Nitrogen Balances in Landfills

R&D Technical Report P1-217/TR

Nitrogen Balances in Landfills

R&D Technical Report P1-217/TR

S. Burton and I. Watson-Craik

Research Contractor:
University of Strathclyde

Publishing Organisation
Environment Agency, Rio House, Waterside Drive, Aztec West, Almondsbury
BRISTOL, BS32 4UD

Tel: 01454 624400 Fax: 01454 624409
Website: www.environment-agency.gov.uk

ISBN : 1844320715

Dissemination status
Internal: Released to Regions
External: Public Domain

Statement of Use
This output is being taken up in the follow-on R&D Project P1-306 to investigate the specific fluxes of ammonia in landfills. The report will be of use to waste management practitioners both on the regulatory and operational side, consultants and scientific specialists and is disseminated for information only.

Key words
Nitrogen, balance, landfill, wastes, biomass, anaerobic, decomposition, ammonia, bacteria, leachate.

Research Contractor
This document was produced under R&D Project P1-217 by:
Department of Bioscience & Biotechnology, University of Strathclyde, Royal College Building, Glasgow, G1 1XW

Tel: 0141 548 2468 Fax: 0141 553 4124

Environment Agency Project Manager
The Environment Agency's Project Manager for R&D Project P1-217 was
Richard Smith, Head Office (formerly managed by Dr Louise de Rome, ETSU)

CONTENTS

EXECUTIVE SUMMARY

This study was commissioned by the former Wastes Technical Division of the Department of the Environment under contract no. EPG 1/7/16. The project transferred to the Environment Agency and became part of the Waste Regulation & Management R&D Programme in April 1996.

Objectives

The objectives of this two year project are:

- to review and report the current understanding of the role and metabolism of nitrogen in the various processes appropriate to landfill wastes.
- to interview local landfill operators and determine their views pertaining to landfill nitrogen and ascertain how the research should be directed to best meet their requirements.
- to investigate the distribution, role and fate of nitrogen within landfill sites using laboratory based landfill models.
- to suggest suitable methods of landfill management likely to lead to the rapid and permanent reduction of the ammonia content of landfill leachate.

Background

The research programme was set against the need for a greater understanding of the basic microbiological processes which occur during the decomposition of landfill refuse and their relevance to the sustainable management of landfills.

Main Findings

A study of the current understanding of the role of nitrogen in the various processes pertaining to landfilled waste was completed. Information was obtained from the current literature and consultation with landfill site operators. The review of the current scientific literature revealed fundamental gaps in our knowledge regarding the role of nitrogen and nitrogenous compounds during refuse decomposition which the research programme addressed. What was previously reported in the literature was that the protein present in the organic components of refuse was the source of the ammonia concentrations in landfill leachate.

The research approached the study of nitrogen in refuse using three different methodologies, by refuse incubation studies, refuse columns and multi-vessel fermenter systems. To quantify the nitrogenous content in each of the systems novel analytical techniques were devised which pioneered the measurement of the nitrogenous content of decomposing waste.

Ammonia is the most important nitrogenous compound in landfill refuse. It persists in landfill refuse because the microbiological and physiological processes which produce it (ammonification) do so at a rate which cannot be met by its utilisation during the growth of microorganisms. Experiments suggested limited uptake of ammonia by bacteria in landfill laboratory models. There was consequently no major route for the removal of ammonia from refuse other than the aerobic treatment of leachate taken from the landfill and treated in aeration lagoons.

The proportion of ammonia in refuse compared to the total organic nitrogenous pool (3.86% N dry weight), the source of ammonia, was relatively small, between 5% to 10%, indicating that by the end of the active phase of ammonia production the bulk of ammonia was retained in the refuse and only a small proportion (5%) was released into the leachate during anaerobic decomposition. Increasing the ammonia concentrations could reduce the methane production by landfill refuse and may be important in determining the rate of refuse decomposition. Acclimatisation of decomposing refuse to high ammonia concentrations was not seen in laboratory experiments but may be operational over longer time scales. Nitrate and nitrite were not found in the refuse studied and presumably have no role in the mineralisation of landfilled refuse.

Treatment of the ammonia in landfill leachate by traditional methods leads to the production of the nitrogenous compound nitrate. Recirculation of nitrate containing leachate could be a viable method for the removal of nitrogen from landfill leachate and may accelerate the decomposition of landfill refuse. Experiments on landfill refuse and nitrate revealed rapid consumption of nitrate and its removal to nitrogen gas after 6 days. The production of methane was inhibited at concentrations of 500 and 1000 mg NO_3^--N l^{-1} but recovered rapidly after the consumption of 50 mg NO_3^--N l^{-1}. The bacterial process of methanogenesis could coexist with the microbial processes which remove nitrate (denitrification) in liquid systems at 5.7 mg NO_3^--N l^{-1}. Nitrate addition encouraged the removal of certain volatile fatty acids and increased the pH of the leachate. Experiments containing 50 mg NO_3^--N l^{-1} revealed that a small proportion of the nitrate (4-7%) was converted back to ammonia and was retained in the refuse.

Main Conclusions

Anaerobically decomposing landfilled refuse rapidly removes nitrate and represents an effective method of removing nitrate from treated nitrified leachate by recirculation, without the expense of secondary anaerobic leachate treatment. The addition of nitrate to a landfill will inhibit methane production in the short term if applied in similar concentrations to nitrified or treated acetogenic landfill leachate. Rates of consumption of nitrate suggest the stratification of refuse into zones of denitrification and methanogensis during the recirculation of nitrified leachate. Concentrations of 50 mg NO_3^--N l^{-1} will allow the rapid recovery of methanogenesis in refuse once the nitrate has been denitrified.

High ammonia concentrations may affect the rates of methane production by refuse. Better rates of methane production and anaerobic degradation could come from controlling the ammonia concentration in refuse.

The anaerobic decomposition by refuse cultures was not phosphate or nitrogen limited.

A reductive nitrogen transformation pathway which converted nitrate to ammonia was found during incubation of refuse cultures. This was capable of reducing 4 -7% of the nitrate added. This may have been caused by the highly reduced conditions of landfill refuse combined with reduction on the surfaces of metals or by microbial enzymes. The reductive pathway competed with the denitrification of nitrate. Greater proportions were reduced when the indigenous bacteria were destroyed by gamma irradiation. Autoclaving prevented significant loss of nitrate following incubation, suggesting that a biotic or cell associated biochemical transformation was involved.

Binding of ammonia to refuse in refuse cultures appeared to be controlled by pH value, suggesting greater leaching at higher pH values.

Incorporation into biomass of the ammonia produced during decomposition appeared insignificant in experiments using heavy isotopes in model landfill systems. Leachate ammonia accumulated during anaerobic decomposition in batch cultures. Nitrogen in refuse did not accumulate significantly during the growth of anaerobic bacteria in refuse columns when supplied with combinations of ammonia and volatile fatty acids. Utilisation of ammonia during bacterial growth is probably not an important route for the nitrogenous components in decomposing landfill refuse.

Acknowledgements

The authors kindly acknowledge Mr. Ian Wilson and colleagues at Cunninghame District Council for access to their landfill site at Shewalton. We would also like to thank Dr Tom Preston and Dr H. M. Banford at the Scottish Universities Research and Reactor Centre for assistance in the analysis of natural nitrogen isotopes and the irradiation of samples. Two students, Marc Jacobs and Cheryl Posnett, helped on aspects of the project whilst studying for their degrees. We also acknowledge the assistance of the staff at the DoE and ETSU.

1. INTRODUCTION

Current legislation requires that landfill operators obtain a certificate of completion prior to the surrender of a site licence (DoE, 1994a,b). One of the key criteria by which landfill sites can be judged to have reached 'Completion Condition' or stabilised is leachate quality. Of the potentially hazardous components in leachate, ammonia (see appendix 1) concentrations, in particular, may remain at elevated levels for many years into the landfill fermentation.

At present our knowledge of nitrogen transformations in landfill, including ammonia generation, is limited. The origins of nitrogenous compounds in leachate such as ammonia, nitrite and nitrate are not well studied. Records of ammonia production from landfills are kept but little is known about the overall nitrogen budget or the expected concentrations of ammonia in the leachate. The effect of leachate management practices, such as recirculation of leachate, on the nitrogen budget have not been sufficiently studied nor has the concept of a nitrogen budget been applied to newly proposed sustainable landfilling practices. Ammonia is both a toxic byproduct of landfill degradation processes and an essential nutrient required by the bacteria responsible for these processes. Too much ammonia may reduce the rate of decomposition and negate the effect of introducing 'sustainable' landfill practices such as recirculation to promote the rapid decomposition of refuse. Optimising the concentrations of ammonia in the leachate may reduce the length of time a landfill take to stabilise and may promote methane production.

Thus the nitrogenous compounds found in refuse components such as kitchen and industrial waste could have an important effect on the progress of the decomposition of landfill refuse. Since these components represent the source of ammonia, one of the most important landfill pollutants (Kristensen, 1992), their study is of chief importance.

1.1 Landfill Degradation Processes

When refuse is first placed and is exposed to air and moisture, aerobic decomposition similar to the process of composting occurs. Bacteria and fungi break down the constituent organic components of refuse such as paper, card and vegetable matter into simpler molecules releasing carbon dioxide gas. Deep within the landfill the supply of oxygen becomes depleted and organic compounds degrade anaerobically through the action of a diverse range of anaerobic and facultatively anaerobic bacteria (see glossary). The absence of oxygen reduces the energy available to bacteria breaking down the refuse and the rates of decomposition are significantly slower than aerobic processes.

In the first phase of anaerobic degradation, biological polymers such as cellulose (found in paper and card), proteins and fats from biological material are converted into smaller soluble sugars, amino acids, long-chain carboxylic acids and glycerol. These are then fermented in subsequent steps to carboxylic acids, alcohols, carbon dioxide and hydrogen. Ammonia is released from the bacterial degradation of proteins and nucleic acids as a means of detoxifying them, allowing further breakdown of the carbon components of these compounds. The production of carboxylic acids such as acetate (also described as volatile fatty acids because of their pungent odour) and carbon dioxide leads to a decrease in the pH of the refuse known as the acetogenic stage. The acetogenic stage is followed by the methanogenic stage of refuse breakdown. Increased activity by the methanogenic bacteria encourages further breakdown by the acetogenic bacteria decreasing the concentrations of carboxylic acids. The partial pressure

of carbon dioxide decreases with a corresponding increase in methane production until the gas produced typically contains 60% methane.

The conversion of longer chain fatty acids to acetate by acetogenic bacteria requires the maintenance of low concentrations of hydrogen (Barlaz *et al.*, 1990). This is maintained by mutualistic associations with hydrogen utilising methanogenic bacteria (Mah *et al.*, 1977; Blaut, 1994), sulphate reducing bacteria (Postgate, 1984) and homoacetogenic bacteria (Ljungdahl, 1986; Diekert & Wohlfarth, 1994). The removal of hydrogen by hydrogen utilising bacteria facilitates the degradation of compounds by acetogenic bacteria enabling greater energy production (Wolin, 1974) and probably an increased overall rate of decomposition.

The mutualistic associations form consortia which can be regarded as a single functional species with the combined metabolic capacity of the member species. These consortia have growth rates, which in the methanogenic consortium is dependent on the hydrogen utilizing step of the methanogenic bacterium (Winter & Wolf, 1980; Archer & Powell, 1985). The anaerobic degradation process therefore involves the cooperation of groups of different bacteria, the requirements of which can be arranged together as seen in Figure 1.1. The complexity of the diagram also illustrates how various groups of bacteria use the same compounds and that the anaerobic degradation is not just a simple progression from larger compounds to smaller ones as often seen in aerobic systems. The key to the process is the maintenance of low pressures of hydrogen gas (H_2) and an adequate moisture content. An abundance of water allows the ready transfer of materials to and from the bacteria and the swelling of the paper waste into a form more susceptible to bacterial attack.

The need for several bacterial groups to function together during anaerobic decomposition is most apparent when the process sours. Failure of the methanogenic bacteria to be sufficiently active allows the build up of large amounts of volatile fatty acids and carbon dioxide during acetogenesis which increases the acidity and reduces the pH. This can inhibit further breakdown which in turn delays the onset of methanogenesis. Once methanogenesis resumes the concentrations of the volatile fatty acids decrease as fermentative bacteria break them down passing the unwanted hydrogen to the methanogens and the pH balance is restored to near neutral.

The anaerobic decomposition of landfill refuse can be neatly separated into stages based on the transition from acetogenesis to methanogenesis first drawn by Farquhar & Rovers (1973) and modified in Figure 1.2.

Paper (Cellulose) ⟶ (1) landfill gas methane CH_4(60%) and carbon dioxide CO_2 (40%) and trace gases and odours such as hydrogen sulphide H_2S.

(2) mixture of carboxylic acids

(3) Hydrogen H_2 (trace)

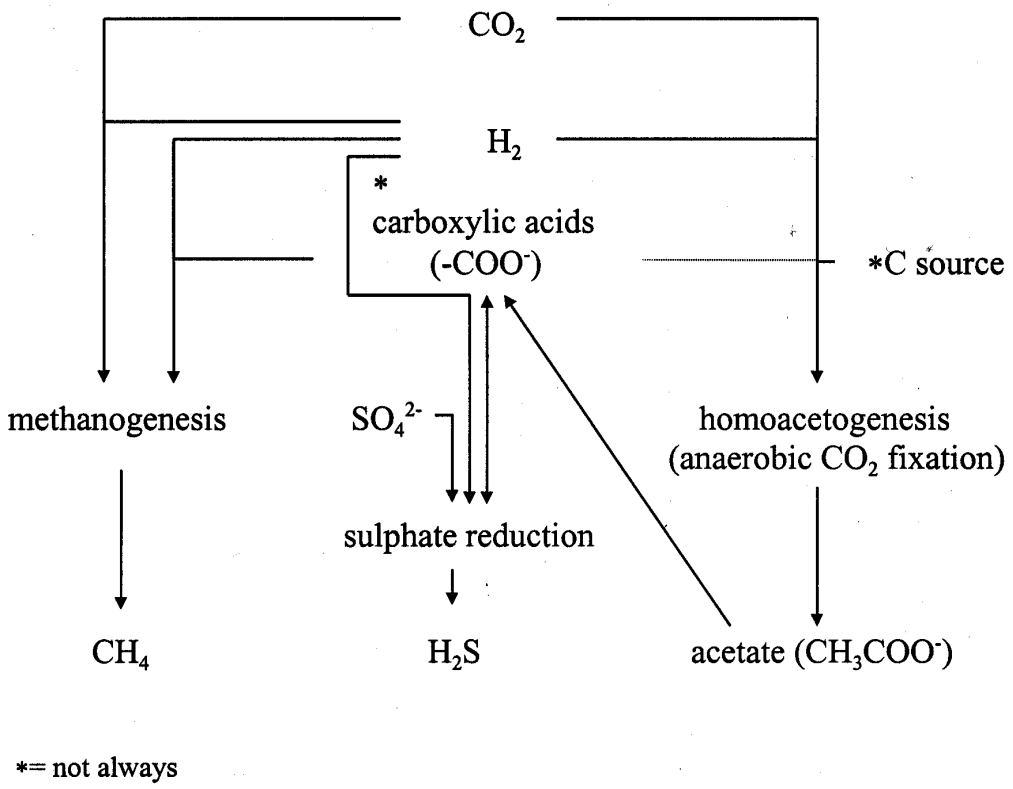


Figure 1.1 The relationship between the tertiary bacterial processes of refuse decomposition and the key products of the anaerobic decomposition of paper waste, CO_2, H_2, carboxylic acids and methane.

