nitrogen. 10 ml of raw slurry were added to 10 ml of ultra pure water and filtered, under vacuum, through a Buchner funnel with a No. 4 (fast) filter paper. The filtrate was then drawn into a clean syringe, through a 0.2 μm syringe filter, and delivered into a glass HPLC vial before crimping on the lid and placing in the autosampler. After running the sample, chromatogram data were collected for a period of 8 minutes before integrating using a personal computer. The procedure was repeated with a standard/water mixture (10 ml/10 ml) and a slurry/standard mixture (10 ml/10 ml) and also with treated slurry in place of raw. In all, five replicates were made.

6.2.1.3 Results and discussion

Slurry + Std.	mean, %	SD	n
raw + NO_3^-	96.6	2.9	5
treated + NO_3^-	101.6	7.6	5
raw + NO_2^-	88.4	11.1	5
treated + NO_2^-	94.2	10.9	5

Table 6.1, Recoveries of standards from raw and treated slurries

Notes: n = number of samples taken.
Full percentage recovery data is located in table A1 of Appendix I.
A sample chromatogram is shown in figure 6.1.

The results (Table 6.1) represent a great improvement over those obtained by the Devarda's alloy method. The recovery of NO_3^- from raw slurry was close to the target of 100 %, with a low standard deviation. The other recoveries were in the order of 90 to 100 %, but had substantially

higher standard deviations. This is particularly worrying for the treated slurry since it is this, rather than the raw slurry, from which one would expect to detect significant amounts of oxides of nitrogen. The high standard deviations were most likely due to inaccuracies in pipetting the slurry, in particular the treated slurry, which may have had variable amounts of foam associated with each pipetting operation.

6.2.2 Recovery of nitrite and nitrate from weighed samples

6.2.2.1 Introduction

To improve the reproducibility of HPLC as a method of NO_2^-/NO_3^- recovery, experiment 6.2.1 was repeated using aerobically treated slurry (4 day treatment, medium aeration) by weighing samples rather than pipetting. Also the number of replicates taken was increased from 5 to 10. Apart from these two modifications, the basic procedure remained unchanged.

6.2.2.2 Method

A mixed standard was made up, containing 100 mg l^{-1} of NO_2^- nitrogen and 100 mg l^{-1} of NO_3^- nitrogen. Solutions were then made up containing the following:

1. 5 g of treated slurry + 5 g of water.
2. 5 g of mixed standard + 5 g of water.
3. 5 g of treated slurry + 5 g of mixed standard.

Filtered aliquots were taken of each in turn, and injected on to the column (conditions as for 6.2.1). The whole procedure was repeated a further 9 times.

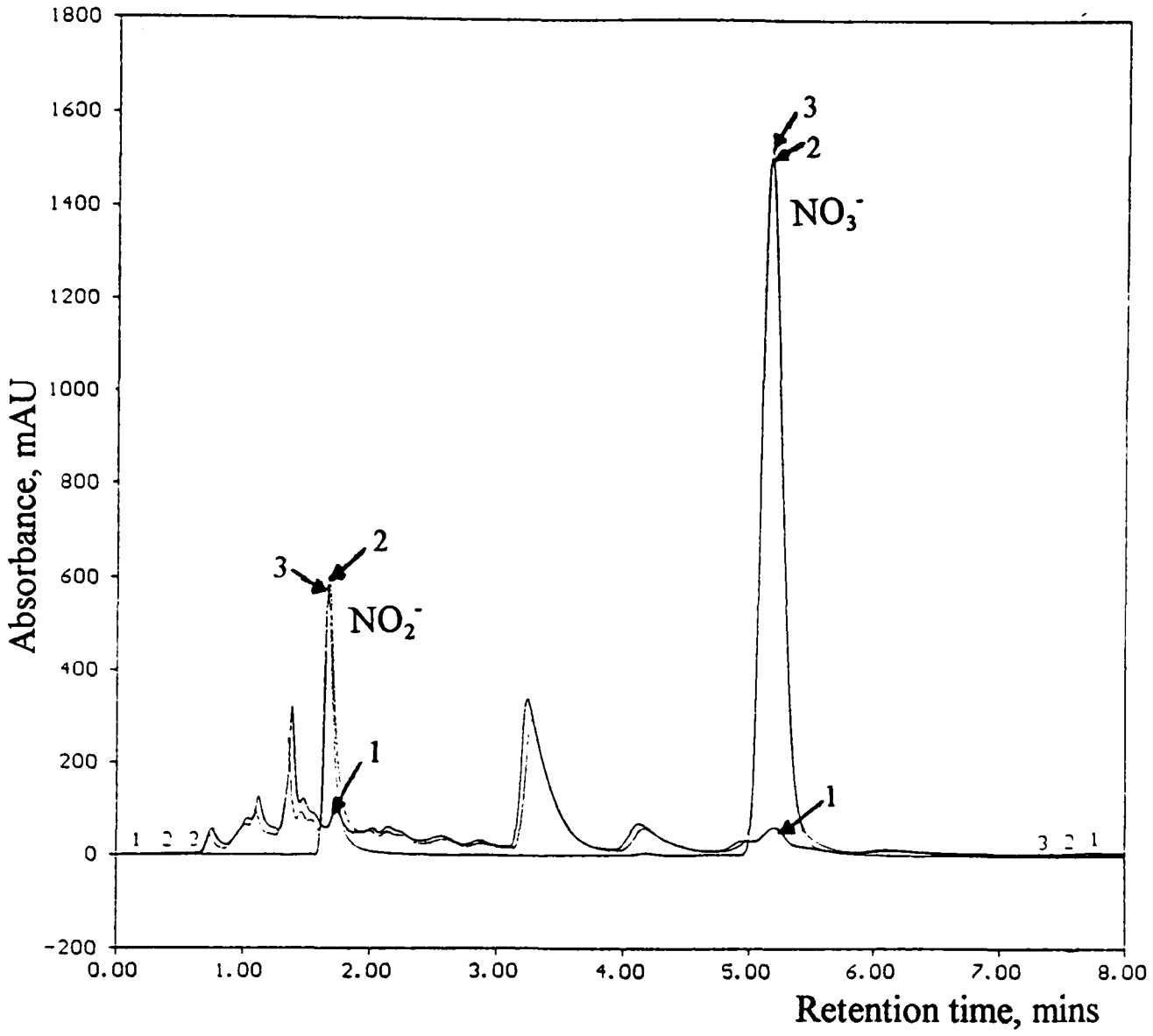


Fig 6.1, Chromatogram comparison of data obtained for a raw slurry sample

Note: Three superimposed chromatograms are shown. Chromatogram 1 corresponds to the raw slurry sample, 2 to the standard and slurry sample mixture, and 3 to the standard alone. Absorbance was measured in arbitrary units: mAU (milli Absorbance Unit).

6.2.2.3 Results and discussion

Slurry + Std.	mean, %	SD	n
treated + NO_3^-	117.2	3.0	10
treated + NO_2^-	118.0	2.8	10

Table 6.2, Recoveries of standards from treated slurry

As expected, weighing samples led to a definite improvement in the standard deviation of the method (NO_3^- SD fell from 7.6 to 3.0, and NO_2^- SD fell from 10.9 to 2.8). The rise in the mean values to substantially greater than 100 % is, however, rather difficult to explain (especially in the light of the reduced SD values). Assuming that the low standard deviations indicate that operator or machine error did not have a significant influence on the mean values, and also bearing in mind the lower mean values obtained in 6.2.1, it seems likely that, once again, some factor in the slurry was responsible for the problem. For example, there may be a component present in slurry which associates itself in some way with the NO_2^- and NO_3^- molecules, and in doing so enhances the absorption of light in the UV/VIS range. This increase in absorption would not be encountered in the standard solution, therefore peak areas would be smaller leading to percentage recoveries of larger than 100.

If one considers that this experiment was carried out more than 6 months after experiment 6.2.1, and that slurry characteristics vary over time, this may explain the difference in recoveries experienced.

6.2.3 Recovery of hydroxylamine from standard solutions

6.2.3.1 Introduction

The aim of this work was to assess the use of HPLC in recovering NH_2OH from standard solutions. According to Cotton et al. (1987), NH_2OH forms a weak base in aqueous solution, i.e.:

$$NH_2OH + H_2O = NH_3OH^+ + OH$$

Therefore, its behaviour is electropositive and retention by HPLC will require a stationary phase with cationic exchange properties (as opposed to the anion-exchange stationary phase used in the NO_2^-/NO_3^- analysis). The stationary phase used in this experiment was Techsphere, 5 µm C18 cation-exchange, supplied in a 250 mm stainless steel column (HPLC Technology, Macclesfield, Cheshire, UK). Since NH_2OH is a polar molecule, a polar mobile phase is desirable. Hence, HPLC grade water was used, at a flow rate of 2.0 ml min^{-1}. Samples were analysed in the UV/VIS spectrum at a wavelength of 214 nm. The effect of lowering pH was also investigated to see if this was necessary for maximum ionisation of the NH_2OH molecule. This was achieved by adding potassium hydrogen phosphate buffer to the mobile phase.

6.2.3.2 Method

Two standard solutions were made up containing 5000 and 10 000 mg l^{-1} respectively of NH_2OH nitrogen. After placing aliquots of the two standards into separate glass vials and crimping on the lids, the solutions were injected onto the column with HPLC grade water mobile phase (injection volume = 50 µl).

The effect of adding different proportions of phosphate buffer to the mobile phase was assessed using the 10 000 mg l^{-1} NH_2OH standard. 50 µl aliquots were injected into mobile phase streams containing the following HPLC grade water/phosphate buffer combinations:

1. Water : Buffer, 100 % : 0 %
2. Water : Buffer, 75 % : 25 %
3. Water : Buffer, 50 % : 50 %
4. Water : Buffer, 25 % : 75 %

The peak areas obtained for each of the above mobile phase conditions was then compared to see if any change in NH_2OH recovery was apparent.

6.2.3.3 Results and discussion

Mobile phase, %/%	Ret. time, mins	Area, mAU*mins	Mobile phase pH
water/buffer 100/0	1.24	8.58	7.0
water/buffer 75/25	1.24	8.52	2.6
water/buffer 50/50	1.24	8.49	2.2
water/buffer 25/75	1.24	8.94	2.3

Table 6.3, Comparison of NH_2OH peak areas for different mobile phase conditions

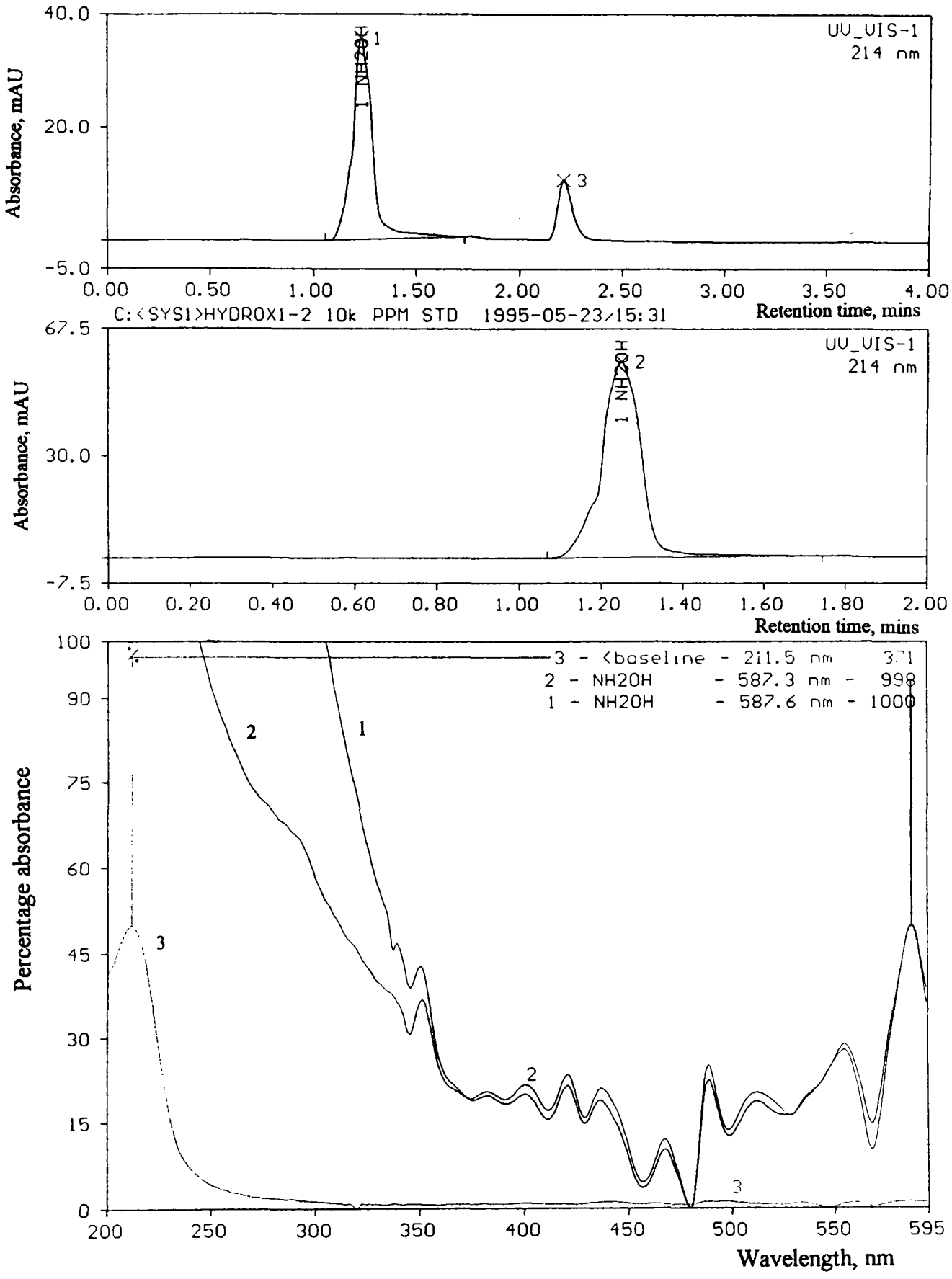


Fig 6.2, UV/VIS spectra comparison of eluted components

Note: Top chromatogram refers to 5000 mg l^{-1} standard, bottom chromatogram refers to 10 000 mg l^{-1} standard. Spectrum **1** corresponds to NH_2OH (5000 mg l^{-1}), **2** to NH_2OH (10 000 mg l^{-1}), and **3** to the contaminant peak at RT=2.23 min.