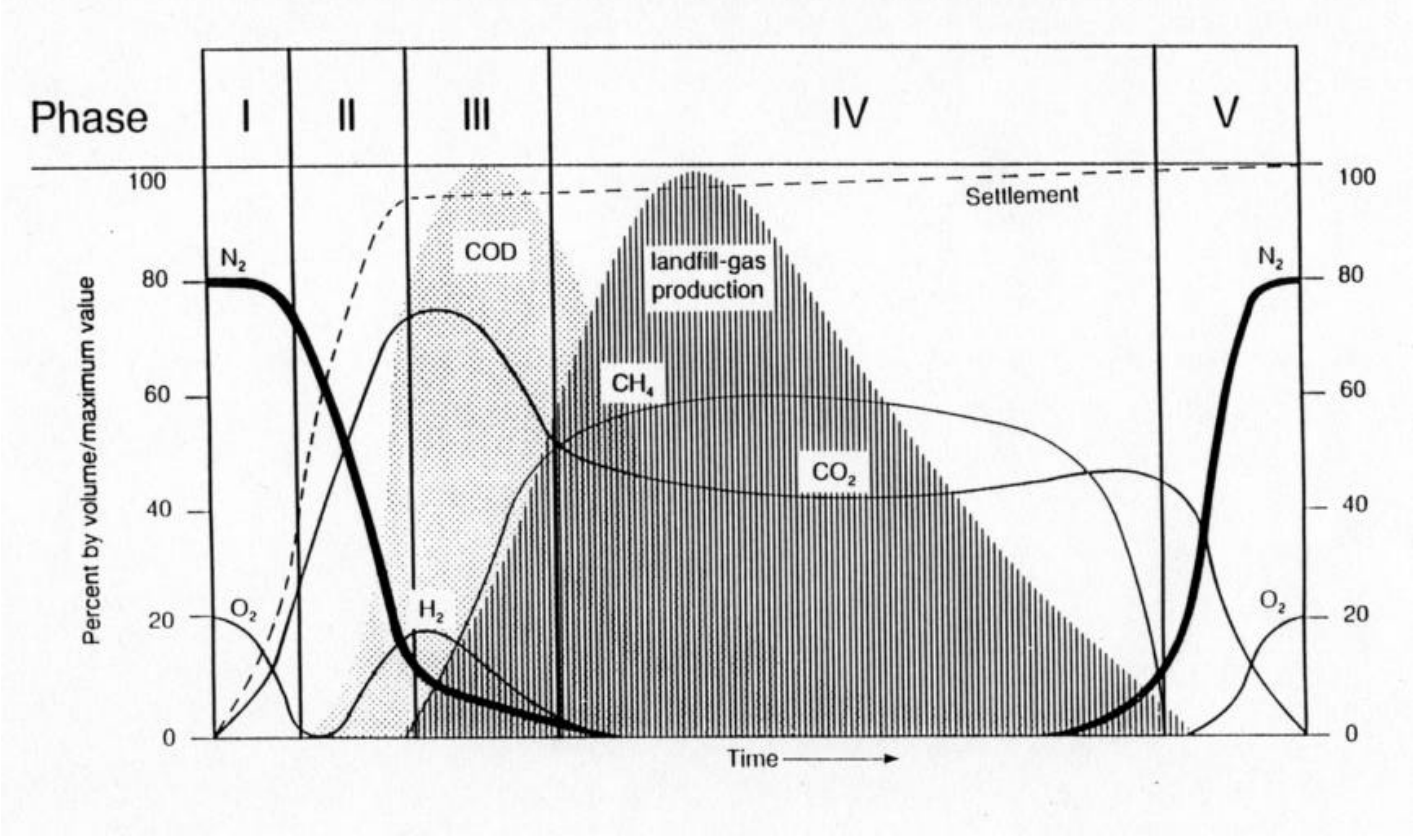


Figure 1.2 Qualitative assessment of gas production from landfilled waste taken from Archer *et al.*, 1995 (after Farquar and Rovers, 1973).

The production of volatile fatty acids and ammonia during anaerobic decomposition produces a toxic leachate high in BOD and COD (see glossary). Considerable quantities of volatile fatty acids (Harmsen, 1983) and ammonia (Ehrig, 1989) are produced which must be treated before discharge.

1.2 Treatment of Ammonia

To meet landfill discharge limits, ammonia and organic components in leachate are typically treated by aeration in lagoons (Robinson & Maris, 1985; Enzminger *et al*., 1987; Robinson *et al*., 1992). This produces the nitrogenous compound nitrate through the bacterial process of nitrification (Knox, 1985). The organic carbon containing leachate components are oxidised and broken down, most escaping as carbon dioxide or remaining as water and microbial sludge. To meet nitrate discharge limits (where required), an anaerobic process, denitrification is employed to reduce nitrate to nitrogen gas (N_2) and some nitrous oxide (N_2O), returning the nitrogen to the atmosphere. The denitrification of nitrate requires a carbon source which is often removed during the aerobic stage of leachate treatment. Successful systems use a carbon supply which is fed to the bacteria in the anaerobic chamber. Economical systems employ carbon rich wastewater which is co-treated in the chamber.

1.3 Sustainable Waste Management

Public concern about the environment has been met by ever more stringent legislation. As awareness of the legacy which we will leave to subsequent generations increases so the waste management industry must adapt and become 'sustainable'. The Brundtland Report (1987) describes the principle of sustainable development as 'development that meets the needs of the present without compromising the ability of future generations to meet their own needs.' The commitment to sustainability was the theme of agreements made by the British Government at the Earth Summit in Rio de Janeiro in June 1992 and has now been incorporated into United Kingdom development strategies and policies. It is in stark contrast to the landfill policy of twenty years ago when the preferred option was to bury the waste in a 'dry tomb', minimising the moisture content and therefore the biological degradation of the waste. Dry sites show little or no degradation for many generations and the pollutive capacity of the waste remains unchanged, rendering the land useless for future generations.

Applying the concept of sustainability to waste management requires the improved use of resources, reduced packaging and optimised recycling. There will always be some refuse for which no recycling option exists and which will require either composting, incineration or landfilling. The most popular method and the more cost effective option in the United Kingdom is landfilling. Benefits of landfilling include the ability to handle large amounts of refuse quickly, the option of co-disposing toxic substances which can be attenuated during the landfill degradation and the collection of landfill gas for power generation. Applying the concept of sustainability to landfilling requires an accelerated decomposition to reduce the pollutive life of the landfill to within one generation. The costs and the treatment of the waste will be met by that generation and potentially hazardous waste will not be left for future generations. Returning the landfill to productive use such as building or farming represents a much greater challenge but once the refuse has reached a stable condition the threat of pollution can be considered to have been lifted and the ground allowed to go fallow or landscaped.

1.4 Sustainable Landfilling Techniques

Sustainable landfilling must employ techniques to reduce the time taken for the refuse to degrade to a stable non-polluting state to within one generation. Considerable advances can be made by increasing the moisture content of the waste and recirculation of leachate has been shown to significantly accelerate refuse decomposition (Robinson *et al.*, 1982, Tittlebaum 1982, Barlaz *et al.*, 1987). Although leachate recirculation offers a reduction in the COD and BOD content in the landfill leachate, residual COD persists and requires dilution or treatment prior to discharge (Barber & Maris, 1992). Hence leachate recirculation does not offer a solution to the treatment of the organic components in leachate. Combining both recirculation and a flushing action and you have the basis of the so called 'wet/flushing bioreactor' (Harris *et al.*, 1994). Anaerobic degradation can be maximised by recirculation and keeping the refuse moist, and toxic leachate components and slowly degradable dissolved compounds flushed out for treatment and discharge (Figure 1.3).

Any landfill designed to operate moist with leachate recirculation and a flushing action such as the proposed 'wet/flushing bioreactor' requires a contained site with accurate moisture control and the effective movement of leachate through the refuse. Leachate collection in pools within the refuse would reduce the effectiveness of recirculation. Careful sorting and packing of the refuse is required to minimise the channeling of leachate through selected

regions of refuse. Of primary concern is the potential for leakage through the liner because of the high moisture content of the refuse and the pollutive capacity of the waste. A wet/flushing bioreactor must therefore be situated away from vulnerable ground water supplies but be close enough to an irrigant supply for flushing when required.

1.5 Aims and Objectives

It was proposed that the ammonia and nitrogen compounds in landfilled refuse be studied both in the context of the bacterial degradation processes and accelerated refuse decomposition. A review of the current state of knowledge pertaining to landfill nitrogen was prepared and published as CWM A125/96 Nitrogen Balances in Landfills (Burton & Watson-Craik, 1996b). Various members of the landfill industry were consulted prior to starting the research programme to see how the research could be tailored to best fit their needs.

Before an attempt is made to accelerate the landfill fermentation, the degradation processes which control the rate of stabilisation must be elucidated. The anaerobic decomposition of refuse leads to the formation of very high ammonia concentrations in landfill leachate which represent a considerable threat to aquifers and drinking water supplies. Considering their toxicity, surprisingly little is known about the formation and concentration of ammonia and other nitrogenous components present in landfill refuse. If a nitrogen budget could be created for refuse decomposition then it would be possible to predict the rate of mineralisation of ammonia from refuse and the facilities required to treat and process it. The project addressed these needs by analysing the nitrogen content of actively decomposing refuse in model landfill systems.

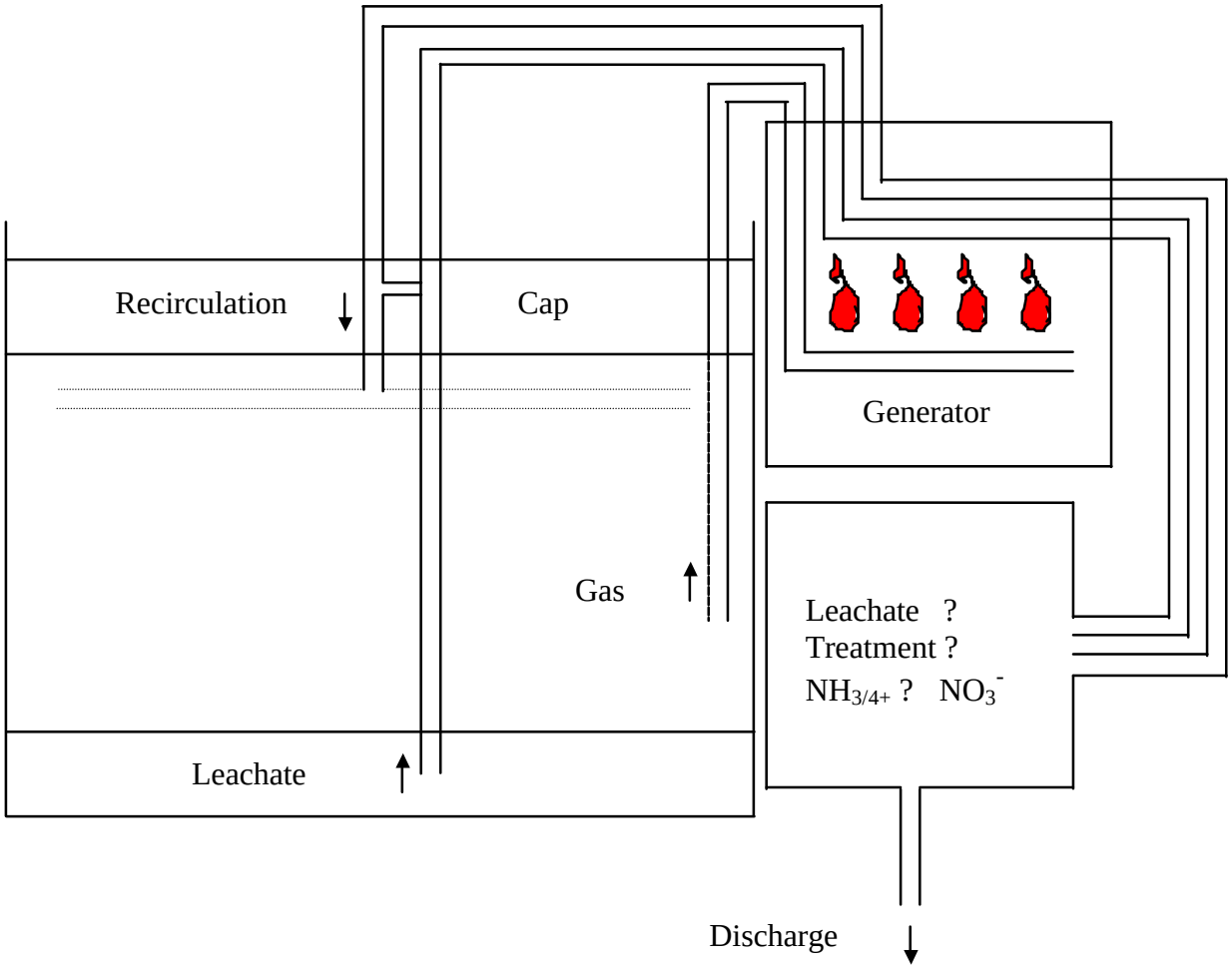


Figure 1.3 Schematic diagram of the wet/flushing bioreactor with combined leachate treatment recirculation

Recirculation of leachate may lead to extremely high and potentially toxic concentrations of ammonia which may adversely affect the degradation processes. The flushing action of the wet/flushing bioreactor will produce large amounts of ammonia in the leachate which will require treatment before discharge. Treatment in aeration lagoons will produce large quantities of nitrate for discharge. If nitrate treatment is required the most economic way of treating nitrified leachate would be use one that employs the anaerobic environment of the landfill to denitrify nitrate to nitrogen gas, avoiding the more costly secondary anaerobic treatment stage. Such a system would bypass the anaerobic stage and return the aerated treated nitrate containing leachate from the lagoon to the landfill. The system would form the basis of a landfill ammonia and nitrate treatment strategy within a sustainable landfill design.

Previous work has indicated that following nitrate addition methane generation from actively methanogenic systems may be reduced. This has potentially serious implications for gas recovery should partially aerated leachate containing nitrate be recirculated. Conversely the reduction in the carbon content of the refuse following nitrate addition may be seen as beneficial in terms of the acceleration of site stabilisation and removal of nitrogen from a

landfill through the process of denitrification. The research studied the effect of nitrate on the landfill decomposition processes and characterised the transformations it underwent in model landfill systems. The research was used to determine whether recirculation of nitrified leachate represented a viable and justifiable method of nitrogen treatment.

The objectives of this two year project were:

- to review and report the current understanding of the role and metabolism of nitrogen in the various processes appropriate to landfill wastes;

- to interview local landfill operators and determine their views pertaining to landfill nitrogen and ascertain how the research should be directed to best meet their requirements;

- to investigate the distribution, role and fate of nitrogen within landfill sites using laboratory based landfill models; and

- to suggest suitable methods of landfill management likely to lead to the rapid and permanent reduction of the ammonia content of landfill leachate.

2. LITERATURE REVIEW

2.1 Conclusions from the Literature Review

The literature review prepared during this contract is available separately as R&D Technical Report CWM A125/96 Nitrogen Balances in Landfills Report (Burton & Watson-Craik, 1996b).

At present our knowledge of nitrogen transformations in landfill, including ammonia generation, is limited. Ammonia remains at high concentrations in leachate long into the life of the landfill and requires treatment before discharge. Little is known of the microbial nitrogen transformations occurring in landfill refuse. Ammonia is assumed to be produced by the ammonification of proteins in the refuse by bacteria (Figure 2.1). The ammonia is released during the detoxification of the nitrogenous content of amino acids which make up proteins. This ammonia appears to be entombed in the refuse. Once the landfill is capped it can only be released through leachate discharge or bound up within growing microorganisms (Figure 2.2). The surface layers of a landfill will almost certainly produce nitrate (nitrify), due to the ubiquitous nature of the bacteria which carry out these transformations, but the overall effect will be insignificant in comparison to what occurs in the anaerobic zones of the refuse. Work in our laboratory has indicated that landfilled refuse retains the ability to produce gaseous nitrogen from nitrate/nitrite(denitrify) despite the absence of nitrate and nitrite in the landfill and leachate (Sinclair, 1994). The rate of assimilation (or use) of ammonia, nitrite and nitrate by bacteria during growth and during refuse degradation is unknown and is important if the nitrogen budget of a landfill is to be quantified and the leaching patterns calculated. Since ammonia accumulates in leachate it seems likely that there are no anaerobic microbially mediated transformations that promote the formation of nitrogen gas from ammonia in landfill refuse.