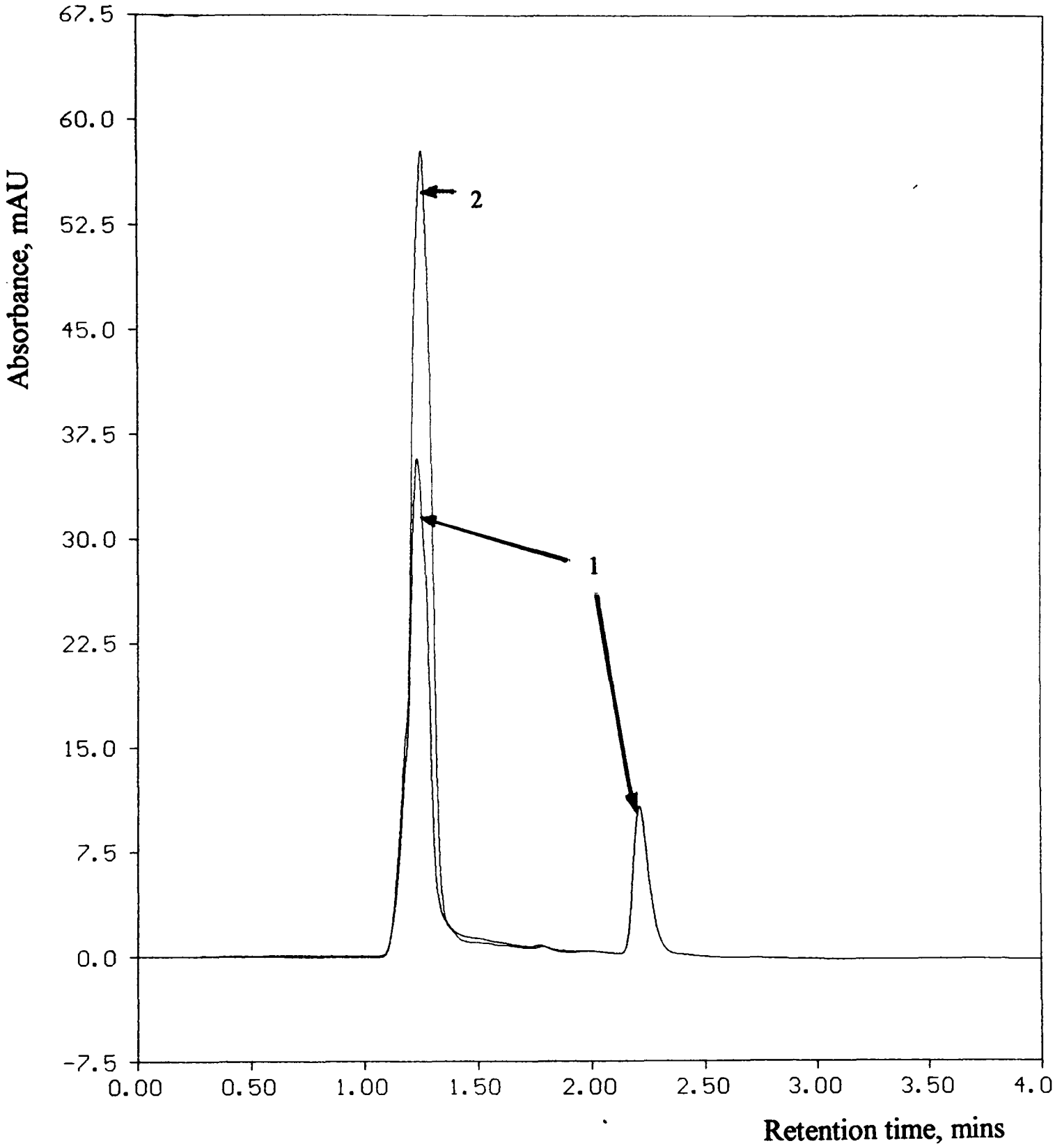


Fig. 6.3, Chromatogram comparison of NH_2OH standard solutions

Note: Peak 1 corresponds to the 5000 mg l^{-1} and peak 2 to the 10 000 mg l^{-1} NH_2OH nitrogen standards.

Figure 6.2 shows two chromatograms, one for each of the two standards, and a comparative plot of the UV/VIS spectra obtained for each peak in both chromatograms. The spectra for the NH_2OH peaks (labelled 1 and 2) are, as expected, almost identical with a 99.8 % confidence level that peak 2 matches peak 1. Peak 3 was identified as a contaminant (rather than an alternative NH_2OH peak) because, firstly, it is absent from the 10 000 mg l^{-1} chromatogram, and secondly, its spectrum is completely different from those of the other two (37.1 % confidence level that peak 3 is a genuine match with peak 1).

The experiment shows that the HPLC equipment is capable of recovering NH_2OH under the conditions employed, but the detector is too insensitive to be of any practical use in slurry analysis: The detector response for the 5000 mg l^{-1} standard is around 35 mAU (refer to figure 6.3) which compares with a response of around 1500 mAU for a 50 mg l^{-1} solution of NO_2^- nitrogen. Since the total nitrogen content of slurry is of the order of 5000 mg l^{-1} anyway, it is reasonable to assume that concentrations of NH_2OH in treated slurry will be below the detectable limit (i.e., 1 mAU) of the instrument.

Lowering the pH of the mobile phase, as can be seen in table 6.3, did not yield any increase in peak area (n.b., the buffer was effective at maintaining a pH of around 2, whether it formed 25 % or 75 % of the mobile phase composition). Therefore it seems that NH_2OH ionisation is already at a maximum under neutral pH conditions, and buffering to a lower pH is not necessary.

6.2.4 Conclusions

Although the mean treated slurry results for the NO_2^-/NO_3^- analysis using HPLC were in excess

of 100 % (sections 6.2.1 and 6.2.2), they still represent a marked improvement over the Devarda's alloy method. Subsequently, treated slurries were analysed by HPLC as it was the most reliable method.

Inspite of the results for the hydroxylamine analysis not yielding a useful method for the work in this project, they indicate that its recovery by HPLC is possible. Hydroxylamine does not exhibit strong UV/VIS absorption characteristics, therefore in the future it may be fruitful to use an alternative mode of detection, such as an ion capture device.

6.3 Identification of raw slurry components

6.3.1 Introduction

It is inconceivable that the only anionic species, detectable in slurry by the method developed in 6.2.1, are nitrite and nitrate. Indeed, if a raw slurry sample is analysed under similar conditions to the nitrite/nitrate analysis, a number of peaks are seen on the chromatogram (as in figure 6.5 on page 100). Therefore, it is clear that HPLC can recover a number of slurry components. Principal among these is a large peak at retention time 2.03 mins, which is notable for its absence from treated slurry (see figure 6.4). It is possible that the unidentified compounds may have either a detrimental or a beneficial effect on the environment (as would be the case with phosphate, for example). Since the aeration process appears to have the ability to degrade some of these components, it was decided to attempt to identify a number of the more prominent ones. Aeration could then be offered as a means of removing these components if they are identified as being environmentally damaging.

Unfortunately, the HPLC software library did not include all the compounds which might be encountered in slurry. Therefore the initial approach adopted was one of trying particular compounds, and then comparing the retention times of solutions of the pure known compounds with the unknown peaks in the raw slurry chromatogram. This approach, however, only had limited success and a sample of raw slurry was later analysed by a gas chromatographic instrument (with a substantial library) to see if this could provide any additional information.

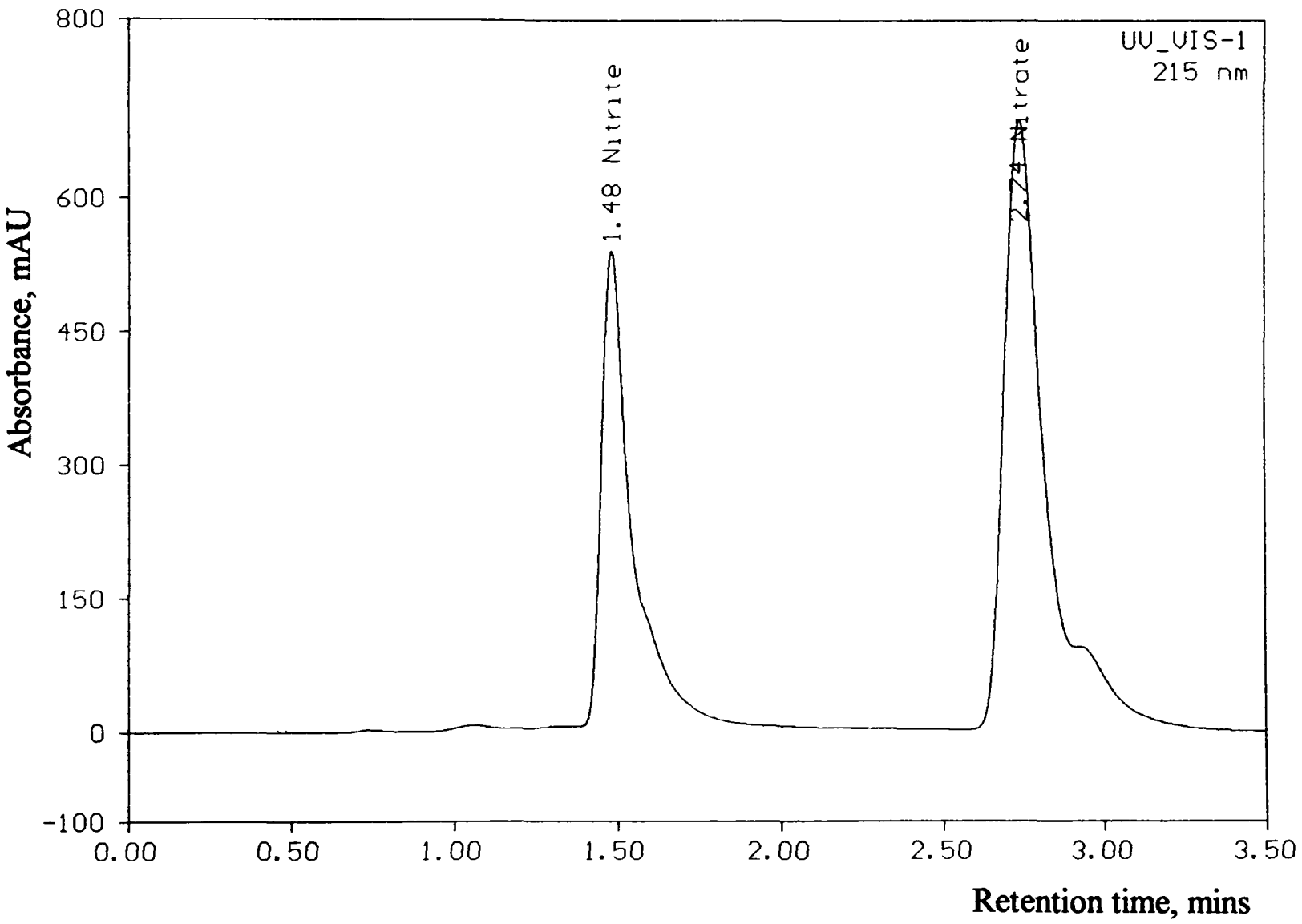


Fig. 6.4, Chromatogram showing nitrite and nitrate peaks in a treated slurry sample

Gas chromatography differs from HPLC in that the liquid samples are first vaporised before being introduced onto the column for separation. Therefore, the technique will only work for those compounds (normally organics) which successfully volatilise under the instrument conditions. A range of easily volatilised, non-polar molecules will also be detected, and these will have to be separated from the polar organic molecules which are most likely to form the anions detectable by HPLC.

6.3.2 Method

Conditions employed were as follows:

Stationary phase: Techsphere 5 μm.

Mobile phase: 10/90 mixture of methanol and 0.05 M phosphate buffer (flow rate: 1.5 ml min^{-1}).

Injection volume: 20 μl.

Wavelength analysed at: 214 nm.

A number of solutions of different species (see results for list) were injected onto the column, and the retention times produced compared with the retention times of unknown peaks in the raw slurry chromatogram depicted in figure 6.5. Following this, a sample of similar raw slurry was analysed using gas chromatography, by the Institute of Grassland and Environmental Engineering, North Wyke. These results are also presented.

6.3.3 Results and discussion

Table 6.4 shows the retention times of the species investigated and compares them with the

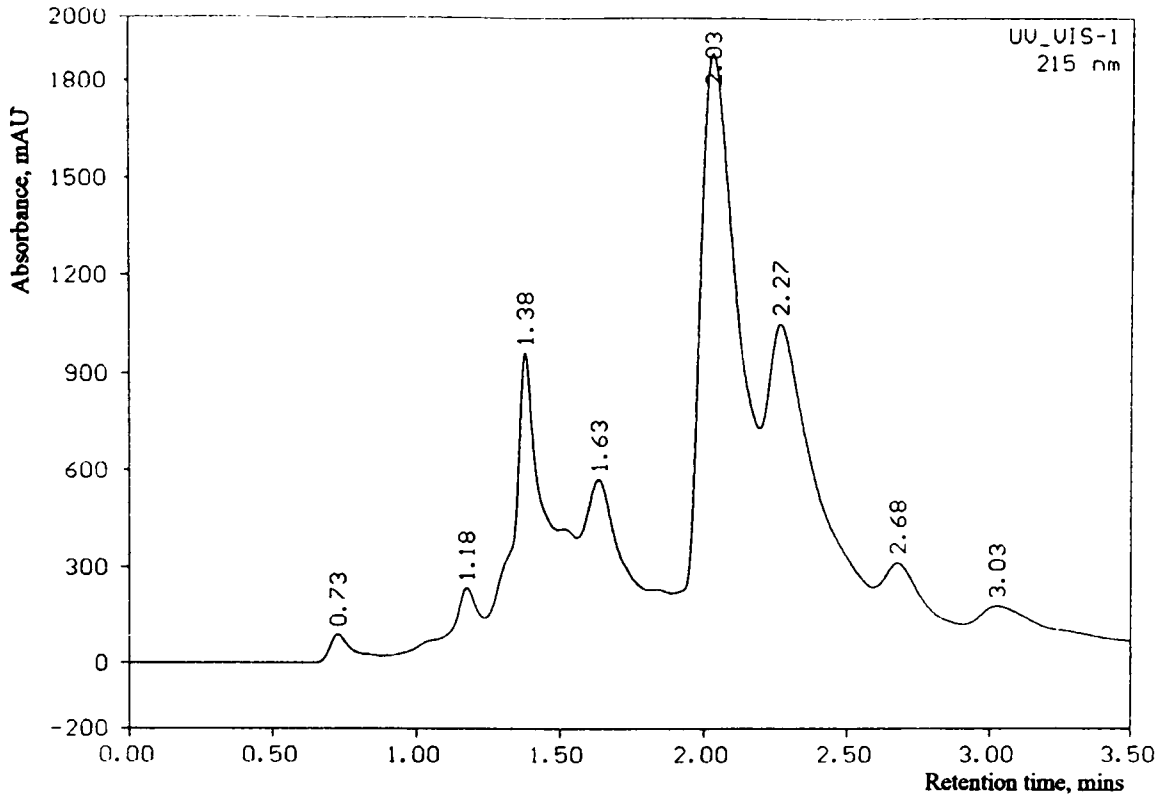


Fig. 6.5, Raw slurry chromatogram

Species	Retention Time, mins	Recognised in Raw Slurry?	Confidence, %
Phosphate	1.22	Possible, at 1.18 mins	81.6
Hypochlorite	2.35 & 2.47	No	-
Chloride	1.25	Possible, at 1.18 mins	97.0
Propionic acid	1.37	Possible, at 1.38 mins	91.4
Lactic acid	1.34	Possible, at 1.38 mins	92.2
iso-Butyric acid	1.43	No	-
n-Butyric acid	1.45	No	-
iso-Valeric acid	1.48	No	-
n-Valeric acid	1.52	No	-
Bromide	2.27	Possible, at 2.27 mins	64.2
Acetic acid	1.31	Possible, at 1.38 mins	93.7

Table 6.4, List of species examined for presence in raw slurry

unknown peaks displayed in the chromatogram in figure 6.5. Several species were found to have similar retention times, therefore the UV/VIS spectrum for each was compared with the spectrum of the raw slurry component to which they were thought to match. This gave the confidence levels in the fourth column of the table. Spectra comparison was also used to check if an exact match in retention time was a genuine one (as in the case of bromide at 2.27 mins).

Two potential candidates were recognised for the peak at 1.18 mins, these being phosphate and chloride. However, when the spectra for each was compared with the unknown spectra, it was found that chloride was the most likely candidate with a match at the 97 % level, against 81.6 % for phosphate.

Three possibilities (propanoic, lactic and acetic acids) were recognised for the peak at 1.38 mins. All had confidence levels in the range 91 to 94 %, with acetic acid having the highest at 93.7 %. Since matches below 95 % ought to be regarded with suspicion, it cannot be stated with a high degree of confidence that this peak actually is acetic acid, and therefore it more properly should remain as unrecognised.

Bromide gave an exact match with the peak at 2.27 mins in terms of retention time. Comparing spectra, however, clearly shows that the unknown peak is not bromide and the level of confidence is low at 64.2 %.

The gas chromatography results in table 6.5 identify a number of organic acids, as well as some heterocyclic compounds, as being present in the raw slurry sample. These include significant

quantities of species which were tested on the HPLC, yet failed to produce a match against the slurry components (e.g., acetic and propionic acids). This suggests that HPLC, with UV/VIS detection, is not the most suitable technique for analysing these sorts of molecules.

It should be noted that the gas chromatograph used was not able to recover inorganic ions, therefore none of these appear in table 6.5.

Species	Area
Acetic acid	6283.6
Propionic acid	3211.4
2-Methyl propanoic acid	554.3
Butanoic acid	788.2
3-Methyl butanoic acid	124.4
2-Methyl butanoic acid	262.2

Species	Area
Pentanoic acid	98.3
Phenol	3.7
4-Methyl phenol	14.8
4-ethyl phenol	3.0
Indole	0.7
3-Methyl indole	1.1

Table 6.5, List of raw slurry species with respective peak areas - as identified by gas chromatography

Reproduced with thanks to Philip Hobbs of IGER, North Wyke.

6.3.5 Conclusions

1. Identification of the peaks in the raw slurry chromatogram proved to be more difficult than at first anticipated, with only chloride being positively recognised at 1.18 mins. It is particularly disappointing that phosphate did not appear to be present in the slurry sample, as this is normally a common slurry constituent.

2. Although some common slurry anions were investigated in this piece of work, it may be that some less obvious species, with very strong absorption characteristics in the UV/VIS spectrum, were responsible for generating the unknown peaks in the raw slurry chromatogram. With out access to a database of appropriate spectra, it will require substantial further work to identify the unknown peaks using the method described here.

7. LABORATORY SCALE TREATMENT STUDIES AND DETERMINATION OF NITROGEN MASS BALANCES

7.1 Introduction

This chapter draws upon the methods covered in previous chapters, in order to construct full nitrogen mass balances around the laboratory scale reactor. Measurements were taken of slurry nitrogen in the influent and effluent streams (i.e., both the raw slurry entering the reactor and the treated slurry sampled from the reactor contents) as well as nitrogen gases emitted into the effluent air by denitrification. The total nitrogen in both streams was then compared to see if all forms of nitrogen could be accounted for by the mass balance. The mass balance studies were divided into two series of trials:

Series 1: Preliminary mass balances constructed around a medium aeration 4 day treatment. Experience gained from these experiments was used to improve techniques for the series 2 trials.

Series 2: The effect of changing residence time on nitrogen transformations was investigated using medium aeration 2, 4 and 8 day treatments.

7.2 Series 1 - Preliminary mass balance trials

7.2.1 Introduction

Slurry nitrogen was determined using the Olsen based method for total nitrogen described in section 5.6.2. No separate analysis was made for NO_2^- and NO_3^- nitrogen. This was because of

the inadequacy of the Devarda's alloy technique and also, at the time the runs were made, the HPLC was unavailable. Chemical oxygen demand (COD) and total solids (TS) were also analysed. COD gave an indication of the level of oxidation of organic material (i.e., how successfully the slurry was being aerated), whilst TS content was monitored as it can sometimes explain prevailing nitrogen flux conditions in a reactor, e.g., poor denitrification may be attributable to low TS and hence low organic substrate concentration (Oleszkiewicz, 1986).

7.2.2 Experimental conditions

The gas flow rate (of the non-redox controlled supply) in all experiments was 0.6 l min^{-1}, and raw slurry was fed into the reactor at a rate of 100 ml per hour. Despite this consistency in gas flow and slurry feed rates, differences in slurry composition inevitably lead to different redox potential ranges during the experiments (table 7.1). The overall duration of the series-1 trials was 7 months.

Run No.	Redox Range $Ag/AgCl_2$ (mV)	pH Range	Temperature Range (^0C)
1	+135 to +175	7.82 to 8.00	21.1 to 21.9
2	+155 to +180	6.10 to 6.11	not measured
3	+95 to +100	6.64 to 6.77	23.1 to 23.3
4	+65 to +80	8.10 to 8.30	25.0 to 25.4

Table 7.1, Ranges of physico-chemical parameters measured in the reactor during series-1 trials

7.2.3 Measurement of nitrogen gases

After flushing the reactor with N_2 free air for 230 minutes, at a gas flow rate of 0.6 l min^{-1}, effluent air from the reactor (as well as samples of N_2 free air to allow determination of the background N_2 dissolved in water) was collected in gas jars, and subsequently analysed for N_2 gas by mass spectrometry. The data produced by the instrument gave N_2 concentrations as percentage values. To incorporate these data more easily into the other mass balance data, it was necessary to convert percentage values into mg of nitrogen per litre of slurry. This was achieved using the following formula:

$$M_N = 4195 \cdot Z \qquad (7.1)$$

Where: Z = the percentage (v/v) of N_2 detected by the mass spectrometer,

M_N = mg of N_2 emitted per litre of slurry,

4195 is calculated from the ideal gas equation for a 3 litre jar and a gas flow rate of 0.6 l min^{-1} (Appendix IV).

Other gas sampling included the use of Dräger tubes to monitor gaseous NO, whilst in runs 3 and 4, N_2O was monitored using an infrared analyzer (ADC, Hoddesdon, UK). Although NH_3 gas was tested for (using Dräger tubes), in all runs it was found to be either absent or present in only trace (< 1 ppm) quantities.

7.2.4 Measurement of feed and treated slurry nitrogen

Treated slurry samples were collected from a sample port in the reactor floor and analysed for TS

(by drying to constant weight at 80 ^{0}C in a drying oven) and COD (by closed reflux method (APHA, 1985)). Raw slurry samples were collected, after gas flushing was complete, from the reactor end of the slurry feed pipe. These were also analysed for COD and TS.

7.2.5 Results

Run no.	N in	N out				Total N out	Percent Out/In
	slurry	slurry	Gases				
	N_{tot} (11)	N_{tot} (19)	N_2 (14)	N_2O	NO	(17)	(28)
1	2360	1940	1410	-	0.04	3350	140
2	2940	1250	1300	-	0.04	2550	90
3	2950	2140	760	8.7	0.00	2910	100
4	3170	1610	2280	9.9	0.18	3900	120

Table 7.2, Comparison of influent nitrogen (N in) against effluent nitrogen (N out) for series 1 trials

Notes:
(i) All values quoted (except percentage Out/In) are mg of N per litre of slurry.
(ii) N_{tot} = total slurry nitrogen by the Olsen based method.
(iii) Out/In refers to the effluent nitrogen streams expressed as percentages of the influent nitrogen streams.
(iv) Numbers in parenthesis are percentage errors (calculated from SD/mean x 100 %).
(v) Detailed results for series 1 trials are in Appendix III.

7.2.6 Discussion

In table 7.2, the data for run 3 indicates that all of the influent nitrogen could be accounted for in the effluent nitrogen, whilst in run 2, it is possible that the inclusion of N_2O data (not measured) may have given closer agreement between influent and effluent streams, although the concentration would have needed to be around 20 times greater than that found in runs 3 and 4. The concentration of N_2O was lower than that found by Burton et al. (1993b). From a slurry

which had been exposed to a 4 day treatment time, and TS comparable with the slurries used in these experiments (approximately 4%), they obtained an N_2O emission of 420 mg of N per litre of slurry.

In runs 1 and 4, the measured effluent nitrogen is respectively 40 and 20 % above that for the influent nitrogen. The most likely explanation for this is an ingress of N_2 into the reactor. This may have been due to poor sealing of the reactor lid early on in its operation (hence the high result for run 1). The reactor was resealed a second time, shortly after run 1 had been completed, and this seems to have been more effective. The high value for run 4 may be explained by ingress of air through worn bearings and seals around the impeller shaft. It should be noted that run 4 was taken six weeks after run 3, and by this time the bearings and, most importantly, the seals had been used without break for 10 months. Dismantling of the bearing housing after completion of run 4 confirmed that not only were the bearings worn, but the lip seal and V ring seal, which isolate the bearing housing from the reactor, had completely failed.

7.3 Series 2 - Trials to investigate the effect of changing residence time on the nitrogen mass balance

7.3.1 Introduction

A total of 6 runs were performed under similar aeration conditions to series 1, but using 3 different residence times, these being 2 day, 4 day and 8 day. Funding constraints limited the number of N_2 analyses by mass spectrometry, thus preventing further runs from being made. Since four runs with a 4 day treatment had already been made in series 1, it was decided that only a further two runs would be made using this treatment, whilst three runs would be made using the

8 day treatment. One run was planned using a 2 day treatment, from which no denitrification activity was expected. This was of particular importance for testing the validity of the gas flushing technique, which would be confirmed if a zero (or very low) concentration for N_2 was returned.

7.3.2 Experimental conditions

The mean operating conditions for the series 2 trials are summarised in table 7.3:

Residence Time (d)	Duration of Trial (d)	Redox $Ag/AgCl_2$ (mV)	pH	Temperature (^0C)	Raw Slurry TS (%)
2	14	45 (34)	7.3 (0.1)	28.9 (1.7)	2.6 (0.1)
4	36	147 (14)	6.1 (0.1)	25.6 (0.5)	2.7 (0.2)
8	54	179 (14)	6.3 (0.1)	23.5 (0.4)	2.6 (0.1)

Table 7.3, Mean values of physico-chemical parameters measured in the reactor for series-2 trials

Notes: Figures in parenthesis represent standard deviations.
More information in Appendix III.

7.3.3 Measurement of liquid phase and gas phase nitrogen

These were generally the same as in sections 7.2.3 and 7.2.4, but with the following modifications:

1. Output from the infra-red N_2O analyser was recorded every 6 minutes by a data logger. This improved the measurement of N_2O, and its subsequent incorporation into the mass balance.

2. The Olsen method for total nitrogen was replaced with separate measurements for Kjeldahl nitrogen and ammonium nitrogen (by steam distillation), and nitrite and nitrate nitrogen (by HPLC). The aim of this was to gain a more detailed picture of the quantities of different forms of nitrogen encountered in aerobic treatment. Also, wherever possible the number of replicates made on each sample was increased to four, rather than the more usual two.

7.3.4 Results

Table 7.4 summarizes the mass balance results by comparing the influent and effluent total nitrogen measured for each of the 6 runs. Table 7.5 contains the nitrogen data for the 2 day treatment, collected only on the day that N_2 was measured. Mean values for the data collected over the duration of this trial are not shown because of the unreliability of earlier data. In tables 7.6 and 7.7, the mean nitrogen data collected over the duration of each of the 2 remaining trials is presented (with pie-charts), whilst table 7.8 contains NO and NO_2 data for each run. Detailed results are presented in Appendix III.