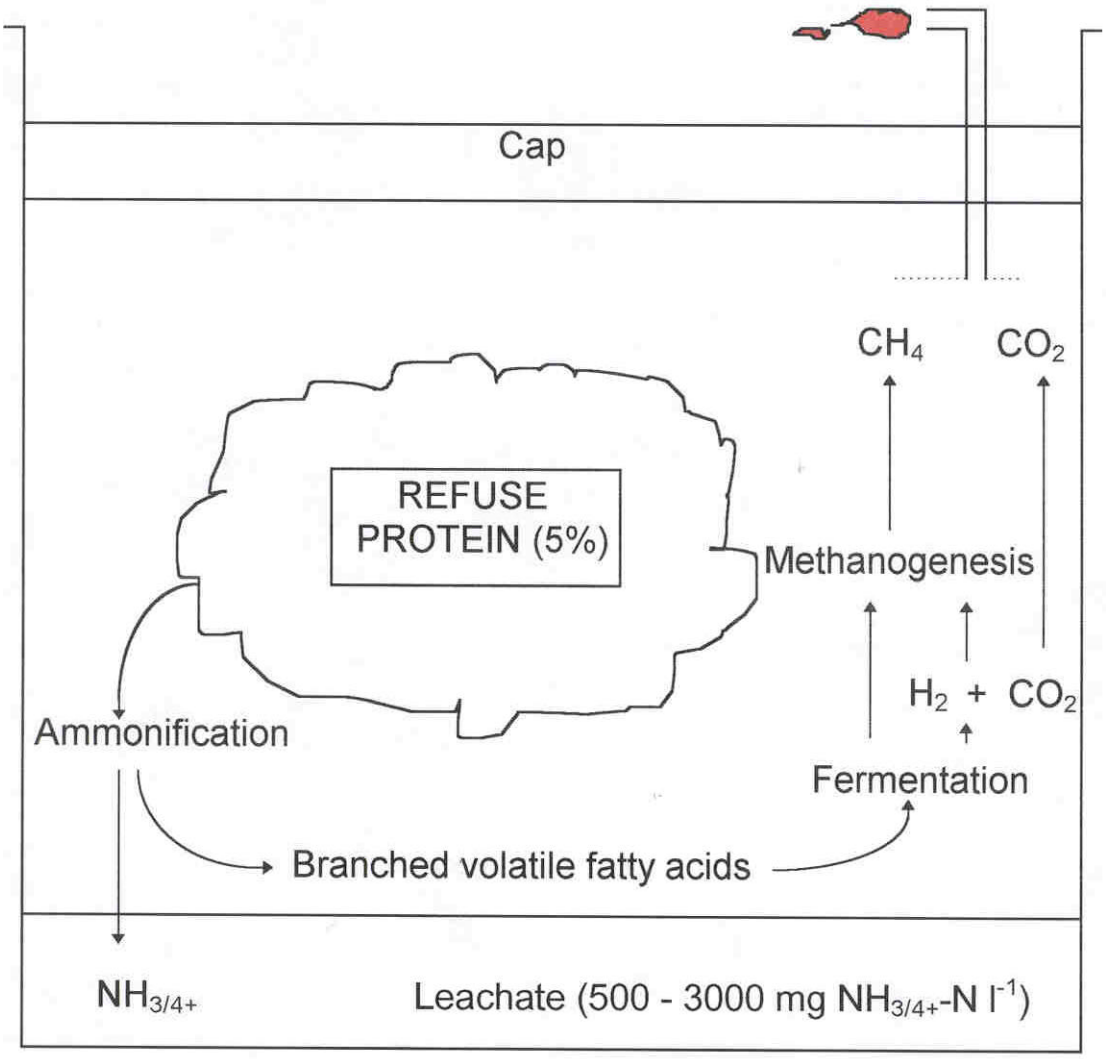


Figure 2.1 Landfill ammonia production

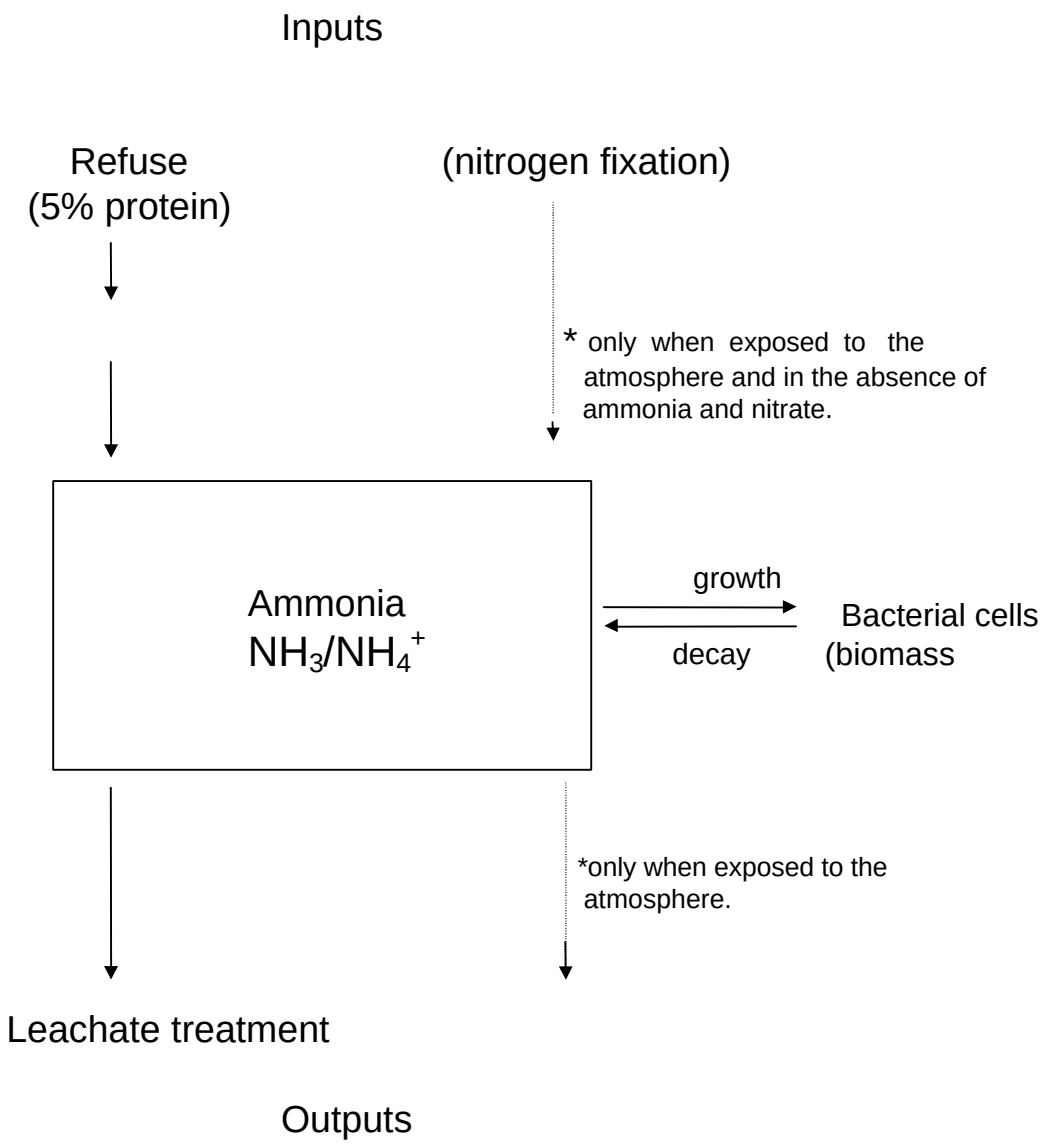


Figure 2.2 The inputs and the outputs to and from the ammonia pool.

The interpretation of reported results for laboratory short-term experimental refuse systems and cultures was hampered by the absence of good measurement data from long-term experiments. Analysis of landfill leachate from experimental test cells was limited to only a short timescale and initial refuse nitrogen concentrations were not provided. This made it impossible to predict the overall nitrogen budget of the systems studied.

The effects of leachate management practices on nitrogen transformations and gas production are poorly understood although previous work in this laboratory has indicated that following nitrate addition, methane generation from actively methanogenic refuse may be reduced. More information is required on the effects of ammonia, nitrite and nitrate on methanogenesis in refuse should leachate recirculatiion be used to promote landfill stabilisation or nitrified leachate (leachate containing nitrate) be added to landfill.

It was apparent that the degradation processes can be enhanced by the addition of moisture to the landfill. The proposed wet/flushing bioreactor would have an increased rate of refuse degradation. Whether this would also increase ammonification in the landfill, creating toxic leachate ammonia concentrations which would reduce the rate of degradation requires further study. Treatment of ammonia by the nitrification of leachate ammonia in aeration lagoons would be a short-term solution to high ammonia leachate concentrations. The treated leachate containing nitrate could be fed back into the landfill where it would be denitrified to nitrogen gas. It is not known whether the recirculation of nitrified leachate would enhance stabilisation in the short term, however the C/N ratio of municipal solid waste, if all the nitrogen was oxidised to nitrate, is insufficient to promote the degradation process significantly in the longterm. Methane production is a major product of the landfill degradation process and could be an important source of power should the flushing bioreactor concept be realised. Addition of nitrate to the landfill may inhibit methanogenesis causing a temporary halt to methane production where nitrate reaches zones of methanogenesis.

2.2 Overall Conclusions

The paucity of information regarding the ammonia and nitrogen content of landfill refuse and the fact that no study had directly addressed the topic of nitrogen balances in landfills led us to believe that this research would be beneficial to the industry.

3. INDUSTRY VIEWS AND PERSPECTIVES

3.1 Introduction

Interviews were conducted with two local landfill site operators and contacts from several consultants within the landfill industry. The meetings were intended as a guide, so that any operational problems associated with leachate nitrogen could be addressed by the research. The operators were also asked whether they felt research into the nitrogen transformations that occur during the decomposition of landfill refuse was important. The authors were particularly interested in opinions on recirculating nitrified leachate (treated leachate containing nitrate) in the context of reducing aqueous nitrogen yields from the site and in the context of the wet/flushing bioreactor. The operators were also asked for their opinions on the wet/flushing bioreactor concept, the future of the landfilling industry and sustainable landfilling.

3.2 Conclusions from the Interviews

3.2.1 Nitrogen Balances in Landfills

Both landfill operators believed that studying the nitrogen transformations that occur during the decomposition of refuse was important. One site had experienced a short term problem with its reed bed and was therefore receptive to suggestions about the possibilities of returning nitrified leachate to the refuse mass as a means of reducing nitrogen outputs. This would not be necessary, however, because this particular site did not have a nitrate discharge limit. Operators at the other site were less responsive to suggestions about recirculating nitrified leachate. They saw recirculating nitrified leachate as prohibitively expensive, possibly because they had no problems with their leachate treatment or output. However, if the levy imposed by the water company is linked to nitrate discharge then there may be an impetus to consider other options involving nitrate treatment, either by new anaerobic treatment facilities or by recirculation.

One site did recirculate leachate but the intention was to store leachate, presumably so that it could be treated in the warmer months of the year. The other site pumped leachate from one cell to another as a way of storing leachate. Both methods will have increased rates of decomposition and gas production. One site had been left uncapped to allow the percolation of rainwater into the site and as a result probably had to cope with high leachate levels in the sump.

Since the current guidelines require the monitoring of landfills after the site has closed it will be interesting to see how the operators propose to deal with the leachate once landfilling is discontinued and whether short term considerations about leachate nitrogen discharge will be as important. The amount of money set aside for this treatment after cessation of landfilling is also of interest. If the bulk of leachable nitrogen remains in the refuse long after landfilling then the current guidelines will impose a considerable legacy on the operators in treating it before discharge. If operators have a legacy of old sites then it seems more likely that they would be more responsive to changes in landfill practices that adopt a long term approach and will reduce costs in the future.

Mr. Howard Robinson, (pers. com.) who at the time was preparing a review for the Department of the Environment on landfill leachate (DoE, 1995) was asked for his findings on leachate ammonia. Howard Robinson had examined the relationship between

chloride and ammonia concentrations in leachate and had concluded that chloride and ammonia would leach in a similar manner from landfills but found unexpected differences between them. The most interesting observation from the review of the literature was the fact that recorded ammonia concentrations never appeared to rise above 2000 mg l^{-1}. This was despite the fact that the described landfills varied considerably in size and therefore had vastly different total nitrogen contents. This suggested self-inhibition of ammonia production at or about the 2000 mg l^{-1} level.

3.2.2 Leachate Recirculation and t he Wet/Flushing Bioreactor Concept

The consultants were asked for their opinions on leachate recirculation. They considered a wet/flushing bioreactor could introduce problems in leachate management, since it would have the effect of concentrating the leachate rather than diluting it, it may cool the landfill and present a pollution hazard by maintaining the landfill at a high moisture content. They saw no reason to return dissolved compounds during recirculation of nitrified leachate to the landfill when effective methods existed to dilute and treat the leachate even though nitrate could be removed by this method.

If the wet/flushing bioreactor concept were to be realised then the refuse must undergo some form of shredding and processing to make the landfill as uniform as possible, otherwise leachate pools may build up. The build up of leachate can produce leachate pools of immense size, enough to confuse the measurement of leachate volumes in the sump.

Recirculation of nitrified leachate would present a way of disposing of nitrogen but the technology was available to treat the nitrogen anaerobically if required. If added moisture is required for the landfill fermentation then tap water might be a better source of water since it would not contain the compounds found in the leachate.

Overall, when questioned, the consultants felt that the nitrogenous components within landfill refuse had not been sufficiently studied. There was a consensus that ammonia was the most important compound found in landfill leachate and for the flushing bioreactor concept to succeed it would require good leachate management.

3.3 Overview

All those questioned felt that the nitrogenous components within landfill refuse had not been sufficiently studied. Ammonia is an important landfill pollutant and a determinant of landfill completion. The industry thus had a requirement for fundamental research on the subject. The limited information in the literature meant that the research phase of the project had little information on which to base the research and methods and protocols had to be devised to analyse the nitrogenous components in refuse and leachate.

4. GENERAL MATERIALS AND METHODS

The project required the development of methods to analyse nitrogen concentrations in landfill refuse in models appropriate and relevant to the anaerobic decomposition of refuse in a landfill site. Most methods were modified from methods used to analyse wastewater.

4.1 Refuse Collection and Characterisation

Refuse was collected on 31st May 1995, 17th November 1995 and 31st November 1996 from the Cunninghame District Council landfill site at Shewalton from a depth of 2-3 m. The site receives domestic and commercial waste which is pulverised before emplacement. On each occasion the refuse was estimated to be 1-2 months old. Large plastic fragments (>1cm), metals (>1cm) and all glass were removed and the remaining clumps of refuse cut into fragments approximately 1 cm diameter before use.

The raw refuse was characterised on 3/10/96 by EnviroCentre Ltd, University of Strathclyde. Mean results from two separate analyses are tabulated below.

Table 4.1 Refuse characterisation

Component	% Weight
Paper/card	32.7%
Putrescibles	24.4%
Dense plastic	8.6%
Ferrous metal	7.9%
Plastic film	7.3%
Glass	6.5%
Textiles	3.7%
Miscellaneous – combustibles	3.6%
Fines	2.2%
Miscellaneous – non-combustibles	1.6%
Non-ferrous metals	1.5%

4.2 Refuse Moisture Content

The moisture content of refuse was determined by incubating three 100 g samples of fresh refuse at 105 ^{o}C for approximately 24 h or until the weight was constant, and the moisture content determined by difference.

4.3 Clarification of the Refuse Sample

Where spectrophotometric analysis was required, the clarification of the liquid sample was carried out prior to performing the assay. A refuse sample (20 g) was adjusted to the appropriate moisture content and volume (70-100 ml) with distilled water and mixed by blending in a liquidiser for 5 min or by shaking in a stoppered flask for 30 or 60 min held on a Griffin flask shaker. The bulk solids were separated by passage through a 5.5 cm Whatman GF/C glass micro-fibre filter (1.2 mm retention) held on a Buchner funnel under vacuum.

Dirty suspensions and culture samples containing sulphide were filtered twice. Bacterial cells were removed by centrifugation at 8000x gravity.

4.4 Analysis of Nitrogenous Compounds in Refuse

All chemicals were obtained from BDH Merck Ltd unless stated otherwise.

4.4.1 Spectrophotometric An alysis of Nitrite

Nitrite ions react with sulphanilamide and N-1-naphthylethylenediamine in orthophosphoric acid to form a red azo-dye whose concentration can be determined spectrophotometrically at 540 nm.

Macro-determination of Nitrite

Clarified samples, not exceeding 40 ml, were decanted into 50 ml volumetric flasks. To each flask 1 ml of sulphanilamide reagent was added, mixed and diluted to 50 ml. The solution was incubated at 25 ^{o}C for 1 h and the absorbance measured at 540 nm and compared to standard aqueous solutions of nitrite (0 - 50 mg NO_2^--N l^{-1}).

The sulphanilamide reagent was prepared by dissolving sulphanilamide (40 g) in a mixture of 100 ml of orthophosphoric acid (85% m/v) and 500 ml distilled water. N-1-napthylethylenediamine dihydrochloride (2.0 g) was then dissolved in the solution which was then made up to 1 l and stored at 4 ^{o}C in an amber bottle.

Micro-determination of Nitrite

To 1 ml of a clarified sample and 1ml distilled water in a 1 cm cuvette was added 40 µl of sulphanilamide reagent. The reagent was prepared by dissolving sulphanilamide (5 g) in a mixture of 12.5 ml of orthophosphoric acid (85% m/v) and 125 ml distilled water. N-1-napthylethylenediamine dihydrochloride (0.25 g) was dissolved in the solution which was then made up to 250 ml and stored at 4 ^{o}C in an amber bottle. The solution was incubated at 25 ^{o}C for 1 h and the absorbance measured at 540 nm and compared to standard aqueous solutions of nitrite (0 - 3 mg NO_2^--N l^{-1}).

4.4.2 Spectrophotometric Analys is of Nitrate (modified from Mullin and Riley, 1955)

Spectrophotometric analysis of nitrate was achieved by reduction of nitrate to nitrite with hydrazine sulphate and subsequent formation of a red azo-dye with sulphanilic acid and 1-naphthylamine, whose absorbance was measured at 520 nm. The following reagents were prepared. Phenol-sodium phenate buffer was prepared by adding 9.40 g of phenol to 200 ml of distilled water, and diluting to 250 ml. 50 ml of the resulting solution was added to a 100 ml volumetric flask, to which 16 ml of 1*N* sodium hydroxide solution was added. The resulting solution was made up to 100 ml with distilled water. A hydrazine-copper reducing agent was prepared by mixing 25 ml of an aqueous hydrazine sulphate solution (4.80g l^{-1}) with 5ml of an aqueous copper sulphate solution (0.393 g l^{-1}) and diluting to 50 ml in a volumetric flask. Sulphanilic acid reagent was prepared by dissolving 0.30 g of sulphanilic acid in 80ml of distilled water in a 100ml volumetric flask and adding 12.9 ml of concentrated hydrochloric acid and diluting to the mark with distilled water.

40 ml of clarified sample was dispensed into a 50 ml Erlenmeyer flask to which phenol-sodium phenate buffer (2 ml) and hydrazine-copper reagent (1 ml) was added and the solution

left for 24 h in the dark. Acetone (2 ml) was then added to complex any residual hydrazine and retard the precipitation of the red azo-dye. The reaction was left to proceed for 2 min and then 2 ml of sulphanilc acid reagent was added whilst shaking. After not less than 5 min 1 ml of aqueous 1-naphthylamine hydrochloride (6 g l^{-1}) was added and then 1 ml of 2 M sodium acetate. The solution was finally diluted to 50 ml and the absorbance read at 520 nm with a spectrophotometer after 15 min. A calibration curve was created with 1-10 mg NO_3^--N l^{-1}. For smaller volume samples (4 ml) the procedure was adjusted by reducing the reagent volumes by 90%.