Residence time, d	Run date	Influent total N, mg l^{-1}	Effluent total N, mg l^{-1}	Recovery, %
2	29/3/95	2798 (5)	2418 (31)	86 (36)
4	13/3/95	2973 (5)	3028 (15)	102 (20)
4	15/3/95	2715 (5)	3393 (15)	125 (20)
8	25/1/95	3032 (15)	3055 (27)	101 (42)
8	31/1/95	2872 (15)	2922 (27)	102 (42)
8	7/2/95	2906 (15)	3254 (28)	112 (43)

Table 7.4, Nitrogen mass balances for series-2 trials

Figures in parenthesis represent percentage errors (calculated from SD/mean x 100 %).

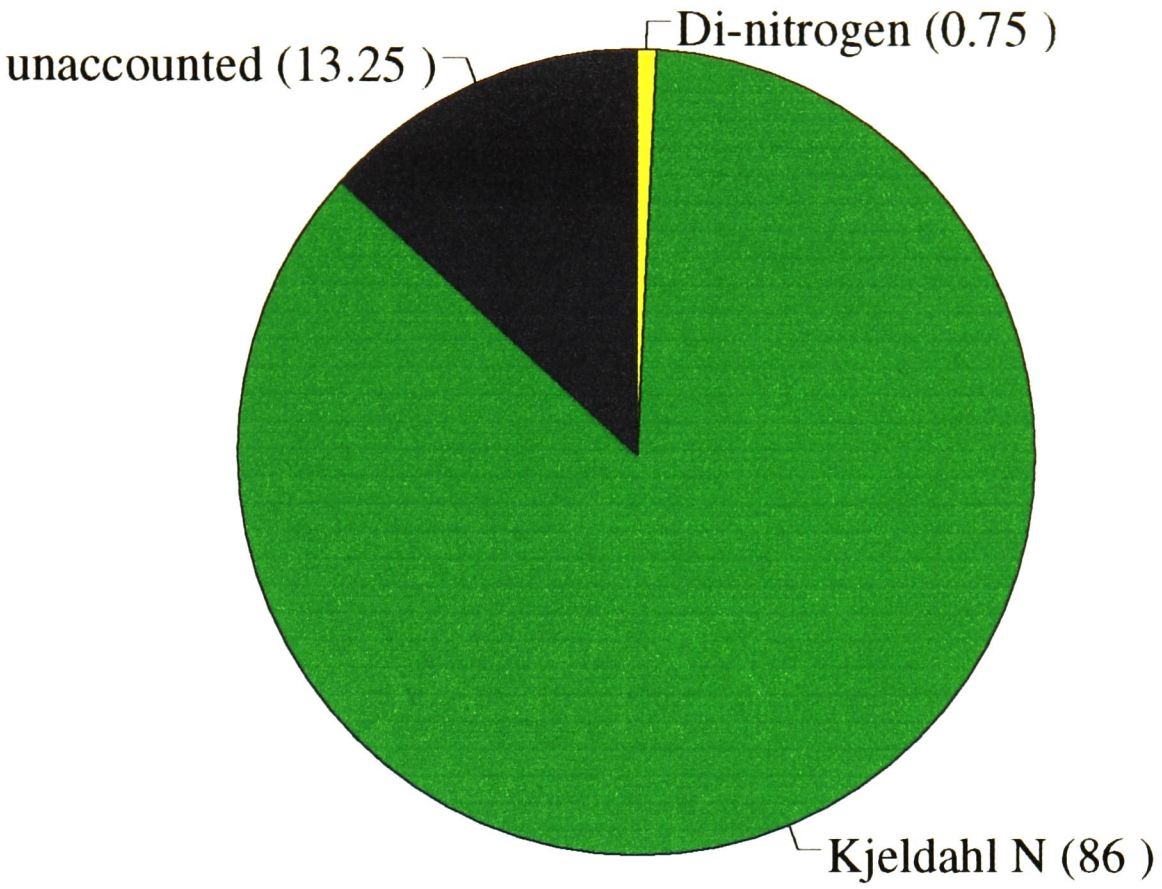


Fig. 7.1, Effluent nitrogen species (2 day residence time)

	Raw	Treated
Di-nitrogen	-	21 (620)
Nitrous Oxide	-	0
Nitrite	0	0
Nitrate	0	0
Ammonium	1979 (6)	1269 (65)
Kjeldahl N	2798 (5)	2397 (26)
TOTAL N	2798 (5)	2418 (31)

Table 7.5, Nitrogen data obtained for 2 day residence time mass balance study

Notes:

(i) Pie-chart shows treated slurry nitrogen values expressed as a percentage of raw slurry total N

(ii) Table units are mg of N per litre of slurry and numbers in parenthesis are % errors

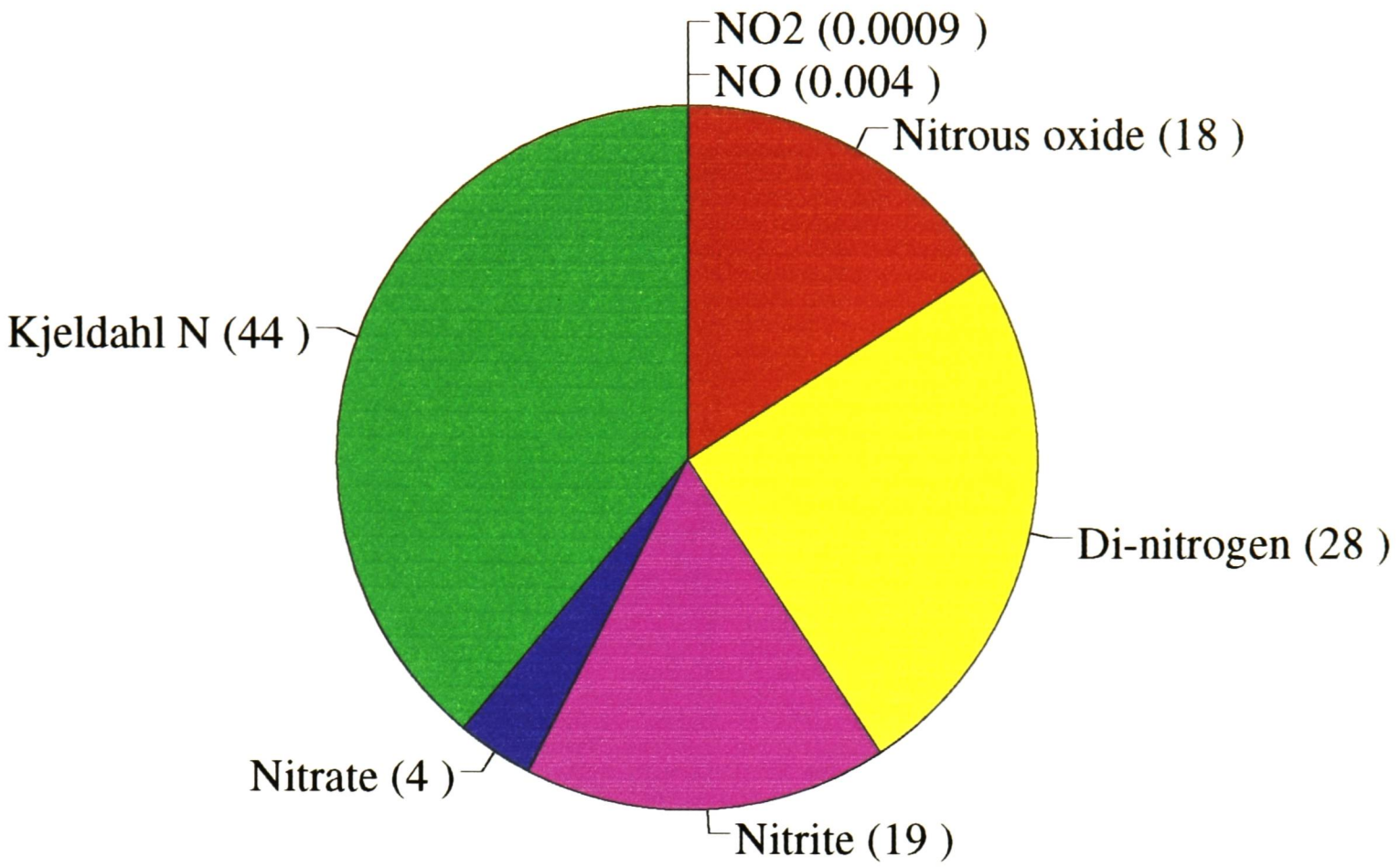


Fig. 7.2, Effluent nitrogen species (4 day residence time)

	Raw	Treated
Di-nitrogen	-	774 (19)
Nitrous Oxide	-	514 (21)
Nitrite	-	516 (4)
Nitrate	-	125 (15)
Ammonium	1941 (5)	204 (10)
Kjeldahl N	2784 (5)	1215 (14)
TOTAL N	2784 (5)	3144 (15)

Table 7.6, Mean nitrogen data obtained for 4 day residence time mass balance studies

Note: Ammonium is included in the Kjeldahl N sector of the pie-chart

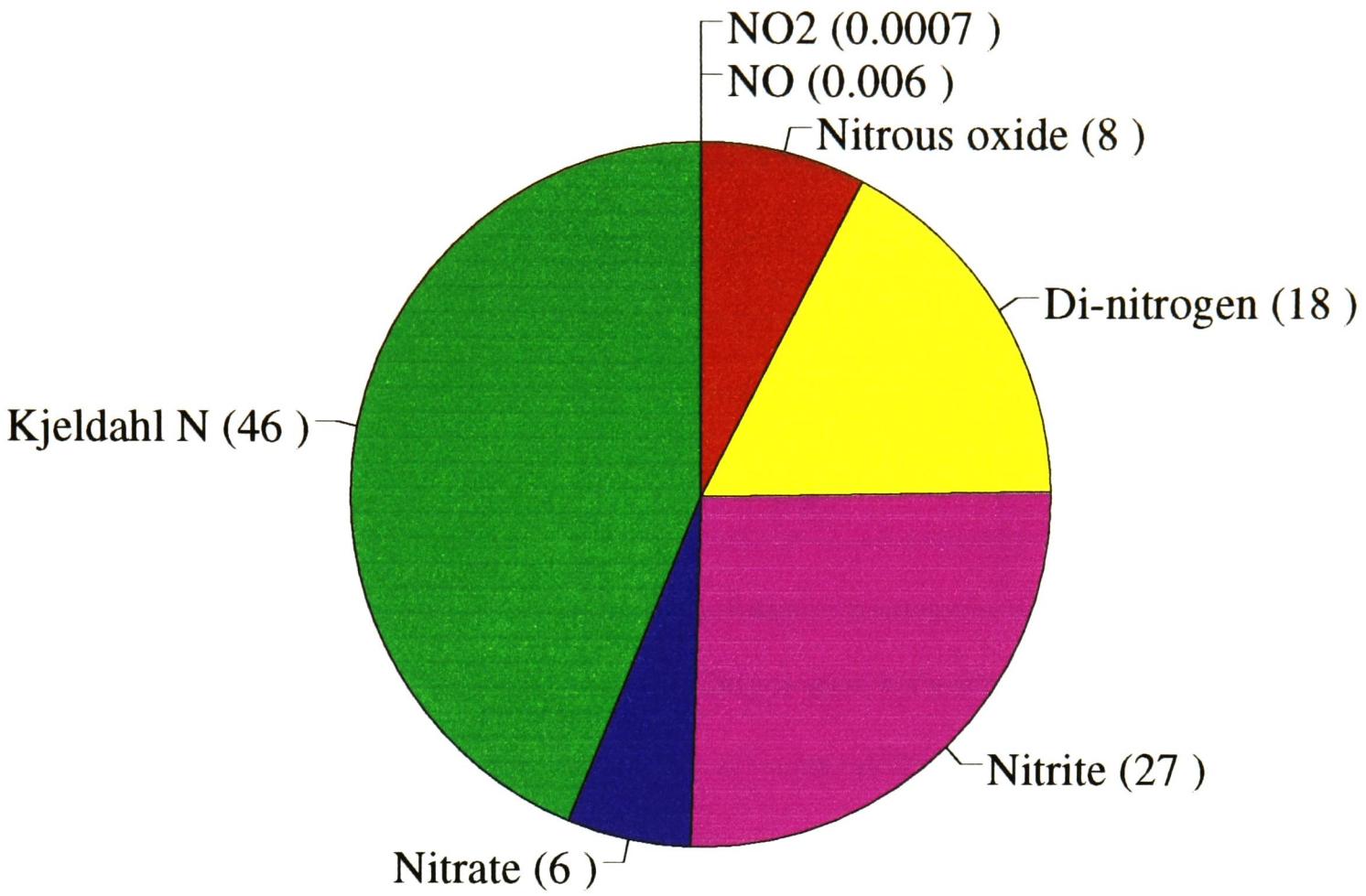


Fig. 7.3, Effluent nitrogen species (8 day residence time)

	Raw	Treated
Di-nitrogen	-	523 (29)
Nitrous Oxide	-	219 (35)
Nitrite	-	763 (12)
Nitrate	-	161 (64)
Ammonium	2199 (8)	282 (34)
Kjeldahl N	2848 (15)	1299 (29)
TOTAL N	2848 (15)	2965 (27)

Table 7.7, Mean nitrogen data obtained for 8 day residence time mass balance studies

Residence time, d	Run date	NO_2-N concentration, mg l^{-1}	NO-N concentration, mg l^{-1}
2	29/3/95	0	0
4	13/3/95	0.01	0.11
4	15/3/95	0.04	0.09
8	25/1/95	0.01	0.29
8	31/1/95	0.03	0.04
8	7/2/95	-	-

Table 7.8, NO_X gas data corresponding to mass balance runs

Notes: NO_X data was not included in the complete mass balances because of the small quantities involved, and small number of samples taken.
Concentrations are mg of N emitted per litre of slurry.

7.3.5 Discussion

7.3.5.1 Errors in liquid slurry analyses

Overall the percentage recoveries of effluent nitrogen compared with influent nitrogen were found to be variable. This appears to be due to the high percentage errors calculated, which (refer to final column of table 7.4) ranged between 20 % for the 4 day treatment and 43 % for the 8 day treatment. The primary source of these errors lay in the treated slurry analyses, where almost without exception errors were above 10 %. Treated slurry errors were also significantly higher than the equivalent raw slurry errors (usually by a factor of more than 2).

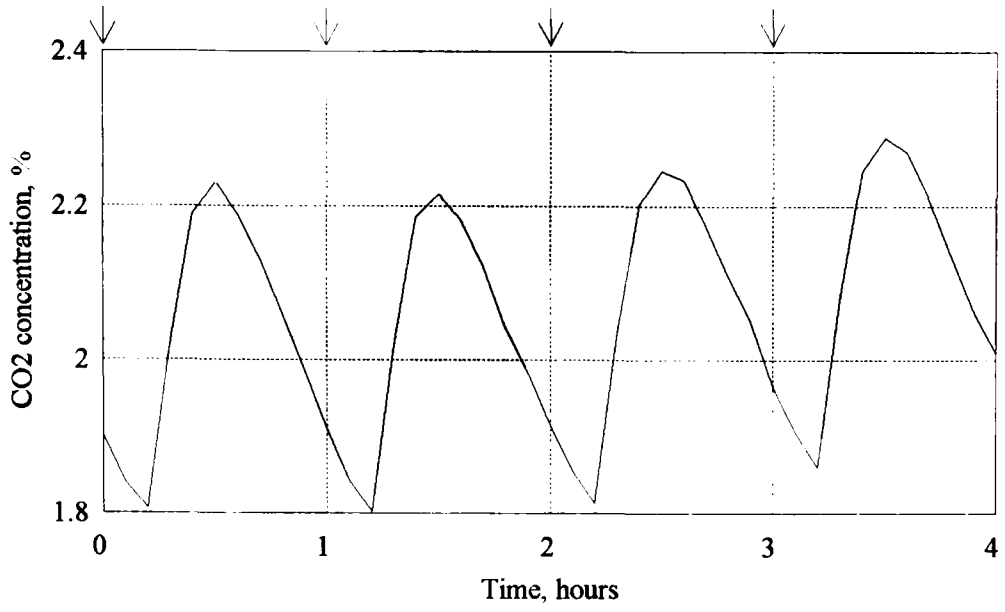
The most probable source of error in analysing slurry (raw slurry included, but treated slurry in particular) is not in the actual collection of samples, where the slurry is usually well agitated during the process, but in the operation of removing aliquots of sample to perform the analyses on. All of the slurry analyses in the mass balance work were carried out on sub-samples which

had been pipetted out rather than weighed. Slurry densities may vary slightly, depending on composition, therefore the assumption that 1 ml of slurry weighs the same as 1 g is not always valid. The problem is particularly acute in aerated (i.e., treated) slurry where the incorporation of trapped gas bubbles can lower density by as much as 10 %.

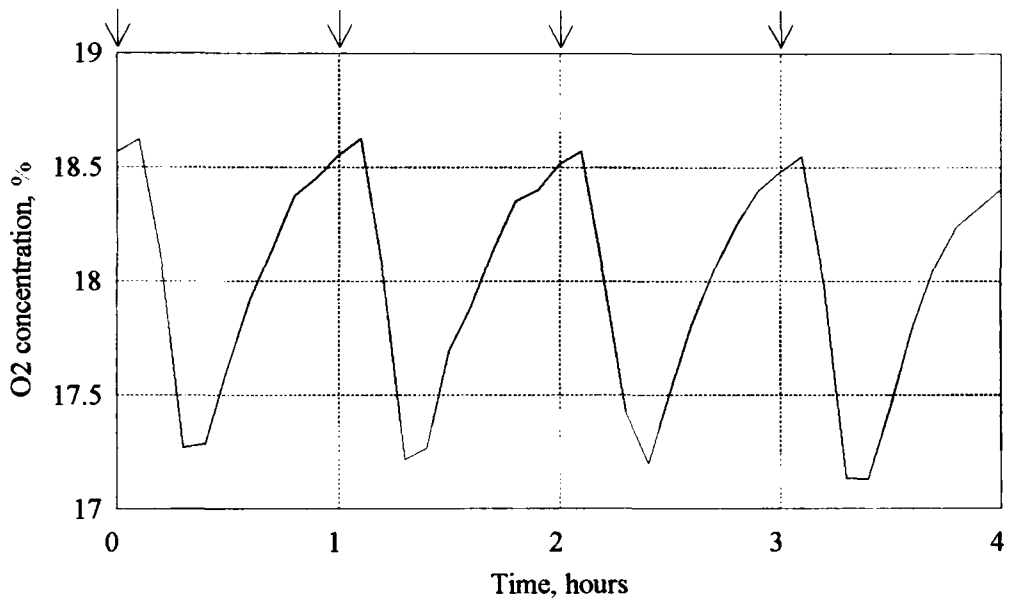
An attempt was made to allow for this problem by measuring the density of each slurry sample, and using the values obtained to correct analyses data. However, this did not lead to a reduction in error, possibly because slurry density measurements were not always made at the same time interval after collection of samples. Therefore, the amount of air trapped in samples of aerated slurry could vary, as bubbles left the liquid phase over differing time spans.

7.3.5.2 Errors in gas analysis

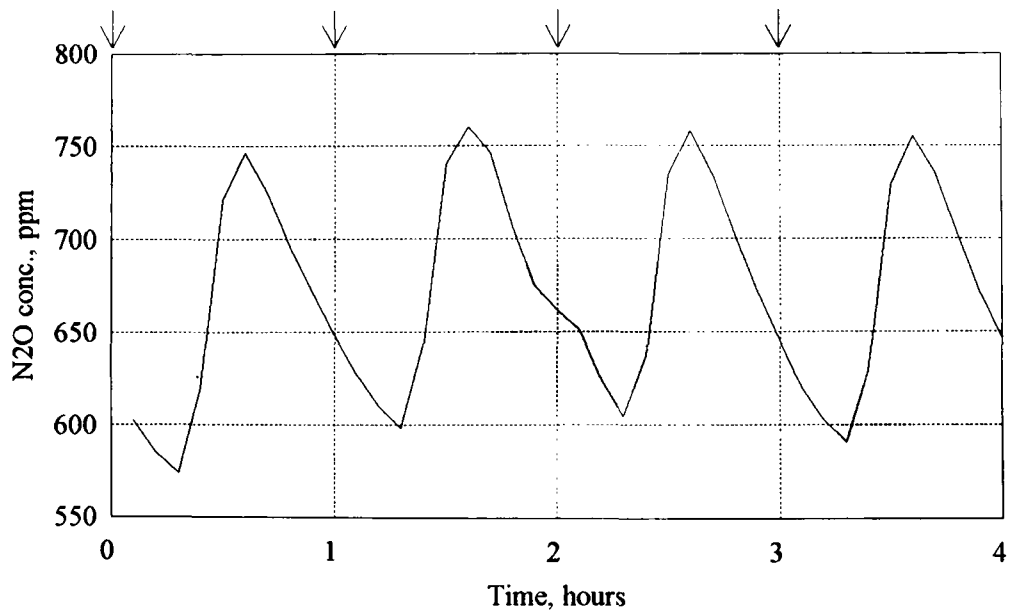
In the gas phase, there are also high percentage errors. However, this has less to do with procedural error and more to do with the fact that concentrations of gases produced by microbial activity in a continuous treatment have a tendency to cycle between maxima and minima. Graphs 7.1 to 7.3 show typical gas cycling, monitored during the 4 day treatment. In the case of N_2 measurements there is also the added error of some residual N_2 in the displaced water contaminating the gas samples. In the case of the 2 day treatment, the high percentage error for N_2 (620 %) is somewhat misleading. If the standard deviations for all the runs are examined in appendix III, it can be seen that the standard deviation for the 2 day treatment is of exactly the same order as that of the other treatments. The problem lies in calculating the standard deviation as a percentage of the mean value, to arrive at the percentage error: The low mean value, which is considerably less than the standard deviation, will therefore return a high percentage error.



Graph 7.1, 4 day treatment - CO_2 cycle



Graph 7.2, 4 day treatment - O_2 cycle



Graph 7.3, 4 day treatment - N_2O cycle

7.3.5.3 Cycling of gases during feed cycle

Graphs 7.1 to 7.3 show typical cycling of CO_2, O_2 and N_2O gases during the treatment trials, all of the graphs being synchronised. Raw slurry was fed into the reactor each hour, at which point, microbial activity increased as the incoming nutrients were oxidised. The demand for oxygen was therefore at its greatest, shortly after a slurry feed and this can be seen by the fall in the O_2 curve. The increased respirative and denitrification activity (because of the increased nutrient loading) lead to increased N_2O and CO_2 production shortly after feeding. As the nutrients were oxidised, the demand for O_2 decreased (therefore the level of O_2 measured began to rise) and respirative activity started to fall (leading to a decline in N_2O and CO_2 production) until the next slurry feed.

7.3.5.4 Comparison of mass balances for the different residence times

Referring to the pie-charts associated with tables 7.5 to 7.7, the 2 day treatment shows a complete absence of oxides of nitrogen due to the failure of nitrifying organisms to establish themselves at a residence time shorter than the 3 days. If nitrification cannot proceed, than obviously there will be an absence of the evidence of any subsequent denitrification activity. The small amount of N_2 displayed is atmospheric in origin and results from sample contamination, rather than denitrification. If this is compared with the di-nitrogen segments in the other pie-charts, the level of sample contamination by atmospheric N_2 is seen to be small in comparison with N_2 produced by denitrification.

The unaccounted for segment forms a noticeable proportion (13.25 %) of the 2 day treatment mass balance. It could not be explained by the stripping of ammonia from the slurry, as Dräger tube tests revealed only traces (< 1 ppm) in the headspace. The most feasible explanation is that

the treated slurry Kjeldahl nitrogen value underestimates, by a large proportion, the true value. The percentage error on this analysis for treated slurry (from table 7.5) is 26 %. An error margin of 15 % would be sufficient to bring the treated slurry value into the range of the raw slurry value, hence error must be the prime consideration in explaining the occurrence of this segment.

Comparing the 4 day treatment pie-chart with the 2 day, there was a significant breakdown in Kjeldahl nitrogen in the slurry from 86 % to 44 % (a breakdown of approximately half the raw slurry Kjeldahl nitrogen). Between the 4 day and 8 day treatments, however, there was no significant difference in treated slurry Kjeldahl nitrogen suggesting that treatments of longer than 4 days (under the redox conditions employed) do not enhance breakdown of this form of nitrogen.

In both 4 and 8 day treatments, the Kjeldahl nitrogen which was broken down, could be accounted for in the products of nitrification/denitrification activity. Again, Dräger tube tests revealed only traces (< 1 ppm) of ammonia in the headspace, so ammonia stripping was not an important source of nitrogen loss. Nitrite accumulation appeared to be a significant process, with both treatments having high proportions. This indicates that the slurry environment inside the reactor was not aerated sufficiently to drive the nitrification process to its full extent.

The 4 day treatment has greater proportions of the gaseous products of denitrification, and a smaller proportion of nitrite than the 8 day treatment. This may be due to increased residence time improving contact between oxygen and the slurry. Therefore, the prevalence of zones in which denitrification could occur were reduced in the 8 day treatment, leading to decreased N_2 and N_2O production. This observation is analogous to the model of Stenstrom & Song (1991)

which predicts that nitrate production at low DO levels is greater at residence times of 6 days than 3 days. However, in this work it is nitrite (because of its accumulation) which is produced in greater quantities at the longer, rather than the shorter residence time.

7.3.5.5 Observation of greater gas production for N_2 compared with N_2O

Another point of interest arising from the mass balance trials is that the concentrations of N_2 were always greater than the corresponding N_2O concentrations. The link could be taken further to include NO since N_2O concentrations were found to be higher than those of NO (although this observation lacks sufficient NO data to draw a firm conclusion). The concentrations of nitrogen gases produced, therefore, appear to increase with increasing relative proportion of N to O in each species. That is to say that, N_2 is produced in greater amounts than NO, which has a smaller ratio of N/O, whilst N_2O, which has an intermediate N/O ratio, is produced in concentrations between that of the other two species.

On the information available, there appears to be a pattern of increasing gas production as nitrogen moves through each step in the denitrification pathway, the expected proportion of each gas being:

$$N_2 > N_2O > NO$$

Burton et al. (1993b) have observed a similar pattern on 4 and 8 day aerobic treatments measuring N_2O and NO. The cause of this pattern may arise from the denitrification process favouring those pathways which terminate in N_2, followed by those which terminate in N_2O.

From the information presented in tables 7.6 and 7.7, the ratio N_2/N_2O was 1.5 : 1 for the 4 day

treatment and 2.4 : 1 for the 8 day treatment. Why the ratios should be different is not clear, and further work is needed to clarify this observation.

Trace quantities of NO_2 gas were also detected in the 4 and 8 day treatments. It is not clear if this is a by-product of nitrification or denitrification activity, or both. Burton et al. (1993b) detected small but significant quantities of NO_2. They found that the peaks in NO_2 production during a feed cycle, coincided with peaks in NO and N_2O production suggesting that the speciation of NO_2 is some how linked to formation of the other oxides of nitrogen, but the exact mechanism is not yet understood.

7.4 Statistical significance of gas flushing as a valid method for N_2 determination

The main purpose of carrying out the 2 day residence time trial was to confirm the validity of the technique for measuring N_2. The 2 day treatment was not expected to provide appropriate conditions for nitrification and denitrification, and thus no N_2 emission should have occurred. Therefore when gas flushing was applied to the 2 day trial, in order for the technique to be proved valid, it would need to return a value for N_2 concentration of the same order as the control (i.e., blank) samples containing N_2 free air.