4.4.3 Analysis of Nitrate with an Ion -Selective Electrode

Nitrate concentrations in refuse suspensions and filtered liquid samples were measured with a Kent Nitrate Ion-Selective Electrode and reference electrode. Samples were buffered with 0.1 M potassium dihydrogen orthophosphate with a sample:buffer ratio of 10:1 (pH 4.5). Samples were compared to a standard curve (10^{-6}-10^{-1} M NO_3^-). Samples containing mineral salts medium were compared to nitrate standards made in mineral salts medium.

4.4.4 Spectrophotometric Analysis of Ammonia

Ammonia concentrations were determined colorimetrically. Stock reagents of phenol: alcohol (10 g phenol in 100 ml of 95% methanol), nitroprusside (0.5 g of sodium nitroprusside in 100 ml of distilled water), alkaline citrate (100 g of sodium citrate plus 5 g of sodium hydroxide in 500 ml of distilled water) and 15% w/v sodium hypochlorite solution were prepared, stored at 4^{o}C and used within 1 month. To 1 ml of a clarifed sample in a plastic cuvette was added, 1 ml distilled water, 40 µl of phenol solution, 40 µl of nitroprusside solution and 100 µl of oxidising solution (prepared by adding 10 ml of alkaline citrate to 2.5 ml of hypochlorite solution). The reaction mixture was incubated at 50 ^{o}C for at least 1 h at 50^{o}C or left overnight before reading the absorbance at 640 nm. A calibration curve was constructed with 0 - 3.0 mg $NH_{3/4+}$-N l^{-1}. Using the same reagents, the assay was adapted for use with 4ml capacity cuvettes in which case 1.5 ml of sample, 1.5 ml of water, 60 µl of phenol reagent, 60 µl of nitroprusside and 150 µl of oxidising solution were used.

4.4.5 Spectrophotometric Analysis of Ammonia Using Nessler's Reagent

The spectrophotometric analysis of ammonia was carried when there were high concentrations of ammonia present but limited concentrations of interfering mineral ions in solution such as ammonia caught by microdiffusion and redissolved in water (Chapter 8). To a clarified sample (2 ml) in a cuvette was added 30 µl of Nessler's Reagent. The mixture was stirred, left for 20 min and the absorbance measured at 425 nm using a spectrophotometer and compared to a calibration curve (0 - 4.0 mg $NH_{3/4+}$-N l^{-1}).

4.4.6 Analysis of Total Nitrogen content by the Kjeldahl Method

Refuse samples (20 g) of known moisture content were adjusted to the appropriate moisture content and volume (70 - 100 ml) with distilled water and mixed by blending in a liquidiser for 5 min. Suspensions equivalent to 2 g refuse (dry weight) were placed in macro-Kjeldahl glass digestion tubes containing 3.67 g Kjeldahl catalyst containing mixed/powdered 10 g K_2SO_4, 1 g $CuSO_4.5H_2O$ and 0.1 g Se. Concentrated sulphuric acid (10 ml) was added and the suspension was digested for 60 min on a Tecator 2020 thermostatically controlled heating block with fume extraction according to the manufacturers instructions. The digested samples were allowed to cool to room temperature, diluted with 50 ml distilled water and distilled on a

Tecator 1026 distilling unit after automatic neutralisation with 50 ml 40% (w/v) NaOH. The distillate was collected in a 250 ml Erlenmeyer flask containing 25 ml boric acid solution (40 g boric acid, 10ml bromocresol green (0.1% w/v in ethanol), 7ml methyl red (0.1% w/v in ethanol and diluted to 1 l with distilled water). The collected distillate was titrated with 0.1 M HCl and the percentage nitrogen present in the sample calculated from the following equation as documented in the manufacturers instructions.

$$\%N = \frac{14.007 \times (\text{ml HCl titrant} - \text{ml HCl titrant blank}) \times \text{molarity of standard acid}}{\text{sample mass} \times 10}$$

The reagent blank was measured using 2 g sucrose of low nitrogen content instead of refuse samples.

4.4.7 Qualitative Analysis of Nitrogenous Compounds in Refuse

Qualitative analysis of nitrate (10-500 mg NO_3^- l^{-1}) was carried out using Merckoquant test sticks (BDH Merck). In addition, qualitative analysis of nitrite (1 mg NO_2^--N l^{-1} and above) could be achieved by adding one drop of Greiss-Ilosvay's reagent 1 and 2 to 0.5 ml of liquid sample. A red colour signaled the presence of nitrite. The method could be modified to measure nitrate in the absence of nitrite by placing a few grains of Zinc powder to the sample for 5 minutes to reduce some of the nitrate to nitrite before addition of the Greiss-Ilosvay's reagents. Ammonia could be detected by adding a drop of Nessler's reagent to 0.5 ml of the liquid sample, a positive result was indicated by an orange colour.

4.5 Sulphate Analysis (Rand *et al.*, 1975)

Sulphate analysis was achieved using the turbidometric method of Rand *et al.* (1975). A clarified sample (10 ml) was decanted into a screw top bottle and oxygen free nitrogen bubbled to expel any sulphides. To this was added 0.5 ml of conditioning reagent (glycerol, 50 ml; concentrated HCl, 30 ml; 95% v/v isopropyl alcohol, 100 ml; NaCl, 75 g; glass-distilled water, 300 ml). A standard spoonful (approximately 0.6 g) of $BaCl_2$ crystals, dry, 20-30 mesh) was added and the resulting solution mixed immediately on a vortex mixer for 60 s. After 4 minutes the solution was poured into a cuvette and the absorbance of the $BaSO_4$ precipitate read at 420 nm with a spectrophotometer.

Sodium sulphate was used as the standard, with a concentration range between 0.1 and 1 mM. A standard curve was derived on each occasion from which the sulphate concentrations of the samples were determined. A reduced volume method was also used successfully in 1.5 ml Eppendorf centrifuge tubes, reducing the sample volume required for each analysis.

Correction for sample coloration and turbidity was achieved by running blanks in which the $BaCl_2$ was not added.

4.6 Measurement of Redox Potential and Conductivity

The redox potential was measured using a BDH redox electrode and Testoterm meter calibrated with an Ingold calibrant solution. Conductivity was measured using a Hanna Conmet conductivity meter.

4.7 Measurement of Volatile Fatty Acids/Carboxylic Acids and Methane

Samples (0.9 ml) of leachate were acidified with 0.1 ml formic acid (Aristar Range, BDH). A µl was injected into Perkin Elmer 8500 Gas Chromatograph linked to a personal computer with a 900 series Perkin Elmer Interface containing a 2 m glass column (internal diameter 2 mm) containing 5% neopentyl glycol sebacate + 1% H_3PO_4 on Chromosorb W - AW (80-100 mesh) (Phase Separations Ltd, Clwyd). The injector and detector temperatures were set at 210^o and 220^oC respectively. The oven temperature was set for 2 minutes at 125^oC with a ramp rate set at $25^oC\ min^{-1}$ to reach a final oven temperature of 160^oC where it was held for 1 minute. Peak areas were compared to a similarly acidified standard of 10 mM solution of acetate, propionate, iso-butyrate, butyrate, iso-valerate, valerate and hexanoate. Linearity of peak area against concentration was confirmed across the concentrations measured. Analysis was confined to these seven volatile fatty acids.

Methane analysis was achieved using the same operating procedure for volatile fatty acids except gaseous sample injections of 50 µl were made and compared with pure methane injections (BOC gases) of 10 µl. The injector, detector and oven temperatures were set at 200^o, 210^o and 80^oC respectively.

4.8 Measurement of Dissolved Methane

Dissolved methane was measured using the method of Watson-Craik & Senior (1989c). Bijoux bottles stoppered with a Suba-Seal were evacuated for 30 s with a Millipore vacuum pump. The leachate sample to be analysed (1 ml) was injected into the bottle with a syringe, which was pricked with a clean needle to remove excess pressure, and allowed to equilibrate with the headspace for at least 1 hour. The headspace methane concentrations were measured by gas chromatography and compared with the headspace of bottles prepared with saturated methane distilled water controls (1ml) at a known temperature. Calculation of the solubility coefficients of methane in water at the measured temperature allowed the calculation of a standard methane concentrations in the water against which the leachate samples were compared (Watson-Craik & Senior, 1989c).

5. STUDY OF THE NITROGEN BALANCES IN LANDFILL REFUSE

5.1 Experimental Rationale

These experiments were carried out to determine the changes in concentrations of the various nitrogenous components in refuse during anaerobic decomposition. The experiments model refuse decomposition in a moist landfill in controlled conditions which could be replicated in future experiments. The addition of nitrate to landfill refuse was carried out to test the effect of nitrate on anaerobic decomposition, simulating the recirculation of nitrified or nitrate containing leachate to anaerobic refuse. To test the effect of nitrate on refuse, which had been actively producing methane, the refuse was incubated prior to the addition of nitrate. The effect of ammonia on methane production was tested by supplementing refuse with ammonia and measuring methane production.

5.2 Introduction

If the decomposition processes are enhanced by increasing the moisture content of the landfill then very high aqueous ammonia concentrations may be produced which could inhibit the refuse degradation. Recirculation of ammonia-laden leachate may be counter-productive, requiring its treatment above ground in aerobic nitrification lagoons before it is returned to the landfill. In such cases much of the ammonia will be oxidised to nitrate which may be denitrified in the landfill to nitrogen gas enabling an aerobic/anaerobic treatment strategy for leachate ammonia recirculation/discharge. The addition of nitrate to the landfill may also have implications for methane production, whose returns will be required to reduce the financial burden of the 'wet/flushing bioreactor' design.

Little is know about the concentration of nitrogenous compounds present in landfill refuse during decomposition. In a series of parallel experiments the nitrogen mineralisation patterns of the inorganic nitrogenous components from 1 - 2 month old landfilled refuse were studied. Nitrate supplements were made to simulate the effect of the addition of nitrified leachate to landfill refuse. The aqueous concentrations of nitrogen in ammonia, nitrite and nitrate were determined during anaerobic refuse degradation and compared with total nitrogen concentrations measured by the Kjeldahl method. Ammonia supplements were made to determine the effect of ammonia on refuse methanogenesis. The effect of phosphate was also determined to make sure that the methanogenic fermentation was not phosphate limited and could not be accelerated by adding more phosphate.

5.3 Materials and Methods

5.3.1 Nitrogen Balances During Anaerobic Decomposition

Refuse (62% moisture content w/wet w) containing exactly 7.6 g dry weight was adjusted to a moisture content of 80% and incubated under a helium atmosphere at 30 ^{o}C in 500 ml bottles stoppered by a Suba-Seal (Merck) and shaken prior to incubation with a Griffin flask shaker and an orbital shaker. The water and solutions were degassed with helium prior to their addition to refuse. The refuse was analysed for methane every 3 - 4 days, using a Perkin Elmer 8500 Gas Chromatograph, after which analyses the bottles were degassed with helium. Triplicate bottles were destructively sampled. After mixing for 1 h with 50 ml distilled water, triplicate slurry samples which represented approximately 2 g dry weight were removed and analysed for total nitrogen with a Kjeltec 20 Kjeldahl digestion system using a mixture of 10 g

K_2SO_4, 1 g $CuSO_4.5H_2O$ and 0.1 g Se as the catalyst (1 h digestion) and a Kjeltec 1026 distilling unit. The analysis of fresh samples enabled their true total nitrogen concentrations to be measured without subjecting the sample to drying in an oven which would remove some ammonia. The remaining slurry was filtered by passage through a Whatman GF/C glass microfibre filter under vacuum and centrifuged to remove suspended sediment. Extract ammonia concentrations were measured spectrophotometrically by reaction to indophenol blue with bleach and phenol. To test the effect of nitrate on the nitrogen mineralisation processes and methanogenesis replicate bottles were prepared with distilled water containing 500 or 100 mg NO_3^--N l^{-1} in the aqueous phase, equivalent to additions of 15.2 and 30.4 mg NO_3^--N to the refuse cultures. The pH of the nitrate solutions added was 7. The nitrate concentrations chosen were representative and double that of concentrations recorded in treated acetogenic landfill leachate. Nitrate and nitrite destruction during incubation was confirmed by spot test with Greiss-Ilosvay's Reagent prior and after reaction with zinc dust and by testing with BDH Merckoquant nitrate test sticks. Samples were removed and analysed for volatile fatty acids and sulphate.

5.3.2 Effect of Nitrate on Methane Production

To measure methane production, similar refuse cultures containing either no added nitrate, 500 or 1000 mg NO_3^--N l^{-1} in the aqueous phase were prepared and analysed for methane every 4 days. To measure the effect of nitrate addition on actively methanogenic refuse, refuse was incubated for 28 days at 30 ^oC and then adjusted to 80% moisture content with distilled water to a total nitrate concentration of either 0, 500 or 1000 mg NO_3^--N l^{-1} in the aqueous phase. To test the effect of the addition of mineral ions to the refuse, KCl (71.4 mM) was substituted for the addition of 1000 mg NO_3^--N l^{-1} (71.4 mM KNO_3) in the aqueous phase.

5.3.3 The Effect of Ammonia on Methane Pro duction

To measure the effect of ammonia supplements on methane production, similar refuse cultures containing no added ammonia, 500, 1000, 3000 and 5000 mg $NH_{3/4+}$-N l^{-1} (NH_4Cl) in the aqueous phase were prepared at 80 and 95% moisture contents and analysed for methane every 4 days. These cultures were compared with similar cultures containing 500, 1000, 3000 and 5000 mg NO_3^--N l^{-1} in the aqueous phase at 95% moisture content. Possible phosphate limitation on methane production was checked for by adding solutions of $NaH_2PO_4.H_2O$ equivalent to 500 and 1000 mg P l^{-1} to similar refuse cultures adjusted to 95% moisture content. The pH of solutions added to the refuse were adjusted to pH 7 with dilute NaOH before addition to the refuse. The pH and nitrate concentrations of these cultures were quantified by removing 100 µl of liquid with a syringe and injecting onto Sigma pH indicators strips, litmus paper and nitrate test sticks.

5.4 Results

5.4.1 Nitrogen Balances During Anaerobic Decomposition

Methanogenesis was observed in all of the refuse cultures adjusted with water but was severely restricted in those cultures supplemented with nitrate. The pH of the extracts decreased to 5.5-5.8 as a result of volatile fatty acid and carbon dioxide production but rose toward the end of the experiment when methane production was at its greatest (Figure 5.1 and 5.2). High volatile fatty acid concentrations were present within the refuse. Measurement of the weights, dry weights and density of the cultures before and after incubation revealed

virtually no loss in weight due to evaporation or decomposition. Nitrate and nitrite concentrations were not detected in the refuse from the landfill or in the unadjusted controls and presumably have no role in anaerobic refuse decomposition.

Release of Ammonia from Refuse and Nitrate Supplemented Refuse

Ammonia concentrations in the aqueous phase increased from 517-565 mg $NH_{3/4+}$-N l^{-1} at the start of the experiment to 935-1130 mg $NH_{3/4+}$-N l^{-1} after 62 days. Leachate draining from area 4 of the Shewalton landfill site from 1984 to 1987, the source of the landfilled refuse for this study, had ammonia concentrations of around 250 mg $NH_{3/4+}$-N l^{-1}, half as what was observed at the start of the incubation (DoE, 1995). Initial ammonia concentrations at 80% moisture content were typical of the leachate ammonia concentrations of many landfills receiving domestic and some commercial waste (DoE, 1995). Squeezing the sorted landfilled refuse at 62% moisture content prior to any incubation studies released a liquid containing approximately 200 mg $NH_{3/4+}$-N l^{-1} similar to leachate measured from the landfill site. Submerging and mixing the refuse during the preparation of cultures allows ammonia to enter solution and encourages a more uniform ammonia concentration to develop unaffected by condensation during storage or variations in the refuse.

Nitrate supplemented refuse cultures maintained their pH above those of the unsupplemented controls, especially during the first 6 days, presumably due to the buffering effect of the process of denitrification and the loss of acidic nitrate ions from solution. No nitrate or nitrite was found in the liquid extract after 6 days indicating extremely rapid denitrification of nitrate to nitrogen gas or nitrous oxide. The rate of the increase in the ammonia concentration bore no relationship to the rapid decline in nitrate concentrations, suggesting that the reduction of nitrate to ammonia was insignificant during the first 6 days. Nitrate addition increased the period till the onset of methanogenesis and reduced both the rate of methane production and the number of refuse cultures which became methanogenic during the experiment. Although the pH decrease during incubation was not as severe as observed in the unadjusted controls the onset of methanogenesis was affected in most of the refuse cultures containing nitrate. Nitrate and nitrite required analysis immediately after extraction as storage of samples, even at $4^{o}C$, led to the accumulation of small concentrations of nitrite and nitrate through the chemical oxidation of ammonia.