A two tailed Student's *t*-test was performed to verify the null hypothesis that there was not a significant difference between the means of sample and blank N_2 data, for the 2 day treatment. The decision to use a *t*-test was based on the following assumptions:

1. Both sample and blank data were normally distributed.

2. The observed variances were both estimates of the same population variance (not unreasonable since both samples and blanks were measurements of the same phenomenon recorded with the same apparatus).

Further statistical analysis, using two tailed *t*-tests, were performed on N_2 data from the other trials. This was to test the alternative hypothesis that there were significant differences between the means of sample and blank N_2 data, as well as significant differences between the mean N_2 concentration of the 2 day treatment and those for the 4 and 8 day treatments. These further *t*-tests were considered important to verify that the sample data obtained for the 4 and 8 day treatments (in both series 1 and 2 trials) was of microbially derived N_2 plus background N_2. If the samples were measurements of only background N_2, then there ought to be no significant difference between the sample and blank means, and in the series 2 trials, no significant difference between the means of the 2, 4 and 8 day treatments.

The different comparisons tested, together with results, are summarized in table 7.9.

7.4.1 Results and discussion

For the 2 day treatment, the *t*-test indicates that there is no significant difference between sample and blank means. The calculated test statistic (t_o) is lower than the appropriate *t*-value, therefore there is very strong evidence that the null hypothesis (i.e., no difference between sample and blank means) should be accepted, hence the validity of the gas flushing technique is confirmed.

Comparison (means in parenthesis)	Calculated test statistic: t_o	Degrees of freedom	Significance level, %	*t*-value (from table)
2 day: samples v. blanks (0.323 v. 0.318)	0.272	8	not significant	1.397
4 day: samples v. blanks (0.524 v. 0.340)	32.9	19	0.2	3.579
8 day: samples v. blanks (0.453 v. 0.328)	290	26	0.2	3.435
4 day v. 2 day (0.185 v. 0.005)	11.30	19	0.2	3.579
8 day v. 2 day (0.125 v. 0.005)	7.80	27	0.2	3.421
Series 1: samples v. blanks (0.784 v. 0.524)	9.47	14	0.2	3.787

Table 7.9, Results of two tailed *t*-test analysis on series 2, N_2 data (results for series 1 also indicated)

The other comparisons, in table 7.9, all show t_o as being higher than the appropriate *t*-value. Therefore, there is very strong evidence that, in each case, the null hypothesis should be rejected at the 0.2 % level and the alternative hypothesis (i.e., there is a significant difference between sample and control means in the 4 and 8 day treatments) should be accepted. In other words, we can be confident that samples collected in the 4 and 8 day treatments (including the 4 day treatment in series 1) are not simply measurements of background N_2, but also include significant proportions of microbially produced N_2.

7.5 Comparison of series 1 and series 2 results

The main purpose of series 1 was to assess the suitability of various techniques and equipment by performing preliminary mass balances. Series 2 was a far more in depth study, carried out on a

greater number of analyses, thus enabling statistical inferences to be made on a much more secure base. Drawing comparisons between series 1 and 2 should be done so very cautiously, especially since they were carried out a year apart. Even so, there appears to be some close agreement between the data obtained for the series 1 trials and the 4 day trials of series 2. For example, the average N_2 recovered in the series 1 trials (1000 mg l^{-1} of N), and that recovered in the 4 day trials of series 2 (774 mg l^{-1} of N) are not too dissimilar, bearing in mind that the average for series 1 may be higher than the true value because of air ingress in some of the experimental runs. Mean treated slurry total nitrogen values for series 1 and 2, were almost identical at 1736 mg l^{-1} and 1856 mg l^{-1} respectively. Also similar raw slurry total nitrogen, and raw and treated slurry COD and TS data were collected in both sets of trials.

7.6 Conclusions

1. Significant quantities of N_2 were measured in the 4 and 8 day trials of series 2, with decreased amounts in the 8 day treatment arising from improved oxygen mass transfer, which lead to reduced denitrification activity. No nitrification or denitrification activity was measured in the 2 day trial, a fact that was used to confirm the validity of the gas flushing technique.

2. The *t*-tests confirmed that measurements made of N_2 during the 4 and 8 day treatments, by the technique of gas flushing, were genuine measurements of N_2 produced by denitrification. This is supported by the fact that under the 2 day treatment, the level of N_2 detected in the headspace was not significantly different to control measurements.

3. The mean Kjeldahl nitrogen levels in the 4 and 8 day trials of series 2 were similar, suggesting that increasing residence time beyond 4 days does not lead to further break down of organic nitrogen.

4. In series 2, the production of gaseous nitrogen species was greater from the 4 day than from the 8 day treatment (46 % against 26 %). If nitrogen is to be retained in slurry for its fertiliser value, the 8 day treatment would be preferable, since this produces a smaller quantity of the environmentally damaging N_2O compared with the 4 day treatment.

5. In the series 1 trials, the amount of N_2 which was emitted through out the study was of the order of 1000 mg of N l^{-1}. Ingress of air into the reactor, during some of the runs, may account for the higher value compared with that for the 4 day treatment in series 2. The results for series 1 emphasized the need to ensure that the reactor is completely air tight. This was successfully achieved in the series 2 work.

6. Inaccuracies were encountered in the liquid phase analysis of slurry. It is recommended that in future, analyses of slurries should be undertaken on weighed, not pipetted, sub-samples. The case for this is especially strong with treated slurry samples.

8. FURTHER WORK

8.1 Further development of N_2 measuring technique

8.1.1 Incorporation of the gas flushing technique for N_2 measurement into further full nitrogen mass balance studies, e.g., comparing low, medium and high aeration regimes, operated on the same residence time. This would enable any nitrogen removal occurring under these regimes to be fully accounted for.

8.1.2 Use of the N_2 measuring technique in the area of quantifying the different ratios of denitrification gases. This would also require the development of an accurate technique for measuring NO and possibly NO_2. The object of the work would be to see if the ratio of concentrations of denitrification gases remains relatively fixed, or if environmental conditions can be used to manipulate it. If the series is susceptible to manipulation, than this could have implications for reducing N_2O emissions from aeration systems.

8.2 Further development of HPLC as a slurry analysis tool

8.2.1 The investigation of the unidentified peaks is a particular area of interest. It would be useful to analyze raw slurries from a variety of sources to see how commonly occurring certain peaks are, or if they were simply indicative of compounds that had contaminated the slurry used in this work. Before this work could proceed however, identification of peaks ought to be a prime concern. The procurement of a UV/VIS spectral library of relevant compounds would greatly speed up this process.

9. CONCLUSIONS

1. The final mass balances indicated greater concentrations of denitrification gases produced from the 4 day treatment than from the 8 day. This is attributed to the improved contact between oxygen and slurry in the 8 day treatment, which reduced the prevalence of zones in which denitrification could take place. No nitrifying activity was expected or measured in the 2 day treatment.

2. The mass balance study showed that the quantity of nitrogen accounted for in the exit streams, compared with that entering the reactor, was on average, 86 % in the 2 day treatment, 114 % in the 4 day, and 105 % in the 8 day. Errors in liquid phase analysis of slurry nitrogen compounds are assigned to the variation in values around the target of 100 %.

3. A technique for the determination of di-nitrogen gas, derived from microbial activity, was designed and successfully implemented in a nitrogen mass balance study. The study indicates that the amount of N_2 emitted from 4 day and 8 day slurry treatments, with TS of 2.6% and medium aeration ($E_{Ag/AgCl}$ in the range +100 to +200 mV) is on average, 774 and 523 mg of N per litre of slurry, respectively.

4. Statistical tests confirm that measurements made of N_2 during the 4 and 8 day treatments, by the technique of gas flushing, were genuine measurements of N_2 produced by denitrification. This is supported by the fact that under the 2 day treatment, the level of N_2 detected in the headspace was not significantly different to control measurements.

5. The relative amounts of N_2, N_2O and NO, in the effluent air, were found to be in a series of decreasing order of concentration, i.e., $N_2 > N_2O > NO$. In the case of N_2/N_2O, the ratio was 1.5 : 1 for the 4 day treatment, and 2.4 : 1 for the 8 day. NO data was not included in this ratio as it was not deemed sufficiently accurate.

6. The Devarda's alloy steam distillation method, for the determination of inorganic nitrogen, was found to be unsuitable for analysing fresh pig slurries. This was due to inhibition of the reduction step in the reaction. The use of HPLC was, by far, the most practical method for quantifying nitrite and nitrate in slurry. The recovery of a nitrate standard from raw slurry was 97%, whilst the best recovery from the Devarda's alloy method was 34%.

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APPENDICES

APPENDIX I, HPLC Results and Background Information

Explanation:

Appendix I consists of 2 parts: The first part contains data obtained from the HPLC analysis of raw and treated slurries for nitrite and nitrate recovery (sections 6.2.1 and 6.2.2). The percentage recoveries of standards are presented in tables A1 and A2. The second part gives background information concerning the technique of HPLC as well as instrument details.

I.I Results

Replicate	Raw NO_3^-, %	Trt. NO_3^-, %	Raw NO_2^-, %	Trt. NO_2^-, %
1	90.9	92.4	76.5	79.2
2	99.0	106.8	92.7	94.1
3	98.4	104.2	87.6	98.6
4	97.9	111.5	107.1	111.6
5	97.0	93.2	78.1	87.3

Table A1, Results of NO_2^- and NO_3^- recoveries from raw and treated slurries using HPLC on pipetted samples (section 6.2.1)

Replicate	Nitrate recovery, %	Nitrite recovery, %
1	121	123
2	120	119
3	111	119
4	114	112
5	117	118
6	117	118
7	120	118
8	117	119
9	119	120
10	115	115

Table A2, Results of NO_2^- and NO_3^- recoveries from treated slurry using HPLC on weighed samples (section 6.2.2)

I.II Background information on the technique of HPLC

(i) Basic principles

Sample analysis by HPLC is similar to that involved in other types of partition chromatography, i.e., a mixture of components can be separated by the different affinities of each component for a mobile phase which moves across a stationary phase. Those components which have a higher affinity for the mobile phase will elute at a faster rate than those with a higher affinity for the stationary phase, thereby effecting a separation.

In HPLC, the stationary phase consists of an inert support (generally small silica particles, of the order of 5 μm) onto which chemical groups are bonded. The type and nature of these groups is

chosen according to the chemical properties of the material under investigation. The stationary phase is packed into a short stainless steel tube, known as 'the column'. The mobile phase is a liquid, again chosen according to the sample's chemical properties, which flows through the tube under high pressure (e.g., 100 bar). The high pressure is necessary to overcome the resistance of the densely packed stationary phase.

For the analysis of NO_2^- and NO_3^-, a strong anion-exchange column was used. In this type of column, the stationary phase consists of quaternary ammonium exchange sites bonded to the inert support. These sites are fully ionised up to a pH of about 10 (Lindsay, 1992). In a simple mixture of NO_2^- and NO_3^-, NO_3^- has a greater affinity for the positive sites, and therefore is retained in the column longer than NO_2^-.

(ii) Detection of components

The components may be detected by a number of techniques, but the one used here was ultra violet/visible (UV/VIS) light detection. This makes use of the different UV/VIS absorption characteristics of the components as they elute from the column (providing that the components actually have the ability to absorb radiation in the UV/VIS spectrum). Modern UV/VIS detectors consist of a photodiode array (PDA) which is able to simultaneously detect all the wavelengths across the specified UV/VIS spectrum.

The PDA detector expresses the size of UV/VIS absorption of eluted components in arbitrary units called milli-Absorbance Units (mAU). As each component is eluted and detected, a chart record, or chromatogram, is produced with each component being represented by a peak, and its

concentration by its peak area (the product of mAU and time). The PDA produces a UV/VIS spectrum, almost instantaneously, for each point on the chromatogram, and this enables the purity of a component to be assessed. For example, if an impurity shows a similar chromatographic response to that of a sample component, it may still be detected using its different UV/VIS absorption characteristics, and from the way it modifies those of the sample component.

The PDA also has the advantage of allowing the optimum wavelength, at which a component absorbs UV/VIS, to be rapidly determined. This enables the best peak resolution on the chromatogram to be obtained.

(iii) HPLC instrument data

The HPLC used was a Gynkotek model 480 with PDA UV/VIS detector and ternary gradient pump, capable of delivering mobile phase flow rates of between 0.001 and 8.0 ml min^{-1} up to a maximum pressure of 414 bar (supplied by Severn Analytical, Macclesfield, Cheshire, UK). Sample volumes in the range of 1 to 250 µl could be autoinjected from an autosampler carousel model GINA 50 (same supplier).

APPENDIX II, Data from experiments to assess methods for slurry nitrogen analysis

Explanation:

Appendix II gives the raw data obtained for each of the steam distillation based methods detailed in chapter 5. Results calculated from the titration volumes are expressed as mg of nitrogen per ml of slurry sample. The calculations were performed using the equation (5.1) in section 5.2.2.