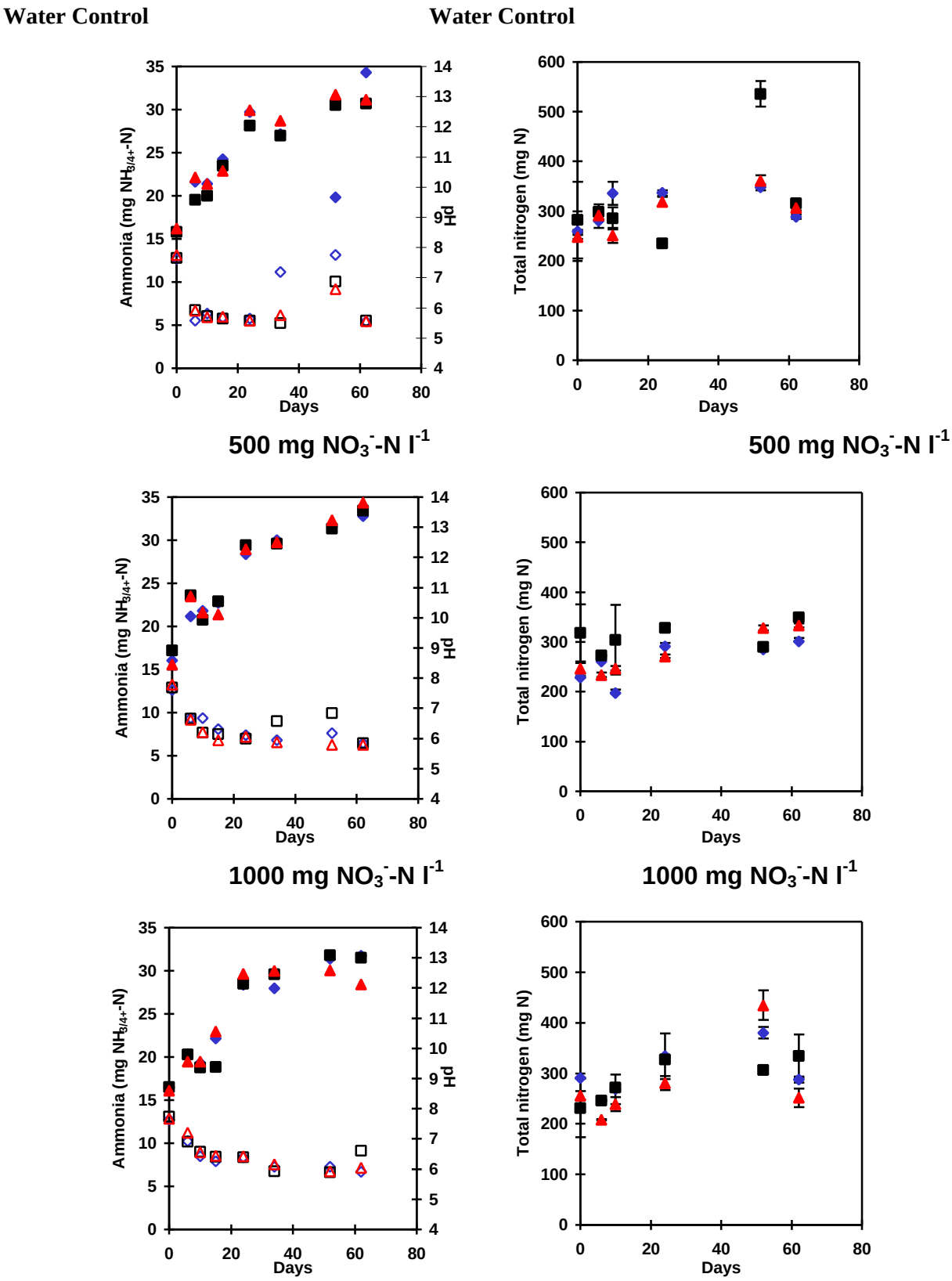


Figure 5.1 Measurement of ammonia and total nitrogen from refuse supplemented with water (control), 500 or 1000 mg NO_3^--N l^{-1} at 80% moisture content. Cultures 1 (◆), 2 (■) and 3 (▲) are identified by symbols. Clear empty symbols denote the pH value of the same cultures. Data points are the means of triplicate analyses. Error bars represent the standard error of the mean of triplicate analyses.

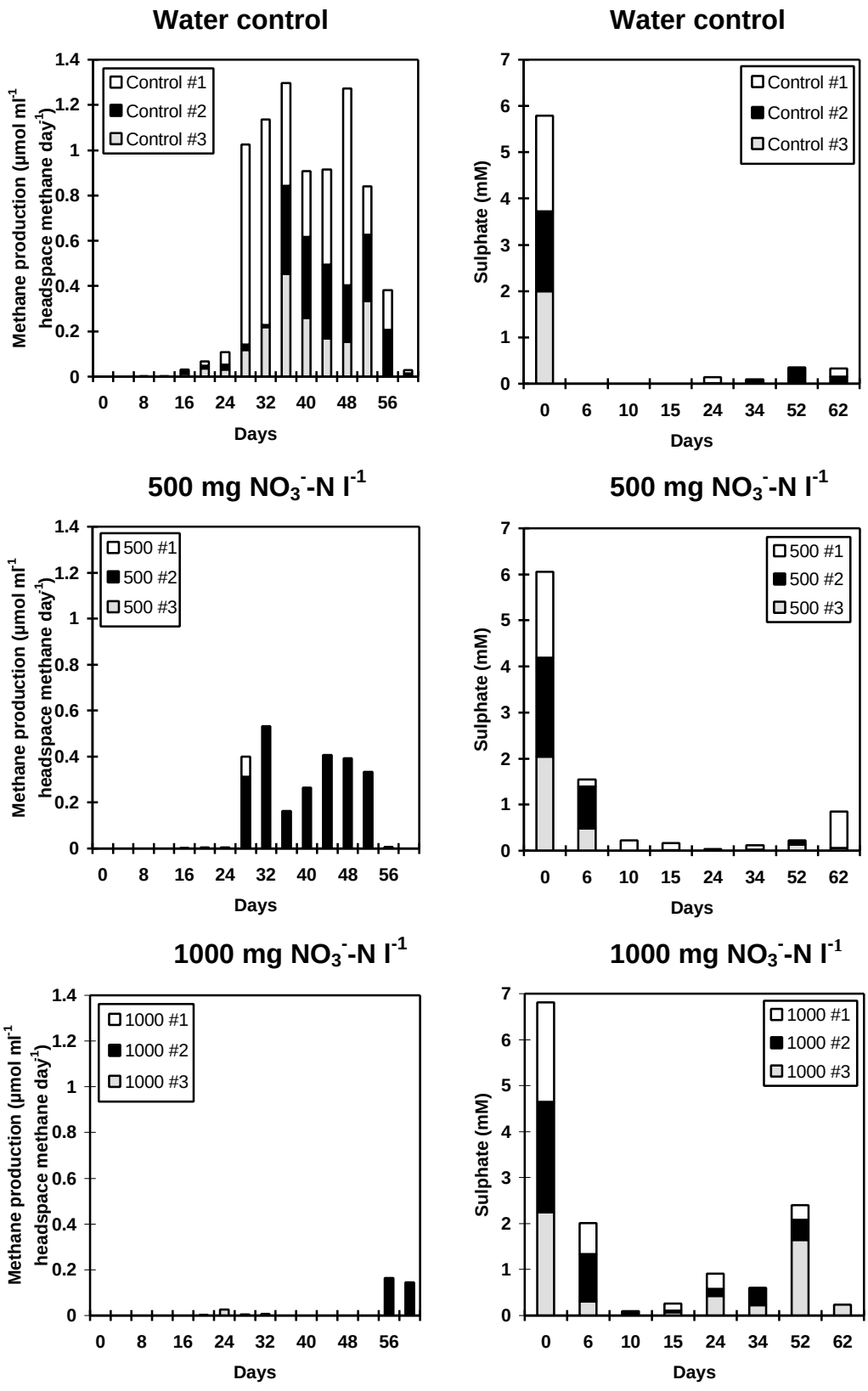


Figure 5.2 Methane production and sulphate concentration from refuse supplemented with water (control), 500 or 1000 mg NO_3^--N l^{-1} at 80% moisture content. Clear, dark and striped shaded areas represent the mean methane production or sulphate concentration of three cultures, 1, 2 and 3 at each time interval. Concentrations expressed are the means of triplicate analyses.

Total Nitrogen Concentrations in Refuse

The total nitrogen content of the refuse remained unchanged at approximately 300 mg N in all the cultures (Figure 5.1). With the exception of the denitrification/removal of nitrate to nitrogen gas and nitrous oxide there was no net loss of total nitrogen from the samples. The addition of nitrate had no effect on the mineralisation of ammonia in relation to total nitrogen content. Owing to the insensitivity of the Kjeldahl method to nitrate and the variability of total nitrogen content it was not possible to see an increase in the total nitrogen content of the refuse immediately after nitrate supplementation. The mean total nitrogen from all the samples analysed were calculated at 3.86% N dry weight (SD 0.8455, SE 0.0664). The amount of ammonia present as a proportion of the total nitrogen in the sample was approximately 5% at the start of the experiment rising to approximately 10% after 62 days incubation in all the cultures.

Volatile Fatty Acid Production

High concentrations of volatile fatty acids, representative of acetogenic leachate, which accumulated during incubation (Figures 5.3, 5.4, 5.5, 5.6, 5.7 and 5.8) contributed to the low pH values measured in the cultures sacrificed for analysis. Only slight differences were seen between the concentrations of volatile fatty acids in the water controls and the nitrate treated cultures. Hexanoate and valerate concentrations were significantly lower on day 10 in the nitrate treated cultures which may have contributed to the slightly lower propionate concentrations observed. The differences between the water controls and cultures prepared at 1000 mg NO_3^--N l^{-1} are shown on Figure 5.9. Neither of these fatty acids are normally associated with the decomposition of glucose or glucose polymers such as cellulose by anaerobes and are more likely to have come from longer chain carbon compounds such as fats. In our laboratory, anaerobic fermentations of the glucose polymer cellobiose by bacteria isolated from refuse have yielded only acetate, propionate and butyrate, though anaerobes operate a branched fermentative chain and can release products according to the chemical environment in which they find themselves rather than producing specific products from one specific substrate. The denitrification of 1000mg NO_3^--N l^{-1} nitrate did not dramatically reduce the concentrations of volatile fatty acids after 6 days.

Release of Sulphate

Sulphate concentrations in the water controls were almost completely exhausted after 6 days incubation. Addition of nitrate delayed the reduction of sulphate and concentrations were measured after 6 days. In only a few cultures was sulphate still detected after day 10 but a greater proportion of the cultures treated with nitrate contained detectable sulphate (Figure 5.2).

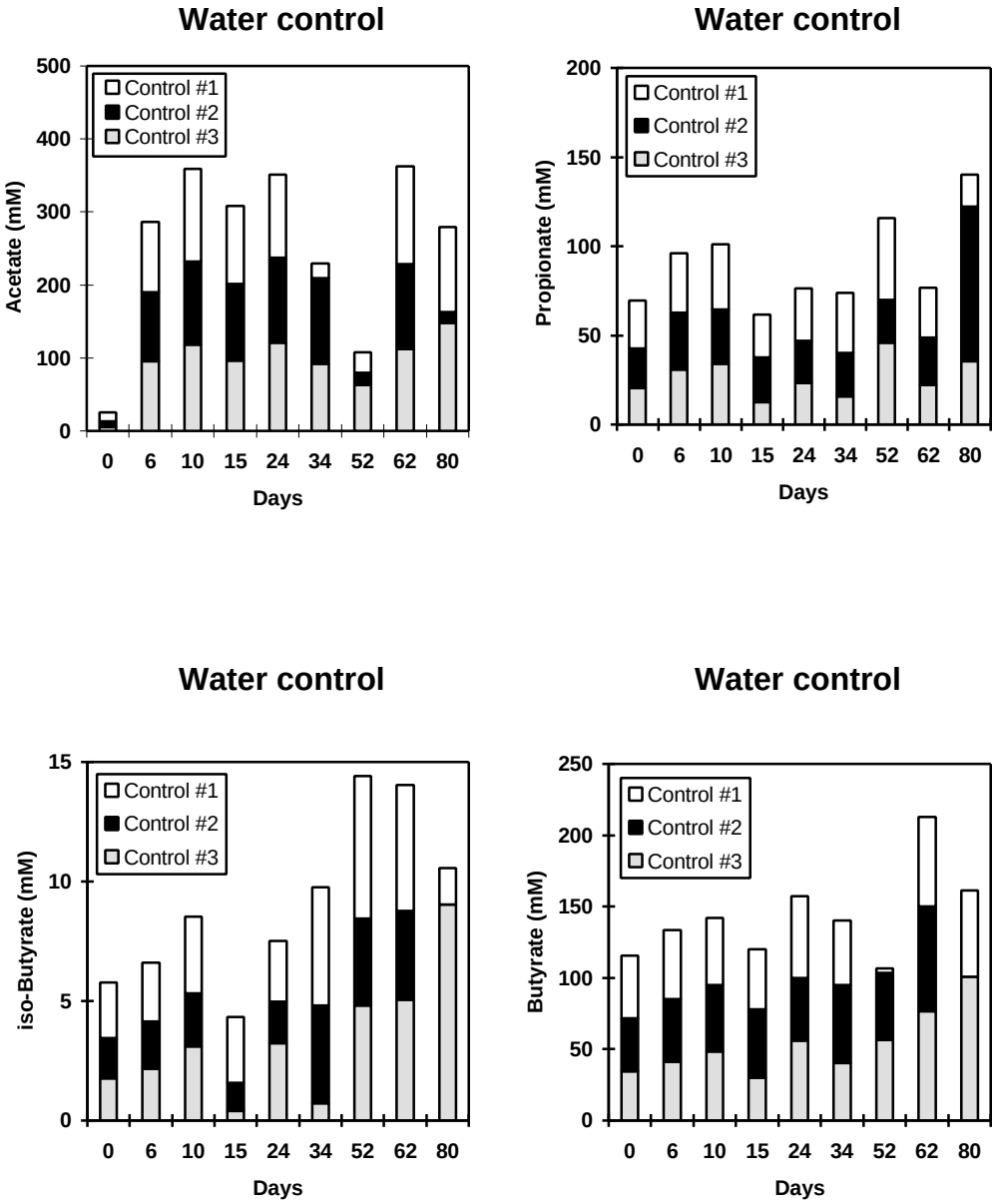


Figure 5.3 Volatile fatty acid production from refuse supplemented with water to 80% moisture content. Clear, dark and striped shaded areas represent the mean volatile fatty acid concentrations of cultures, 1, 2 and 3, destructively sampled at each time interval. Concentrations expressed are the means of duplicate analyses.

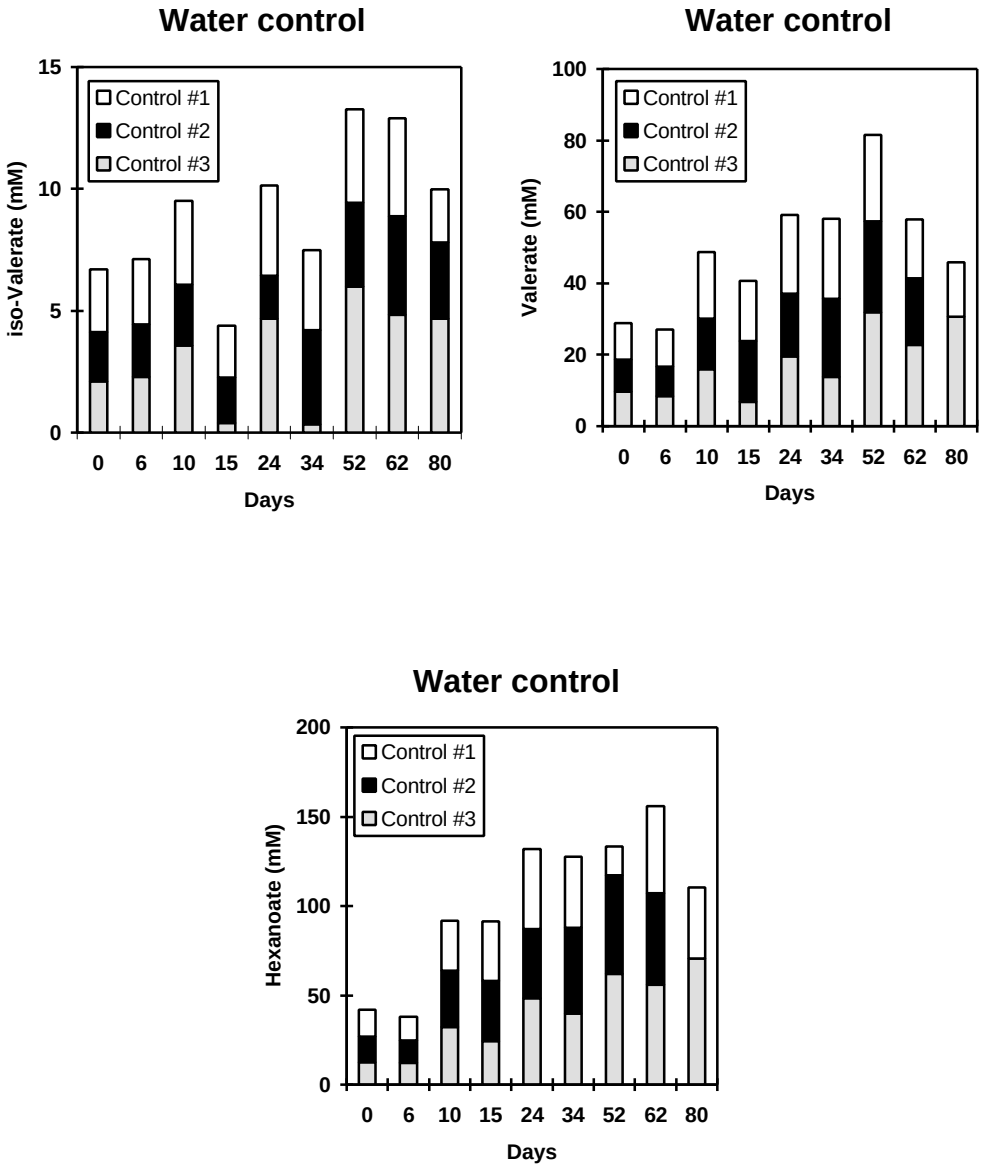


Figure 5.4 Volatile fatty acid production from refuse supplemented with water to 80% moisture content. Clear, dark and striped shaded areas represent the mean volatile fatty acid concentrations of cultures, 1, 2 and 3, destructively sampled at each time interval. Concentrations expressed are the means of duplicate analyses.

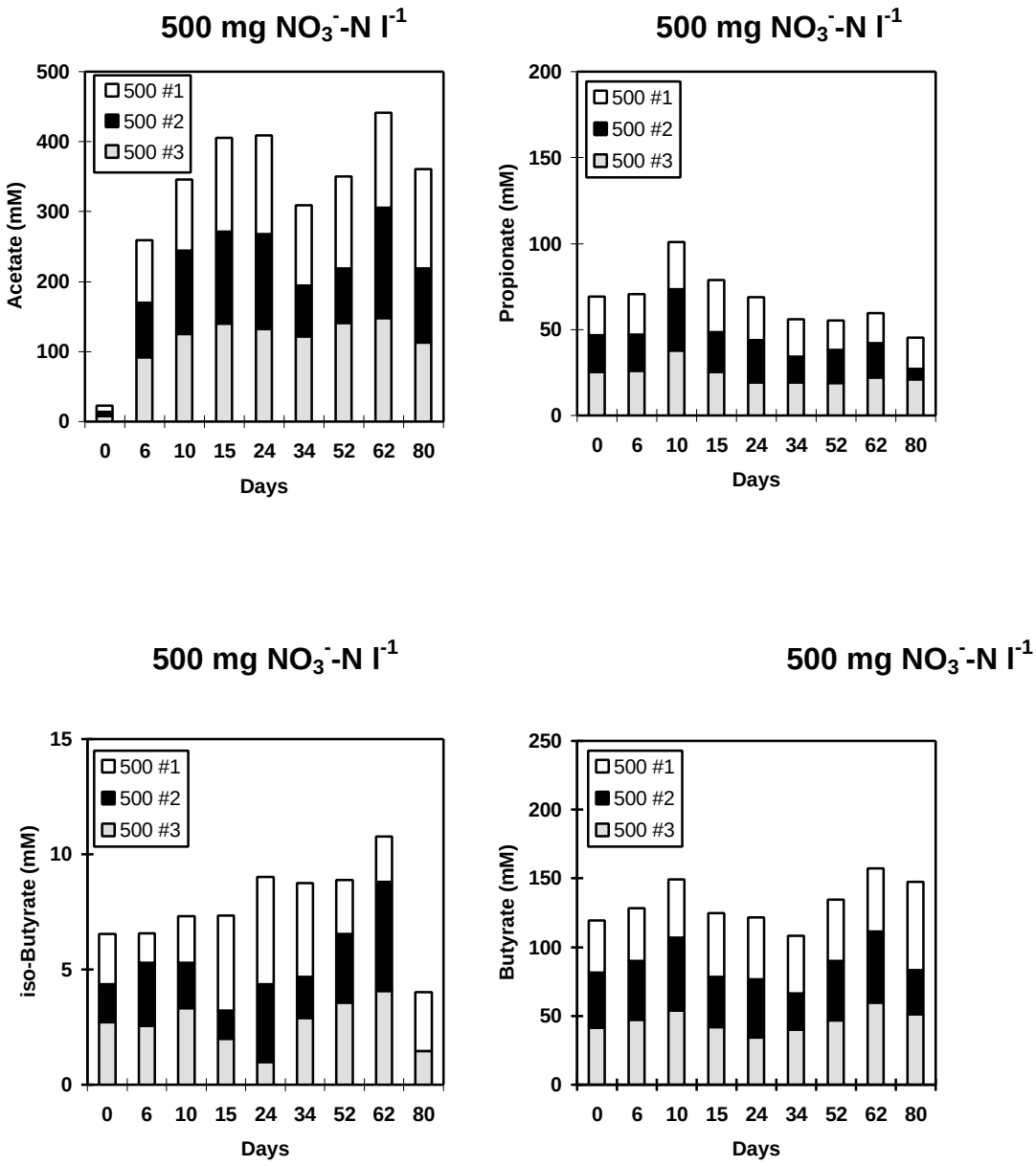


Figure 5.5 Volatile fatty acid production from refuse supplemented with 500 mg NO_3^--N l^{-1} at 80% moisture content. Clear, dark and striped shaded areas represent the mean volatile fatty acid concentrations of cultures, 1, 2 and 3, destructively sampled at each time interval. Concentrations expressed are the means of duplicate analyses.

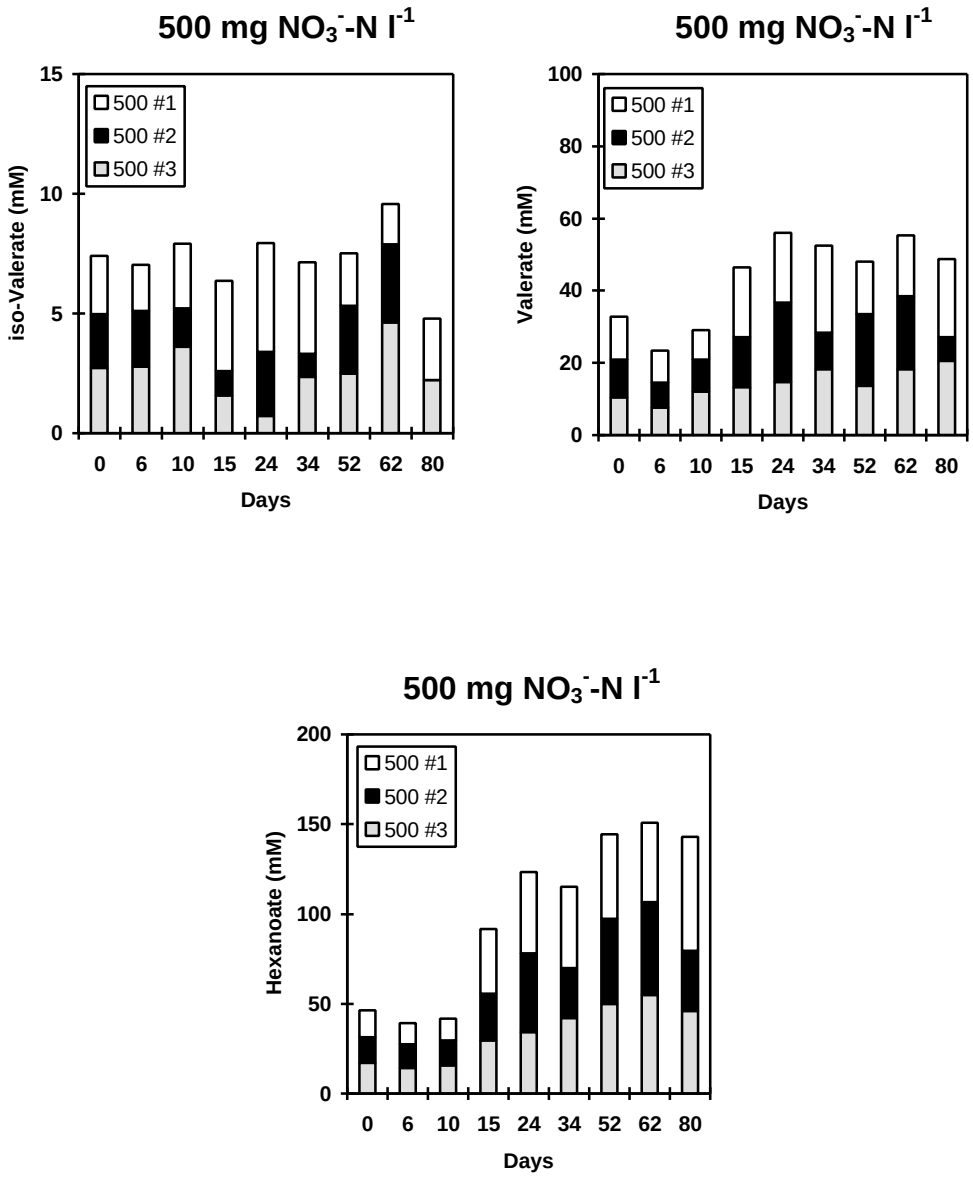


Figure 5.6 Volatile fatty acid production from refuse supplemented with 500 mg NO_3^--N l^{-1} at 80% moisture content. Clear, dark and striped shaded areas represent the mean volatile fatty acid concentrations of cultures, 1, 2 and 3, destructively sampled at each time interval. Concentrations expressed are the means of duplicate analyses.

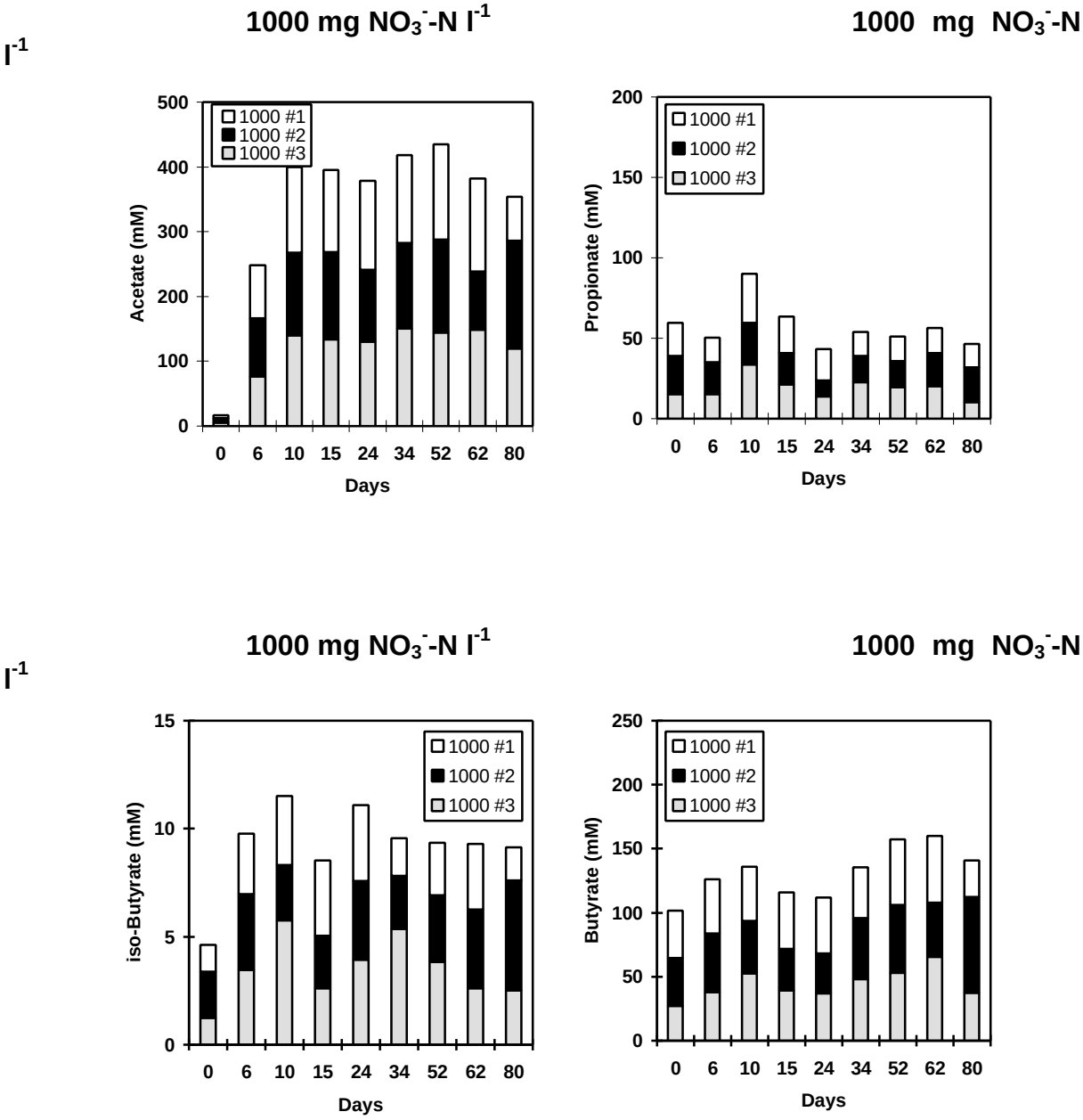


Figure 5.7 Volatile fatty acid production from refuse supplemented with 1000 mg NO_3^--N l^{-1} at 80% moisture content. Clear, dark and striped shaded areas represent the mean volatile fatty acid concentrations of cultures, 1, 2 and 3, destructively sampled at each time interval. Concentrations expressed are the means of duplicate analyses.

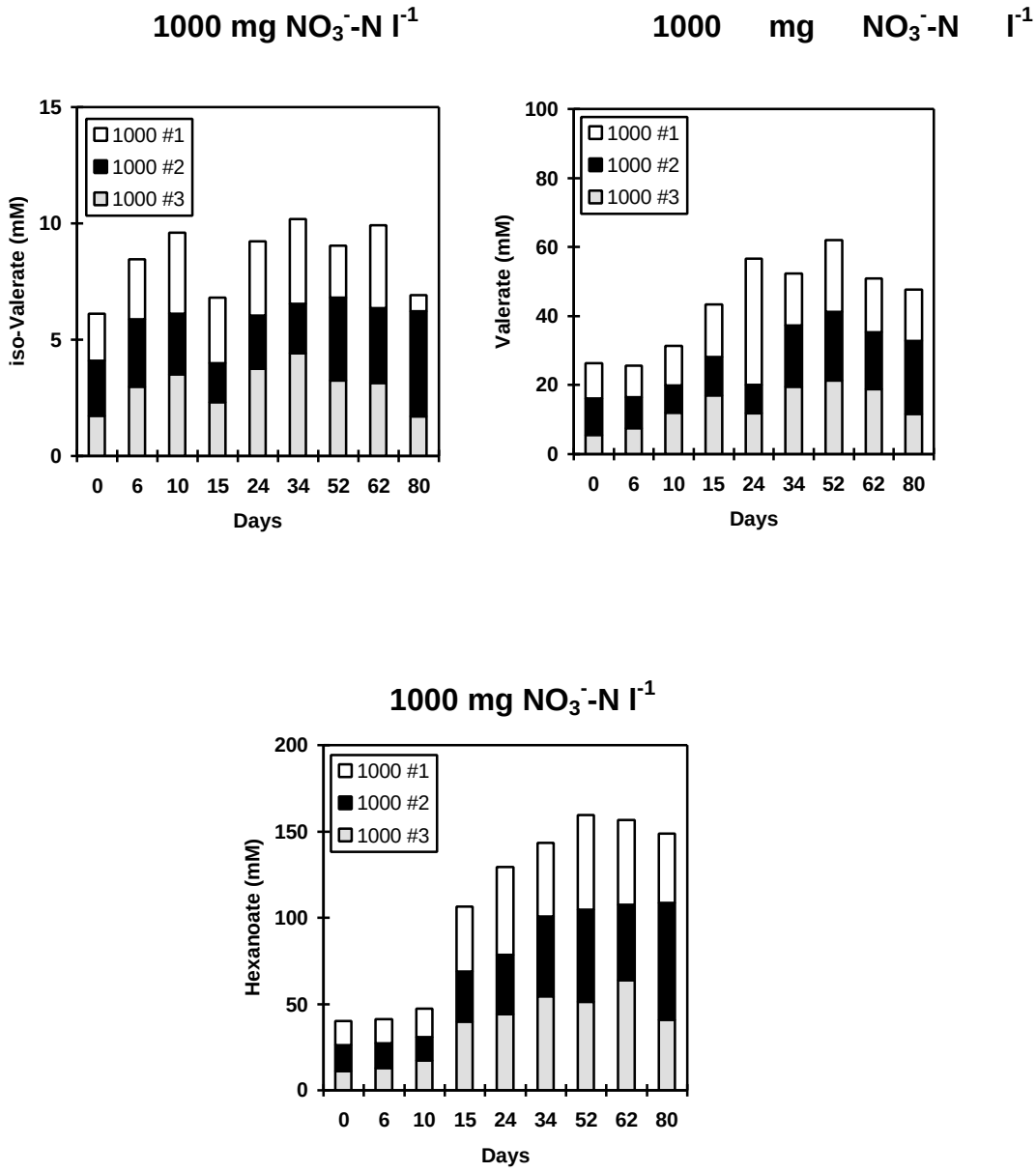


Figure 5.8 Volatile fatty acid production from refuse supplemented with 1000 mg NO_3^--N l^{-1} at 80% moisture content. Clear, dark and striped shaded areas represent the mean volatile fatty acid concentrations of cultures, 1, 2 and 3, destructively sampled at each time interval. Concentrations expressed are the means of duplicate analyses.