Sample	Titre, ml	NH_4^+-N, mg ml^{-1}
blank 1	0.50	-
blank 2	0.50	-
raw 1	1.45	0.27
raw 2	1.48	0.28
raw 3	1.52	0.29
raw 4	1.48	0.28
treated 1	10.13	2.78
treated 2	9.79	2.68
treated 3	10.01	2.74
treated 4	10.04	2.75

Table A3, Ammoniacal nitrogen content of raw and treated slurries (section 5.2.3)

Sample	Titre, ml	N_k, mg ml^{-1}
blank 1	0.50	-
blank 2	0.38	-
1	4.16	1.20
2	4.47	1.29
3	4.47	1.29
4	4.40	1.27

Table A4, Kjeldahl nitrogen (N_k) results for treated slurry (section 5.3.3)

Sample	Titre, ml	N_k, mg ml^{-1}
blank 1	0.41	-
blank 2	0.36	-
1	10.44	3.01
2	9.85	2.84
3	10.61	3.06
4	10.23	2.95

Table A5, Kjeldahl nitrogen (N_k) results for raw slurry (section 5.3.3)

	Titre 1, ml	Titre 2, ml	Mean Titre, ml	N, mg ml^{-1}	% Std. Recovery
blanks	0.37	0.39	0.38	-	-
NO_2^- std.	3.46	3.44	3.45	0.92	92
NO_3^- std.	3.38	3.47	3.43	0.91	91
slurry	14.53	14.16	14.35	4.19	-
NO_2^-+slu.	15.04	15.06	15.05	4.40	20
NO_3^-+slu.	14.79	14.52	14.66	4.27	9

Table A6, Recoveries of NO_2^- and NO_3^- standards from raw slurry by Devarda's alloy method (section 5.4.3)

	Blanks	Standard	Slurry	Std. + Sl.
titre1, ml	0.36	3.47	15.27	15.45
titre2, ml	0.35	3.52	15.56	15.32
titre3, ml	0.34	3.58	15.47	15.69
mean, ml	0.35	3.52	15.43	15.49
N, mg ml^{-1}	-	0.95	4.52	4.54
% recovery	-	95	-	2

Table A7, Standard NO_3^- recovery by Devarda's alloy method after increasing distillation time (section 5.5.2 method (a))

	Blanks	Standard	Slurry	Std. + Sl.
titre1, ml	0.68	1.31	3.68	3.78
titre2, ml	0.70	1.21	3.54	3.51
titre3, ml	0.68	1.26	3.57	3.63
mean, ml	0.69	1.26	3.60	3.64
N, mg ml^{-1}	-	0.85	4.36	4.42
% recovery	-	85	-	6

Table A8, Standard NO_3^- recovery by Devarda's alloy method after increasing pH and Reducing sample volume to 1 ml (section 5.5.2 method (b))

Sample	Titre, ml	N_{tot}, mg ml^{-1}
blank	0.45	-
1	2.71	1.63
2	2.66	1.59
3	1.08	0.45
4	2.64	1.58

Table A9, Total nitrogen (N_{tot}) results for treated slurry (section 5.6.3)

Sample	Titre, ml	N_{tot}, mg ml^{-1}
blank	0.52	-
1	2.48	-
2	4.96	3.20
3	5.10	3.30
4	4.87	3.14

Table A10, Total nitrogen (N_{tot}) results for raw slurry (section 5.6.3)

	Blanks	NH_2OH Std.	Slurry	Std. + Sl.
titre1, ml	0.47	3.57	13.38	14.67
titre2, ml	0.44	3.68	13.55	14.46
mean, ml	0.455	3.625	13.47	14.565
mg N ml^{-1}	-	0.95	3.9	4.23
recovery, %	-	95	-	33

Table A11, Recovery of NH_2OH standard by Devarda's alloy method (section 5.7.3)

APPENDIX III, Data from Mass Balance Experiments

Explanation:

Appendix III contains data from the mass spectrometric analysis of gases for nitrogen, and data from the laboratory analyses of raw and treated slurries. Also included are the physical parameters and N_2O concentration recorded by data logger in the series 2 trials. The appendix is organised as follows:

1. Data collected for series 1 trials.

2. Data collected for series 2 trials (i.e., 2, 4 and 8 day treatments)in the order of:

   (a) Di-nitrogen gas data,

   (b) raw and treated slurry data,

   (c) nitrous oxide gas data,

   (d) temperature, pH and redox data.

Table A12: Run 1 (16/3/94)			
Sampling time, mins	N2 concentration, %		
	samples, A	blanks, B	A-(mean B)
230	0.812	0.494	0.318
240	0.798		0.304
245	0.884		0.390
MEAN	0.831	0.494	0.337
SD	0.038	-	0.038

Table A13: Run 2 (20/4/94)			
Sampling time, mins	N2 concentration, %		
	samples, A	blanks, B	A-(mean B)
225	0.746	0.482	0.264
230	0.827		0.345
240	0.779		0.297
245	0.821		0.339
MEAN	0.793	0.482	0.311
SD	0.033	-	0.156

Table A14: Run 3 (31/5/94)			
Sampling time, mins	N2 concentration, %		
	samples, A	blanks, B	A-(mean B)
240	0.781	0.545	0.166
250	0.752	0.574	0.137
255	0.723		0.108
260	0.706		0.091
MEAN	0.741	0.615	0.126
SD	0.029	0.079	0.029

Table A15: Run 4 (11/7/94)			
Sampling time, mins	N2 concentration, %		
	samples, A	blanks, B	A-(mean B)
226	1.212	0.666	0.531
231	1.199	0.672	0.518
236	1.224	0.704	0.543
MEAN	1.212	0.681	0.531
SD	0.010	0.017	0.010

Series 1: Di-nitrogen gas data

Table A16: Raw slurry			
DATE	TS (%)	COD (mg/l	Total N (mg/l)
16/3/94	2.22	33719	2415
(Run 1)		31416	2307
mean	2.22	32568	2361
20/4/94	2.63	36207	2950
(Run 2)		38704	2936
mean	2.63	37456	2943
31/5/94	2.68	37806	2834
(Run 3)		41418	3071
mean	2.68	39612	2953
11/7/94	3.93	43403	3200
(Run 4)		40509	3140
mean	3.93	41956	3170
MEAN	2.87	37898	2857
Std. Dev.	0.64	3465	300

Table A17: Treated slurry			
DATE	TS (%)	COD (mg/l	Total N (mg/l)
16/3/94	2.26	13614	1867
(Run 1)		14766	2012
mean	2.26	14190	1940
20/4/94	2.02	17229	1154
(Run 2)		17978	1348
mean	2.02	17604	1251
31/5/94	2.10	21239	2098
(Run 3)		18349	2185
mean	2.10	19794	2142
11/7/94	2.58	20576	1630
(Run 4)		24434	1590
mean	2.58	22505	1610
MEAN	2.24	18523	1736
Std. Dev.	0.21	3045	338

Series 1: Liquid phase data for raw and treated slurry

Table A18: 2 Day residence time (29/3/95)			
Sampling time, mins	N2 concentration, %		
	samples, A	blanks, B	A-(mean B)
230	0.312	0.310	-0.006
239	0.364	0.317	0.046
248	0.323	0.328	0.005
257	0.311		-0.007
266	0.371		0.053
275	0.304		-0.014
284	0.276		-0.042
MEAN	0.323	0.318	0.005
SD	0.031	0.007	0.031

Table A19: 4 Day residence time (13/3/95)			
Sampling time, mins	N2 concentration, %		
	samples, A	blanks, B	A-(mean B)
230	0.614	0.418	0.230
239	0.580	0.387	0.196
248	0.545	0.375	0.161
257	0.530	0.357	0.146
266	0.561		0.177
275	0.588		0.204
284	0.594		0.210
MEAN	0.573	0.384	0.189
SD	0.027	0.022	0.027

Table A20: 4 Day residence time (15/3/95)			
Sampling time, mins	N2 concentration, %		
	samples, A	blanks, B	A-(mean B)
230	0.449	0.279	0.153
239	0.474	0.311	0.178
248	0.513	0.297	0.217
257	0.528		0.232
266	0.503		0.207
275	0.464		0.168
284	0.396		0.100
MEAN	0.475	0.296	0.180
SD	0.042	0.013	0.042

Table A21: 8 Day residence time (25/1/95)			
Sampling time, mins	N2 concentration, %		
	samples, A	blanks, B	A-(mean B)
230	0.506	0.330	0.178
235	0.485	0.347	0.157
240	0.435	0.312	0.107
245	0.444	0.291	0.116
250	0.467	0.343	0.139
255	0.423	0.345	0.095
260	0.403		0.075
MEAN	0.452	0.328	0.124
SD	0.033	0.020	0.033

Table A22: 8 Day residence time (31/1/95)			
Sampling time, mins	N2 concentration, %		
	samples, A	blanks, B	A-(mean B)
230	0.517	0.330	0.189
238	0.495	0.347	0.167
246	0.477	0.312	0.149
262	0.487	0.291	0.159
270	0.42	0.343	0.092
278	0.412	0.345	0.084
286	0.384		0.056
MEAN	0.456	0.328	0.128
SD	0.046	0.020	0.046

Table A23: 8 Day residence time (7/2/95)			
Sampling time, mins	N2 concentration, %		
	samples, A	blanks, B	A-(mean B)
230	0.469	0.330	0.141
246	0.439	0.347	0.111
262	0.507	0.312	0.179
270	0.463	0.291	0.135
278	0.434	0.343	0.106
286	0.423	0.345	0.095
294	0.454		0.126
302	0.414		0.086
MEAN	0.450	0.328	0.122
SD	0.028	0.020	0.028

Table A24: Combined averages of N2 data for each residence time			
Res. time	Mean, %	SD	Error,%
2 day	0.005	0.031	620
4 day	0.185	0.035	19
8 day	0.125	0.036	29

Series 2: Di-nitrogen gas data

DATE	TS (%)	COD (mg/l)	Nkj (mg/l)	NH4-N(mg/l)	NO2-N(mg/l)	NO3-N(mg/l)
23/3/95	2.57	46528	2573	1768	0	0
		45270	2575	1759		
		46528	2601	1866		
		46528	2616	1900		
mean	2.57	46214	2591	1823	0	0
24/3/95	2.56	44767	2598	1952	0	0
		51055	2601	1927		
		51055	2590	1938		
		46025	2601	1898		
mean	2.56	48226	2598	1929	0	0
28/3/95	2.44	38403	2800	2036	0	0
		37148	2901	2140		
		38403	2855	2114		
		39658	2915	2128		
mean	2.44	38403	2868	2105	0	0
29/3/95	2.70	45842	2636	1987	0	0
		47094	2889	2019		
		47094	2731	1863		
		45842	2936	2048		
mean	2.70	46468	2798	1979	0	0
MEAN	2.57	44828	2714	1959	-	-
Std. Dev.	0.09	4096	138	114	-	-
% Error	4	9	5	6	-	-

Table 25, Series 2: Raw slurry data (2 day residence time)

DATE	TS (%)	COD (mg/l)	Nkj (mg/l)	NH4-N(mg/l)	NO2-N(mg/l)	NO3-N(mg/l)
23/3/95	2.59	12157	1167	276	483	97
		13170	1341	273		
		14183	1347	273		
		14183	1478			
mean	2.59	13423	1333	274	483	97
24/3/95	2.49	20920	1519	209	492	66
		19857	1525	212		
		18844	1508	209		
		20367	1522	203		
mean	2.49	19997	1519	208	492	66
28/3/95	2.30	21773	2354	854	0	0
		22781	2401	927		
		22781	2365	936		
		22781	2368	933		
mean	2.30	22529	2372	913	0	0
29/3/95	2.76	31822	2374	1197	0	0
		30815	2426	1313		
		29808	2402	1284		
		30815	2385	1281		
mean	2.76	30815	2397	1269	0	0
MEAN	2.54	21691	1905	692	-	-
Std. Dev.	0.17	6268	487	447	-	-
% Error	7	29	26	65	-	-

Table 26, Series 2: Treated slurry data (2 day residence time)

DATE	TS (%)	COD (mg/l)	Nkj (mg/l)	NH4-N(mg/l)	NO2-N(mg/l	NO3-N(mg/l
06/3/95	2.76	37368	2952	2021	0	0
		39859	2957	2018		
		41105				
		39859				
mean	2.76	39548	2955	2020	0	0
09/3/95	2.60	45045	2778	1958	0	0
		43794	2778	1963		
		42542				
		42542				
mean	2.60	43481	2778	1961	0	0
10/3/95	2.82	39493	2586	1990	0	0
		40742	2985	2095		
		38242				
		40742				
mean	2.82	39805	2786	2043	0	0
13/3/95	3.05	42754	2976	2120	0	0
		44012	2970	2123		
		42754				
		45269				
mean	3.05	43697	2973	2122	0	0
15/3/95	2.62	40206	2670	1828	0	0
		38950	2670	1842		
		37693	2778	1886		
		41463	2741	1888		
mean	2.62	39578	2715	1861	0	0
17/3/95	2.60	37690	2763	1851	0	0
		37690	2841	1937		
		38946				
		37690				
mean	2.60	38004	2802	1894	0	0
20/3/95	2.56	41341	2585	1823	0	0
		38835	2784	1938		
		41341	2582	1772		
		42593	2723	1883		
mean	2.56	41028	2669	1854	0	0
MEAN	2.72	40734	2784	1941	-	-
Std. Dev.	0.16	2289	134	102	-	-
% Error	6	6	5	5	-	-

Table 27, Series 2: Raw slurry data (4 day residence time)

DATE	TS (%)	COD (mg/l)	Nkj (mg/l)	NH4-N(mg/l)	NO2-N(mg/l	NO3-N(mg/l
06/3/95	2.44	20741	1406	209	513	143
		20741	1415	200	481	139
		22816			515	142
		21778			514	142
mean	2.44	21519	1411	205	506	142
09/3/95	2.30	16241	1246	232	514	*0
		16241	1243	237		
		17256				
		17256				
mean	2.30	16749	1245	235	514	*0
10/3/95	2.27	14729	1240	203	532	*0
		16746	1245	226		
		18763				
		18763				
mean	2.27	17250	1243	215	532	*0
13/3/95	2.37	22309	1231	223	486	94
		23323	1265	223		
		24337				
		22309				
mean	2.37	23070	1248	223	486	94
15/3/95	3.50	16211	1344	225	519	104
		16211	1344	225		
		17224	1361	225		
		16211	1364	228		
mean	3.50	16464	1353	226	519	104
17/3/95	2.04	19386	839	222	530	114
		18366	742	145		
		17345				
		17345				
mean	2.04	18111	791	184	530	114
20/3/95	2.35	17313	1104	214	560	120
		19351	1128	216		
		17313	1157	216		
		19351	1196			
mean	2.35	18332	1146	215	560	120
MEAN	2.47	18785	1215	204	516	125
Std. Dev.	0.44	2531	174	20	21	18
% Error	18	13	14	10	4	15

Table 28, Series 2: Treated slurry data (4 day residence time)