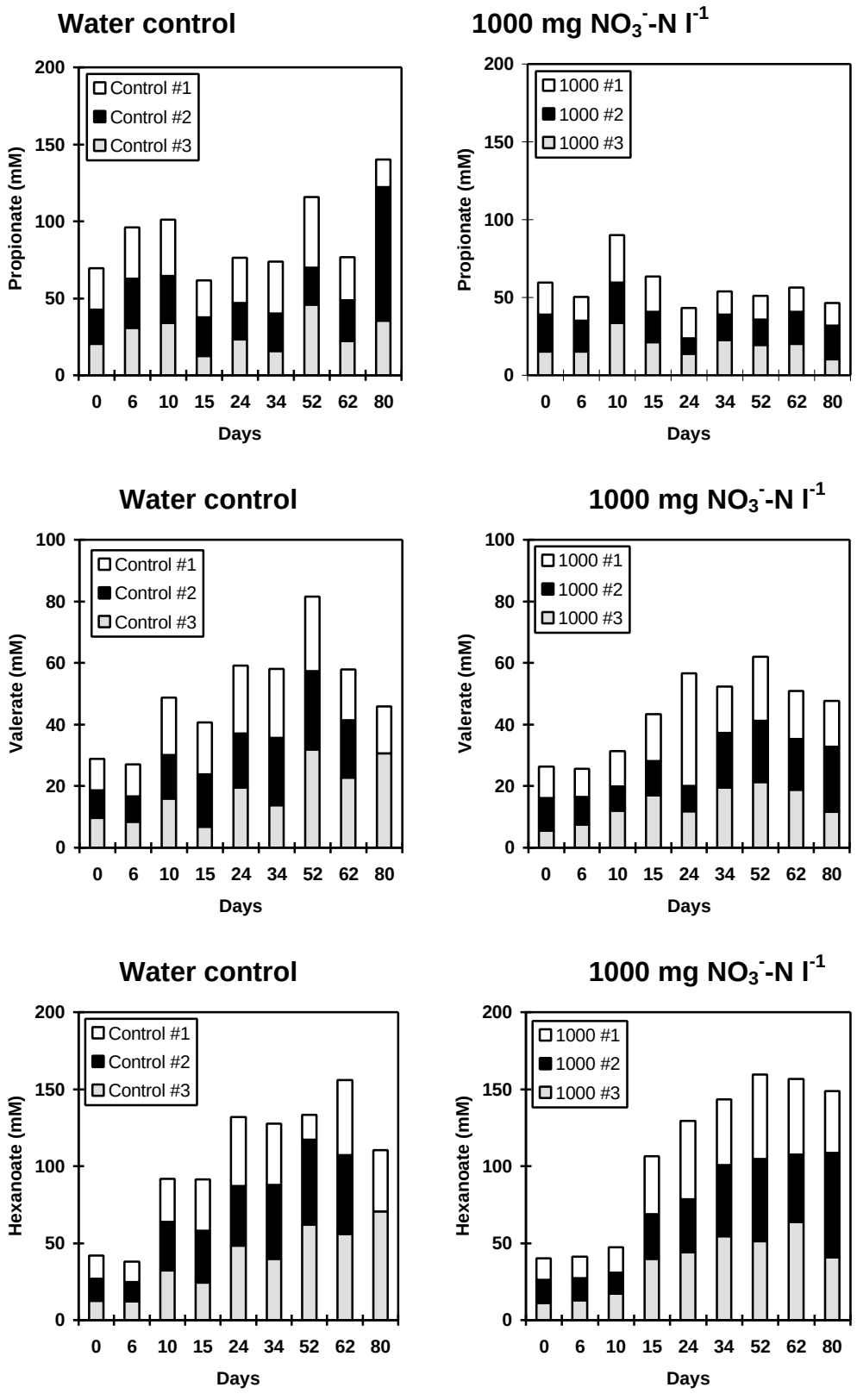


Figure 5.9 Volatile fatty acid production from refuse supplemented with water or 1000 mg NO_3^--N l^{-1} at 80% moisture content. Clear, dark and striped shaded areas represent the mean volatile fatty acid concentrations of cultures, 1, 2 and 3, destructively sampled at each time interval. Concentrations expressed are the means of duplicate analyses.

5.4.2 Measurement of Methane Production

Measurement of methane production from individual refuse cultures produced similar rates of methane production in the water controls compared to cultures sacrificed during extraction towards the end of the experiment. However methanogenesis began earlier and showed little variation between culture bottles (data not presented). Cultures which contained nitrate at the start of incubation had an increased lag phase before the onset of methanogenesis, of between 4 to 8 days in comparison to water controls (Figure 5.10). The final pH values of the cultures after 60 days were similar at 8.48 (SD 0.0379, SE 0.0219), 8.41 (SD 0.111, SE 0.0639) and 8.40 (SD 0.129, SE 0.0742) at initial nitrate concentrations of 0, 500 and 1000 mg NO_3^--N l^{-1} respectively. Volatile fatty acid concentrations in the controls at the end of the experiment were representative of methanogenic leachate showing mean acetate and propionate concentrations of 5.67 mM, (SD 2.40, SE 1.39) and 17.3 mM (SD 17.8, SE 10.8) respectively. Cultures adjusted to 500 mg NO_3^--N l^{-1} in the aqueous phase contained acetate and propionate concentrations of 4.99 mM acetate (SD 2.04, SE 1.18) and 48.7 mM propionate (SD 47.4, SE 27.4) with the cultures adjusted with 1000 mg NO_3^--N l^{-1} containing 42.1 mM acetate (SD 25.1, SE 14.5) and 35.2 mM propionate (SD 9.33, SE 5.39) and a variety of C_4-C_6 acids representative of acetogenic leachate.

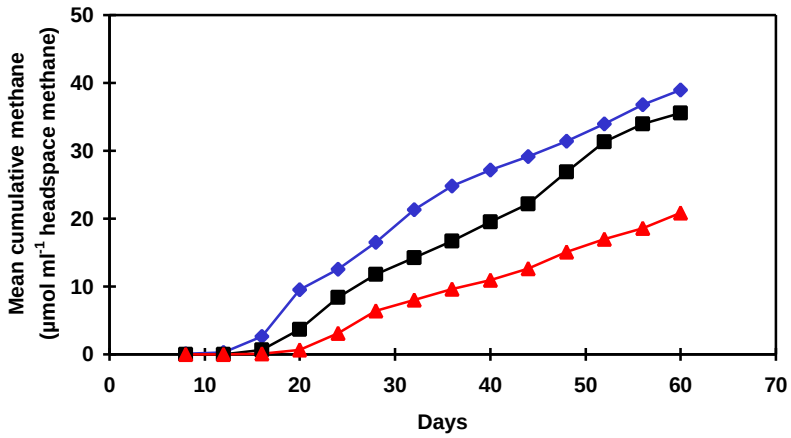


Figure 5.10 Measurement of methane production from refuse supplemented with water (◆), 500 (■) and 1000 (▲) mg NO_3^--N l^{-1} to 80% moisture content. Data points are the mean of triplicate cultures.

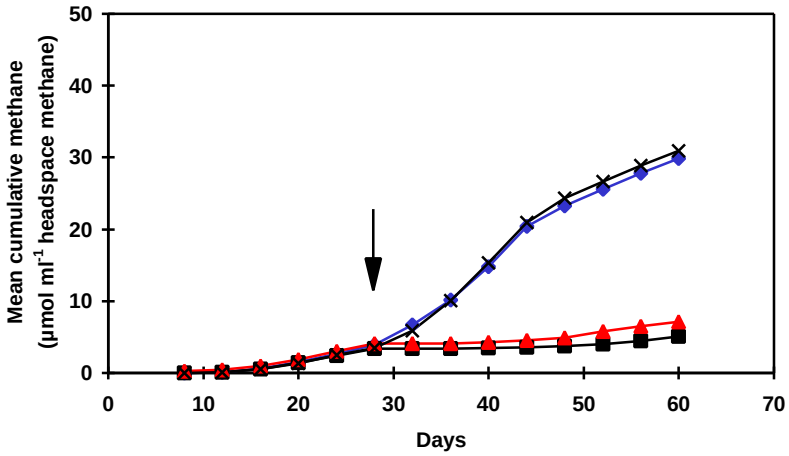


Figure 5.11 Measurement of methane production from refuse (62% moisture content) supplemented after 28 days with water (◆), 71.4 mM KCl (X), 500 (■) and 1000 (▲) mg NO_3^--N l^{-1} to 80% moisture content. The point at which the adjustments were made is indicated with an arrow. Data points are the mean of triplicate cultures.

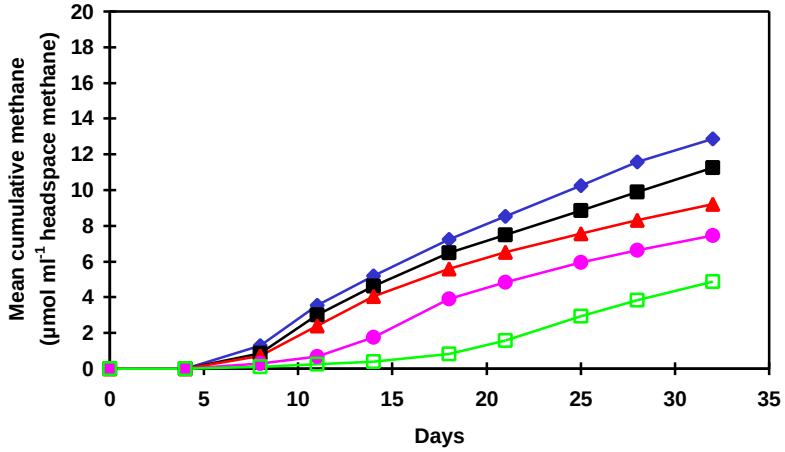


Figure 5.12 Measurement of methane production from refuse supplemented with water (◆), 500 (■) and 1000 (▲), 3000 (O) and 5000 (□) mg $NH_{3/4+}$-N l^{-1} to 80% moisture content. Data points are the mean of triplicate cultures.

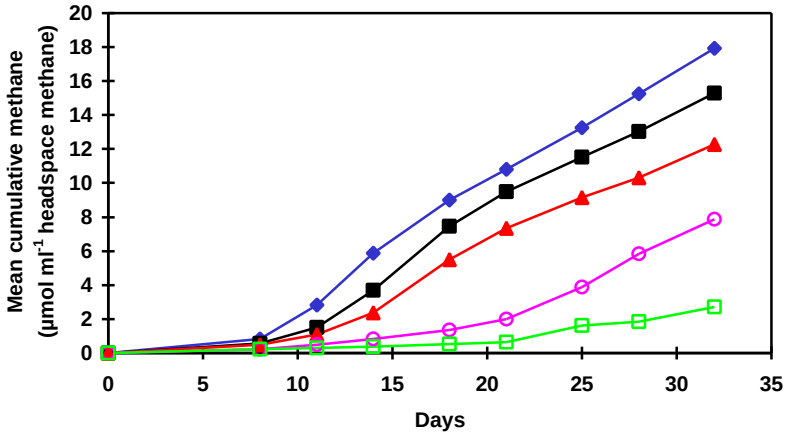


Figure 5.13 Measurement of methane production from refuse supplemented with water (◆), 500 (■) and 1000 (▲), 3000 (O) and 5000 (□) mg $NH_{3/4+}$-N l^{-1} to 95% moisture content. Data points are the mean of triplicate cultures.

5.4.3 The Effect of the Addition of Nitrate to Methanogenic Refuse

Addition of nitrate to methanogenic refuse inhibited methane production for 20 days and methane production upon recovery failed to reach those of the water controls (Figure 5.11). The similarity of the rate of methane production from distilled water controls to those containing KCl suggest that the small changes in ionic strength due to nitrate supplementation did not contribute to changes in the rate of methanogenesis. The higher pH of the cultures adjusted with 1000 mg NO_3^--N l^{-1} was probably a result of the denitrification of the larger amounts of nitrate in these cultures. Volatile fatty acid concentrations were higher in the nitrate treated cultures after 60 days incubation. Volatile fatty acid concentrations in the water controls and the KCl treated cultures were representative of methanogenic leachate and contained acetate at 3.20 mM (SD 1.586, SE 0.915) and 8.17 mM (SD 0.286, SE 0.202), and propionate at 34.83 mM (SD 6.73, SE 3.88) and 43.1 mM (SD 19.4, SE 13.7) respectively. Cultures adjusted with 500 and 1000 mg NO_3^--N l^{-1} were representative of acetogenic leachate containing C_4-C_6 acids and contained acetate and propionate concentrations at 132 mM (SD 17.9, SE 10.4) and 118 mM (SD 50.3, SE 29.1) acetate and 27.8 mM (SD 8.26, SE 4.77) and 22.9 (SD 3.20, SE 1.85) propionate respectively.

The final mean pH values after 60 days were significantly lower in the cultures adjusted with nitrate at 6.11 (SD 0.368, SE 0.212) and 7.00 (SD 0.994, SE 0.574) after additions during methanogenesis of 500 and 1000 mg NO_3^--N l^{-1} respectively. The pH values of the water and KCl controls were 8.37 (SD 0.0361, SE 0.0208) and 8.14 (SD 0.262, SE 0.185) respectively. The high concentrations of volatile fatty acids and the inhibition of methanogenesis, a process which increases pH, in the nitrate adjusted cultures probably contributed to their lower pH values.

5.4.4 The Effect of Ammonia and N itrate on Methane Production

The amount of ammonia reduced the yield of methane produced in the cultures (Figure 5.12 and 5.13). Approximately 20% less methane was produced at 80% moisture content in comparison to 95% moisture content (Figure 5.12 and 5.13), a feature of the increased

hydrolysis (cleavage in water) of paper waste and an increase in the interchange of fermentable substrates to different bacteria and a dissolution of toxic compounds at higher moisture contents. Phosphate concentrations had no effect on the methane yield and therefore the methanogenic fermentation of refuse can not be considered to be phosphate limited under these conditions (Figure 5.14). The pH of these cultures remained relatively constant at approximately 7.5-7.8.

A comparison with similar concentrations of nitrate at 500, 1000, 3000 and 5000 mg NO_3^--N l^{-1} in the aqueous phase at 95% moisture content revealed inhibition of denitrification at or around 1000 mg NO_3^--N l^{-1} in the aqueous phase. No nitrate was detected after 18 days incubation in the cultures treated with 500 mg NO_3^--N l^{-1} and one of the triplicate cultures treated with 1000 mg NO_3^--N l^{-1} indicating complete denitrification within this time. These cultures were the only ones to become methanogenic indicating the inhibition of methanogenesis by nitrate. Cultures treated with higher concentrations of nitrate maintained their concentrations of nitrate above 113 mg NO_3^--N l^{-1} for the entire 31 days incubation. One of the cultures treated with 1000 mg NO_3^--N l^{-1} showed continued denitrification after 18 days, with final nitrate concentration of approximately 56 mg NO_3^--N l^{-1} after 31 days incubation. The pH values in the cultures adjusted with nitrate increased their pH value from 7.5-7.8 at the start of the experiment to approximately pH 8.5 after 21 days. Those cultures which failed to denitrify all their nitrate maintained their pH values at approximately pH 9 indicating the possible alkaline inhibition of denitrification or lack of suitable oxidant. Small quantities (1-2 mg NO_3^--N l^{-1}) of nitrite were also detected in cultures supplemented with 3000 and 5000 mg NO_3^--N l^{-1} in the aqueous phase indicating incomplete denitrification to nitrogen gas and nitrous oxide and the accumulation of intermediates of denitrification such as nitrite.

Considerable quantities of gas were produced from the cultures supplemented with nitrate at 95% moisture content. Cultures bubbled rapidly during the first few days of incubation whose odor resembled an earthy smell characteristic of grassland soil.

5.4.5 Identification an Actinomycete Growing Anaerobically on Landfill Refuse

White surface microbial growth was observed after 80 days in refuse cultures (80% moisture content), which under the microscope bore similarities to *Actinomyces,* showing filamentous and dipheroid cells. The authors are unaware of any previous reports of actinomycetes growing anaerobically in methanogenic landfill refuse. The organism regularly grew in surface clumps with a nitrogen or helium headspace at redox potentials of -200 mV. The occurrence in the cultures was almost uniform and bore no resemblance to any of the treatments imposed including long periods (2-4 months) exposed to landfill gas (approximately 60% methane and 40% carbon dioxide) in heavy isotope experiments and enrichments (Chapter 6).

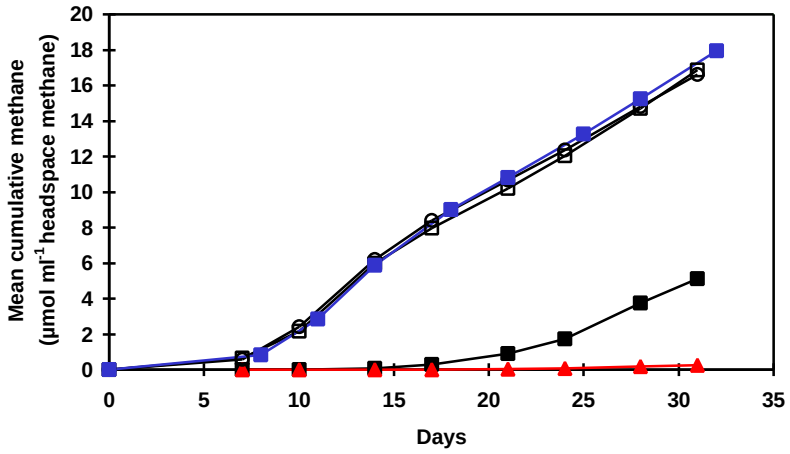


Figure 5.14 Measurement of methane production from refuse supplemented with water (◆), 500 (■) and 1000 (▲) mg NO_3^--N l^{-1} (one culture shown), and 500 (0) and 1000 (□) mg PO_4^{3-}-P l^{-1} to 95% moisture content. Data points are the mean of triplicate cultures.