Note: Asterisked values excluded from mean and SD calculations

DATE	TS (%)	COD (mg/l)	Nkj (mg/l)	NH4-N(mg/l)	NO2-N(mg/l)	NO3-N(mg/l)
23/1/95	2.62	40627	1508	1889	0	0
		39841	1482	2224		
		41143		2200		
		41143		2417		
mean	2.62	40689	1495	2183	0	0
24/1/95	*6.71	46517	3109	2250	0	0
		46517	3069	2218		
		45247	3080	2198		
		46517	3040	2232		
mean	*6.71	46200	3075	2225	0	0
25/1/95	2.55	38983	3146	2512	0	0
		42755	2999	2386		
		38983	3011	2621		
		38983	2970	2572		
mean	2.55	39926	3032	2523	0	0
26/1/95	2.62	42134	3305	2242	0	0
		43396		2251		
		42134		2248		
		40873		2271		
mean	2.62	42134	3305	2253	0	0
31/1/95	2.62	42200	2780	1958	0	0
		44728	2895	2065		
		42200	2904	2051		
		41948	2909	2074		
mean	2.62	42769	2872	2037	0	0
03/2/95	2.59	55059	2837	1955	0	0
		52557	2895	2085		
		47551	2909	2183		
		48803	2901	2163		
mean	2.59	50993	2886	2097	0	0
07/2/95	2.61	33724	2933	2046	0	0
		36222	2895	2112		
		33724	2987	2095		
		33724	2809	2210		
mean	2.61	34349	2906	2116	0	0
09/2/95	2.75	33777	2897	2134	0	0
		31275	2937	2122		
		31275				
		32526				
mean	2.75	32213	2917	2128	0	0
MEAN	2.62	41159	2848	2199	-	-
Std. Dev.	0.06	5813	414	168	-	-
% Error	2	14	15	8	-	-

Table 29, Series 2: Raw slurry data (8 day residence time)

DATE	TS (%)	COD (mg/l)	Nkj (mg/l)	NH4-N(mg/l)	NO2-N(mg/l)	NO3-N(mg/l)
23/1/95	2.23	22155	63	179	767	84
		25190	61	176	778	85
		18816		170	779	85
		20030		173	780	85
mean	2.23	21548	62	175	776	85
24/1/95	2.19	25760	1451	211	635	73
		24445	1405	213	645	74
		25760	1448	208	655	75
		24445		208	664	76
mean	2.19	25103	1435	210	650	75
25/1/95	2.24	22816	1197	314	769	99
		20742	1291	260	774	100
		20742	1290	257	775	100
		21779	1273	263	778	100
mean	2.24	21520	1263	274	774	100
26/1/95	2.85	20575	1531	229	635	86
		18929	1520	232	635	85
		18929	1537	226	646	86
		18929		229	652	88
mean	2.85	19341	1529	229	642	86
31/1/95	2.13	21631	1090	196	793	148
		19610	1315	305	782	146
		20620	1306	325	762	142
		20620	1312	314	746	139
mean	2.13	20620	1256	285	771	144
03/2/95	2.24	22289	1551	196	871	130
		23302	1531	178	904	131
		24315	1479	366	901	130
		22289	1514	377	891	128
mean	2.24	23049	1519	279	892	130
07/2/95	2.07	16098	1381	440	885	362
		17104	1451	437	876	358
		17104	1430	431	886	360
		16098	1439	423	902	368
mean	2.07	16601	1425	433	887	362
09/2/95	2.22	9061	1489	473	716	306
		11075	1406	447	729	312
		11075			672	294
		10068			725	311
mean	2.22	10320	1448	460	711	306
MEAN	2.27	19763	1299	282	763	161
Std. Dev.	0.23	4407	374	97	89	103
% Error	10	22	29	34	12	64

Table 30, Series 2: Treated slurry data (8 day residence time)

Table 31: 4 Day residence time					
DATE	Concentration, ppm			Concentration, mg/l	
	mean	SD	n	mean	SD
6/3/95	863	114	240	569	75
9/3/95	669	69	240	441	45
10/3/95	749	109	240	494	72
13/3/95	618	130	240	407	86
15/3/95	1004	203	240	662	134
17/3/95	1017	198	110	670	131
20/3/95	541	351	170	357	231
MEAN	780	168		514	111

Table 32: 8 Day residence time					
DATE	Concentration, ppm			Concentration, mg/l	
	mean	SD	n	mean	SD
23/1/95	306	212	240	202	140
24/1/95	183	173	240	121	114
25/1/95	604	260	160	398	171
26/1/95	679	137	105	448	90
31/1/95	325	34	200	214	22
3/2/95	256	27	130	169	18
7/2/95	103	37	148	68	24
9/2/95	206	49	240	136	32
MEAN	333	116		219	77

Series 2: Average nitrous oxide data monitored in reactor headspace

Note: n = number of data points recordered during each logging period

Table 33: 2 Day residence time												
DATE	Slurry temp., deg. C			Ambient temp., deg. C			pH			Redox, mV		
	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
23/3/95	28.8	0.6	88	21.1	1.1	88	6.2	0.0	88	99	4	286
24/3/95	29.4	0.4	100	21.6	0.8	100	6.2	0.0	100	102	4	322
28/3/95	27.5	3.7	76	17.2	1.4	76	8.0	0.1	76	-48	59	244
29/3/95	30.3	3.1	86	18.4	1.1	86	8.0	0.1	86	-28	88	278
30/3/95	28.5	0.7	100	19.7	0.7	100	8.2	0.1	100	101	13	324
MEAN	28.9	1.7		19.6	1.0		7.3	0.1		45	34	

Table 34: 4 Day residence time												
DATE	Slurry temp., deg. C			Ambient temp., deg. C			pH			Redox, mV		
	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
6/3/95	25.1	0.3	79	17.1	0.4	79	6.1	0.0	79	133	5	256
9/3/95	25.4	0.4	90	18.1	1.4	90	6.1	0.1	90	147	6	291
10/3/95	26.2	0.3	96	18.8	0.8	96	6.1	0.1	96	158	7	310
13/3/95	24.3	0.4	81	17.7	0.4	81	6.2	0.1	81	141	7	262
15/3/95	26.2	0.4	88	18.7	1.3	88	6.1	0.1	88	149	7	286
17/3/95	26.5	0.5	48	18.8	1.4	48	6.1	0.0	48	153	5	156
20/3/95	25.5	1.0	93	18.6	1.3	93	6.2	0.0	93	150	61	300
MEAN	25.6	0.5		18.3	1.0		6.1	0.1		147	14	

Table 35: 8 Day residence time												
DATE	Slurry temp., deg. C			Ambient temp., deg. C			pH			Redox, mV		
	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
23/1/95	21.3	0.8	106	16.5	0.9	106	6.5	0.1	106	117	58	341
24/1/95	22.8	0.2	74	16.9	0.5	74	6.4	0.1	74	168	5	240
25/1/95	22.8	0.4	93	16.7	0.4	93	6.3	0.1	93	177	6	293
26/1/95	22.7	0.3	93	16.0	0.4	93	6.3	0.1	93	185	4	303
31/1/95	24.6	0.6	101	18.5	1.0	101	6.2	0.1	101	193	7	325
3/2/95	24.6	0.5	95	18.6	0.4	95	6.1	0.1	95	199	16	305
7/2/95	25.7	0.2	79	19.2	0.4	79	6.1	0.1	79	203	8	255
9/2/95	23.8	0.5	89	16.5	0.4	89	6.1	0.0	89	193	6	287
MEAN	23.5	0.4		17.4	0.6		6.3	0.1		179	14	

Series 2: Average physical conditions monitored in the reactor environment

APPENDIX IV, Miscellaneous

Explanation:

Included here are calculations of two values used in equations in the thesis (relevant section numbers are indicated). Also included is a detailed drawing of the mechanical foam breaker described in section 3.4.1.

1. Calculation of the figure 4195 in equation 7.1 (section 7.2.3)

Explanation:

4195 is the factor used to multiply the percentage volume (Z) of N_2 gas (recovered during the gas analysis by mass spectrometry) in order to calculate the mass of N_2 emitted per litre of slurry (M_N). It was calculated as follows:

Volume of gas sample jars is 3 litres. Therefore the number of moles of gas (n) contained in this volume can be calculated from the ideal gas equation, assuming a temperature of 20 ^{0}C and 1 atmosphere (1.013×10^5 N m^{-2}).

$$n = \frac{PV}{RT}$$

$$= \frac{1.013 \times 10^5 \times 3 \times 10^{-3}}{8.314 \times (273 + 20)}$$

$$\therefore \; n = 0.1248 \text{ moles}$$

Let Z = the percentage of N_2 gas detected in 3 litres,

$$\therefore \quad \mathbf{n\ of\ N_2} = \frac{0.1248\ \mathbf{x}\ \mathbf{Z}}{100}$$

$$= 1.248\ Z \times 10^{-3} \quad \text{moles}$$

$\therefore$ given that the molecular mass of N_2 is 28.014, the mass of N_2 in 3 litres = 0.03496 Z g

Flow rate of flush gas = 0.6 l min^{-1}, $\therefore$ 3 litres of gas are collected every 5 minutes.

1 litre of raw slurry is fed every 10 hours, $\therefore$ in 10 hours, mass of N_2 released is:

$$M_N = 12 \times 10 \times 0.03496\ Z \quad g$$

$$= 4.915\ Z \quad g\ l^{-1} \text{ of slurry}$$

$$= \underline{4195\ Z \quad mg\ l^{-1} \text{ of slurry}}$$

2. Calculation of the number of mg of N which are equivalent to T ml of 0.05 M standard H_2SO_4 (see equation 5.1, section 5.2.2)

$$H_2SO_4 + 2NH_4^+ + 2OH^- \rightarrow (NH_4)_2SO_4^{2-} + 2H_2O$$

i.e., 1 mole of H_2SO_4 ≡ 2 moles of NH_4^+ (NH_4^+ being that which is collected in boric acid during steam distillation).

1 ml of 0.05 M H_2SO_4 contains 5×10^{-5} moles,

$\therefore$ 1 ml of 0.05 M H_2SO_4 ≡ 10×10^{-5} moles of NH_4^+

≡ 1.8×10^{-3} g of NH_4^+

$\equiv 1.8 \times 10^{-3} \times 14/18$ g of N

(where 14/18 is the proportion of N in NH_4^+)

$\equiv 1.4 \times 10^{-3}$ g of N

$\therefore$ <u>1 ml of 0.05 M $H_2SO_4 \equiv 1.4$ mg of N</u>

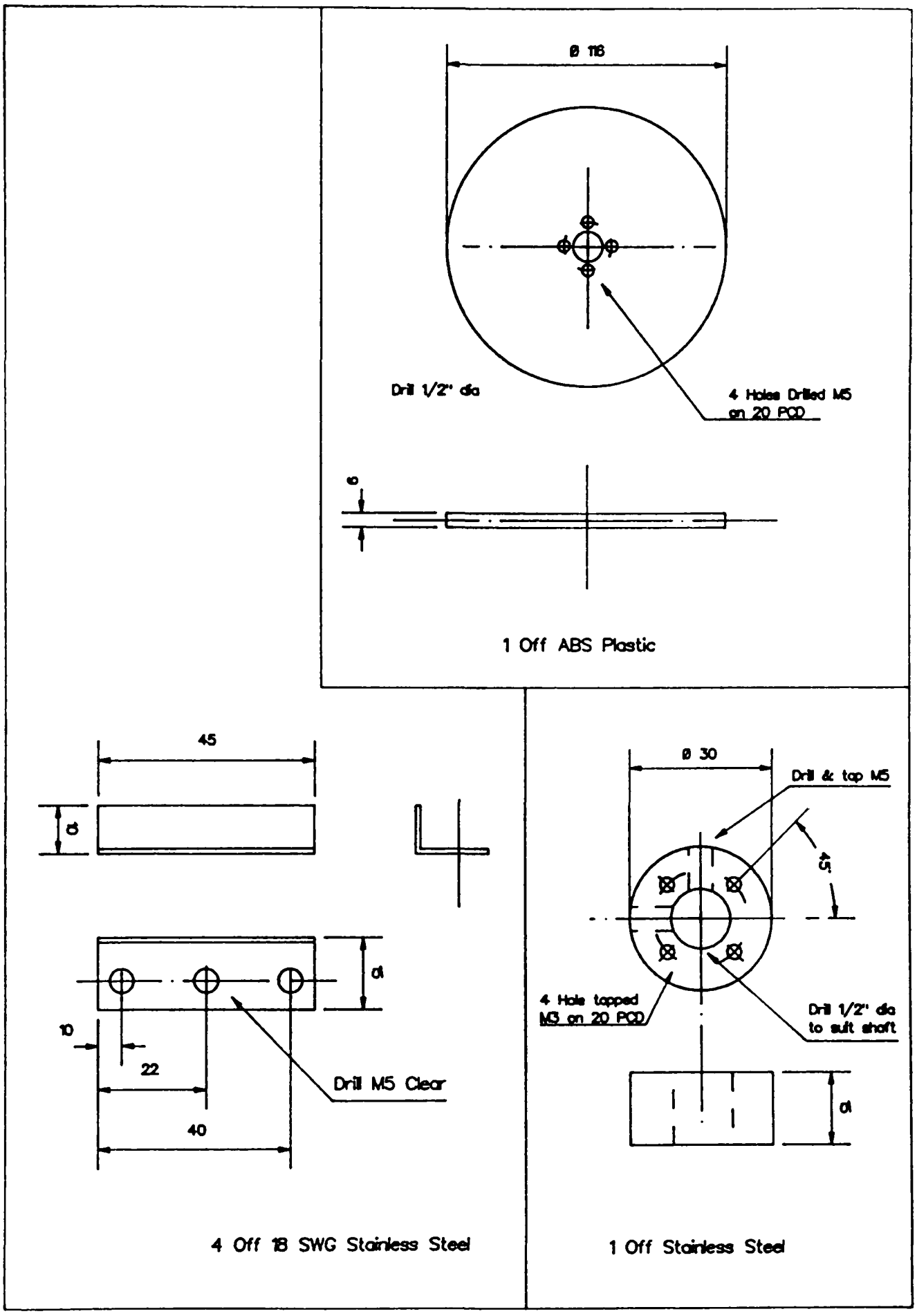

Fig.A1, Detail of mechanical foam breaker