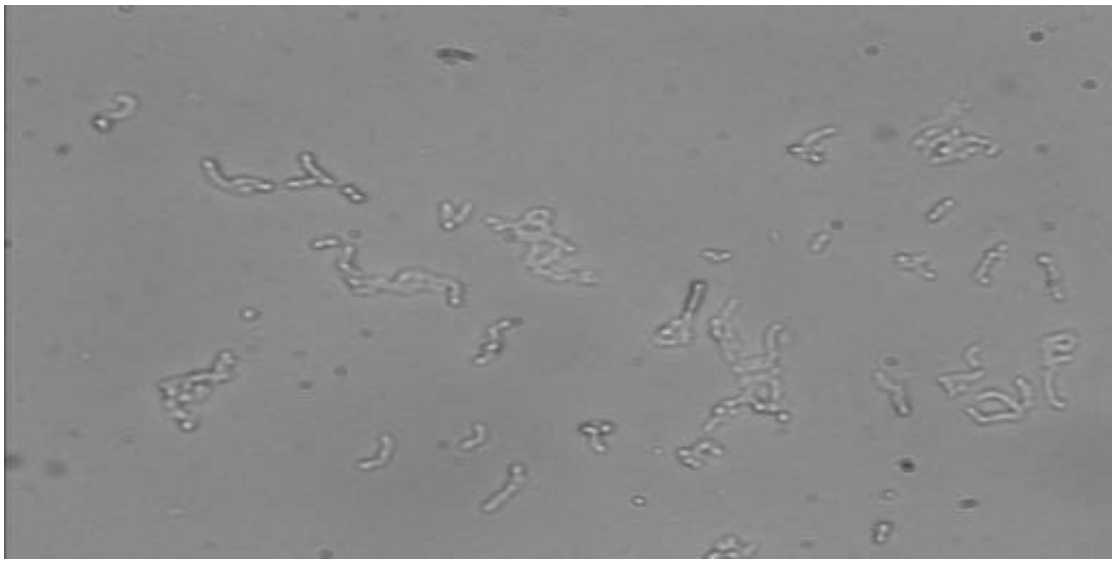


Figure 5.15 Filamentous bacteria (x1000)
Diperoid cells approximately 1µm diameter

5.5 Discussion

Refuse incubation studies at 80% moisture content revealed the release of ammonia during methanogenic refuse decomposition, through the deamination/removal from the organic nitrogenous components in the refuse and the movement of bound ammonia bound to negatively charged refuse particles. Ammonia concentrations increased but the total concentrations of nitrogen including the ammonia pool remained constant indicating the retention of nitrogen in refuse.

The proportion of ammonia-N compared to the total organic nitrogenous pool, the source of ammonia, was relatively small, between 5% to 10%, indicating that by the end of ammonia production the bulk of ammonia was retained in the refuse and only a small proportion (5%) was released into the aqueous pool. No significant loss of nitrogen from the system was measured during the incubation indicating that without the movement of leachate, ammonia will be retained in the refuse and that no biological or chemical process was observed which mediates the conversion of ammonia to nitrogen gas.

Rees (1980) gave the nitrogen and phosphorus requirements for the anaerobic fermentation of glucose (found in cellulose) to methane and carbon dioxide based on the theoretical yields of Pirt (1978). A kilogramme of refuse will contain about 0.47 kg of glucose which can theoretically be fermented into 26.3 g dry biomass. The nitrogen and phosphorus content of dry bacterial biomass is approximately 12% and 1.5% which will constitute 3.2 g and 0.4 g nitrogen and phosphorus respectively. Thus demands for nitrogen can be met by the 3.86% N dry weight analysed in the refuse.

Few analyses of refuse by the Kjeldahl method have been made. Bogner (1990) gave total Kjeldahl nitrogen measurements of between 800-9000 ppm dry weight (0.08-0.9% N). These measurements are lower than in the present study but are comparable adjusting for the fact that the non-biodegradable proportion such as glass, large plastics and metals have been removed from the refuse before the cultures were prepared. Samples in the National Household Waste Project (DoE 1994cd) contain approximately between 0.4 and 1.2% N wet weight at 28 to 41% moisture content, calculated to between 0.575 and 1.82 % N dry weight including the presence of glass, large plastics and metals.

The introduction of treated leachate, which has been aerated in a lagoon, may represent a viable method for the removal of aqueous nitrogen concentrations without the need for an anaerobic leachate treatment stage, in addition to stimulating refuse decomposition by the process of denitrification. Challenging the refuse with nitrate allowed the biological conversion of nitrate to nitrogen gas and nitrous oxide and the loss of nitrate and nitrogen from the system. Effects of nitrate on the anaerobic decomposition processes were many, delaying the onset of methanogenesis, long chain volatile fatty acid production and sulphate reduction. It could not be ascertained what proportion of the carbon pool was being consumed during denitrification although one effect was the lowering of the overall propionate concentrations.

Additions of nitrate at 80% moisture content delayed or inhibited methanogenesis, reducing both the amount of methane produced and in the acetogenic cultures (Figure 5.2) the quantity of refuse samples that became methanogenic. Nitrate could not be detected after 6 days incubation but the onset of methanogenesis was inhibited for a further 2 and 12 days in refuse samples treated with nitrate at aqueous concentrations of 500 and 1000 mg NO_3^--N l^{-1} respectively, with only one third of the treated samples producing methane within the 64 day

period. Methanogenesis recovered after longer incubation times but the concentrations of methane produced were much reduced, indicating either residual toxicity below the detection limit of 1 mg NO_3^--N l^{-1} or a poisoning of the methanogenic association at the start of the experiment. Both these effects could have induced longer lag phases for methanogenesis and therefore allowed other bacterial associations to use some of the available reductant and carbon substrate previously earmarked for methanogenesis. The addition of nitrate to landfilled refuse had a buffering effect. Nitrate supplemented cultures had a slightly higher pH during incubation than the water controls since the denitrification process is known to increase pH (Drtil *et al.*, 1995).

The denitrification of nitrate at concentrations of 500 and 1000 mg NO_3^--N l^{-1} at 80% moisture content by refuse was extremely rapid, explaining the absence of nitrate in landfilled refuse. The rate of increase in ammonia concentration bore no correlation to the rapid decline in nitrate concentrations suggesting that reduction of nitrate to ammonia was insignificant during the first 6 days and that most if not all the nitrate was evolved as nitrogen gas and nitrous oxide. Thus the introduction of treated leachate will not recycle significant concentrations of ammonia.

The addition of nitrate to cultures before incubation and during the incubation (Figures 5.10 and 5.11) produced different times before the onset of methanogenesis. These cultures were incubated and prepared at the same time and were more methanogenic than cultures sacrificed and tested for denitrification with relatively little variation between the methane concentrations. The onset or return of methane production was slower in cultures given nitrate during methanogenesis indicating a poisoning of the methanogens in these cultures, presumably by nitrate or an increase in the redox potential. Other factors may have contributed by the addition of nitrate such as volatile fatty acid concentration and pH since the final pH values were lower and volatile fatty acid cultures higher in these cultures. The lower pH would have slowed the initiation of methanogenesis and the activity of the methanogens and the recovery of methanogenesis.

Extremely high concentrations of nitrate at 95% moisture content inhibited the complete denitrification of nitrate and the onset of methanogenesis, though these high concentrations would not be experienced if landfill leachate was nitrified to nitrate owing to the lower potential nitrate concentrations. It was not possible to determine the limiting factor associated with incomplete denitrification of nitrate. Since there was still paper waste present within the refuse, the rate of denitrification may have been inhibited by the increase in pH associated with the loss of nitrate from the culture. The rate at which the paper was being degraded may have also lead to a lag in the denitrification of the nitrate and prolonged incubation may have led to complete denitrification. The higher concentrations of nitrate may have also had an effect on the release of nitrogen gas (N_2) favoring the release of nitrous oxide (N_2O) (Blackmer & Bremner, 1978) especially at the lower moisture content when there was less opportunity for the N_2O to dissolve.

In comparison to 80% moisture content the cultures prepared at 95% moisture content contained considerably more nitrate owing to the increase in the volumes of liquid used in their preparation. At 80% moisture content the refuse cultures prepared with 500 and 1000 mg NO_3^--N l^{-1} in the aqueous phase contained a total of 15.2 and 30.4 mg NO_3^--N respectively in addition to 7.6 g dry refuse. At 95%, prepared with 500 and 1000 mg NO_3^--N l^{-1} in the aqueous phase, the total nitrate available to the culture rose to 72.2 and 144.4 mg NO_3^--N respectively. Complete loss of nitrate was only observed at or below 1000 NO_3^--N l^{-1} in the aqueous phase at

95% moisture content. The maximum amount of nitrate that can be consumed by 7.6 g of dry refuse at 95% moisture content over a period of 31 days is thus 144.4 mg NO_3^--N, equivalent to 18.9 g NO_3^--N kg^{-1} of dry refuse.

The strong influence of ammonia concentration on methane production at 80% and 95% moisture contents demonstrated the importance of ammonia concentrations in influencing the rate of anaerobic decomposition. There was a reduction in the rate of methane production between 500 and 1000 mg $NH_{3/4+}$-N l^{-1} and a visible lag in the production of methane at higher concentrations of ammonia at 80% and 95% moisture contents. Raising the moisture content of the refuse from 80% to 95% will reduce the aqueous concentrations of ammonia considerably which may also contribute to the increase in methane production by refuse cultures at higher moisture contents. The lack of an effect by phosphate on the methanogenic fermentation suggested that the methanogenic fermentation was not phosphate limiting.

The identification of novel forms of actinomycetes in refuse was unexpected and an indication that there is still much to learn about the microbiology of landfilled refuse. The large amount of cellular material produced in these cultures by this organism is extremely interesting and of considerable academic interest. Its contribution to the refuse ecosystem will be considerable owing to its rapid growth in anaerobic environments. Its growth requirements were clearly gaseous owing to its occurrence on the surface of the refuse and the walls of the containers. The actinomycete was a strange and peculiar organism, of relevance to the microbiology of extreme environments and the origins of life. Gas collection pipes in landfill fur with microbial slimes. Videos taken within buried gas pipes reveal microbial slimes attached to the walls of the pipes restricting gas flow (Brain Latham pers. com.), organisms like this actinomycete may be involved.

5.6 Conclusions

- The proportion of free ammonia-N compared to the total organic nitrogenous pool (3.86% N dry weight), the source of ammonia, were relatively small, between 5% to 10%, indicating that by the end of the active phase of ammonia production the bulk of ammonia was retained in the refuse and only a small proportion (5%) was released into the leachate during anaerobic decomposition.

- Nitrate and nitrite were not found in the refuse studied and presumably have no role in the mineralisation of landfilled refuse.

- The reduction of nitrate to ammonia was insignificant and would not lead to increasing leachate ammonia concentrations if recirculation of nitrified leachate is attempted.

- The addition of nitrate to a landfill will inhibit methane production in the short term if applied in concentrations similar to those in nitrified or treated acetogenic landfill leachate. Methanogenesis will only recover once the nitrate has been denitrified.

- High ammonia concentrations had a considerable effect on the rate of methane production. Better rates of methane production and anaerobic degradation may result from controlling the ammonia concentration in refuse.

- The anaerobic decomposition of refuse was not phosphate or nitrogen limited.

6. STUDY OF THE NITROGEN TRANSFORMATIONS THAT OCCUR IN LANDFILLED REFUSE

6.1 Nitrification Potential of Landfilled Refuse

6.1.1 Experimental Rationale

Refuse taken from the surface and from 1-2 m deep was tested for the ability to nitrify, i.e. to produce nitrate by the bacterial process of nitrification. Refuse was inoculated into liquid media favourable for the growth of nitrifying bacteria to determine whether these bacteria are present in refuse.

6.1.2 Introduction

The literature review written prior to this report (Burton & Watson-Craik, 1996b) suggested that nitrification (see glossary), a bacterial process which produces nitrate from ammonia, may be operational in the upper surfaces of a landfill. Fresh refuse prior to being landfilled contains nitrifying bacteria, the bacteria responsible for the process of nitrification, owing to the soil and garden waste contained within it. The potential for nitrification and its contribution in the upper layers of a landfill are unknown but the effect is probably only significant in the top 10 cm of refuse. Movement of leachate in the surface layers, or leachate recirculation, may contribute to the dissolution of nitrate in the landfill and so lead to denitrification (see glossary) in anaerobic zones. Volatilisation of ammonia may also operate at the surface. Volatilisation and combined nitrification and denitrification will probably be the only mechanisms of ammonia loss from landfills outside of leachate treatment. Increases in pH to slightly alkaline pH values will encourage both nitrification and ammonia volatilisation as it facilitates the formation of free un-ionised ammonia (NH_3). However, the slow growth of nitrifiers suggests that daily covering with new refuse will not permit significant nitrification. Similarities exist between ammonia oxidising and methane oxidising bacteria and there is the opportunity for methane oxidisers to oxidise ammonia. Over two weeks exposure to the atmosphere will allow the proliferation of nitrifying bacteria and measurable ammonia oxidation. Since these processes will only be operative at the surface their effects will be minimal in the lower layers of the landfill. Capping will reduce their effect even further making the processes almost negligible. Once buried the nitrifiers are starved of oxygen and probably cease to function.

The presence or absence of nitrifying bacteria in refuse was tested by enrichment in liquid media preferential for the growth of ammonia oxidising bacteria.

6.1.3 Material and Methods

Refuse collected at 2-3 m depth from the landfill site (Chapter 4.1) was tested for the activity of nitrifying bacteria. Sterile Skinner & Walker (1961) medium (100 ml) containing 0.235 g $(NH_4)_2SO_4$, 0.2 g KH_2PO4, 0.04 g $CaCl_2.2H_2O$, 0.5 mg $MgSO_4.7H_2O$, 0.5 mg $FeSO_4.7H_2O$, NaEDTA 0.5 mg, 0.5 mg phenol red in 1 l distilled water was autoclaved for 20 min at 121^oC, 2 kg cm^{-2} in 250 ml Erlenmeyer flask enclosed by a non-absorbent cotton wool bung. Landfilled refuse (Chapter 4.1) or leachate was collected in sterile Bijoux bottles from 2-3 m deep from an uncovered area approximately 5 m^2. The refuse (0.5-1 g) or leachate (1 ml) was after 1 day inoculated into sterile Skinner & Walker (1961) medium adjusted to pH 7-7.5 with

sterile 5% (w/v) Na_2CO_3 and incubated at 30°C for 2-3 months, a method used for the enrichment and isolation of ammonia oxidising bacteria. Repeated inoculations (10) failed to identify ammonia oxidation or nitrification by nitrite or nitrate production from any of the refuse collected for experimentation or refuse collected from two other occasions but stored in black plastic bags at 4°C for 6-9 months. Refuse collected from the surface produced nitrite and nitrate in medium during incubation inferring the activity and presence of nitrifying bacteria which oxidise ammonia to nitrite and nitrate. The rate at which these samples responded to enrichment was similar to soil samples (Burton, 1993) producing small quantities of nitrite (>1 mg NO_2^--N l^{-1}) and detectable concentrations of nitrate in the medium during incubation after 3-5 weeks. The pH value also fell to 5-6 turning the phenol red indicator and the medium yellow.

6.1.4 Conclusions

The enrichments suggested the stratification of nitrification, and its restriction to the upper layers of the landfill. The absence of nitrifiers in the deeper zones suggested that the bacteria were not able to proliferate or able to penetrate to this depth after 1-2 months potential growth at the surface. Results also suggested the attenuation of nitrifying bacteria in deeper layers, since soil, a source of nitrifying bacteria, was present in the refuse collected at 2-3 m depth. Enrichments of sediments nearly always identify nitrification, even in deeper zones (Burton, 1993). Landfilled refuse is unusual in that it is absent of viable nitrifying microorganisms.

6.2 Identification of Nitrogen Transformations by the Use of Heavy Isotopes

6.2.1 Experimental Rational e

Two experiments using a heavy and detectable form of nitrogen ^{15}N were carried out. The first involved the addition of traceable nitrate ($^{15}NO_3^-$) to refuse samples to determine whether there was microbial transformation to ammonia ($^{15}NH_{3/4+}$) in refuse. If so, nitrate added to a landfill may be transformed to ammonia recycling ammonia back into the landfill. Although previous experiments (Chapter 5) failed to identify transformation of nitrate to ammonia, small amounts may be produced which can only be identified by heavy isotope techniques. The second experiment measured turnover of ammonia within refuse samples using heavy ammonia ($^{15}NH_{3/4+}$). The concentrations (pool sizes) of heavy ammonia ($^{15}NH_{3/4+}$) in comparison to normal ammonia ($^{14}NH_{3/4+}$) were determined periodically, since a reduction in the aqueous concentration of heavy ammonia ($^{15}NH_{3/4+}$) would indicate bacterial uptake and transformation to other compounds during refuse decomposition, and binding to refuse particles.

6.2.2 Introduction

Until the industrial manufacture of nitrogenous fertilisers the world's nitrogen supply was controlled entirely by bacteria. Nitrogen fixing bacteria convert gaseous nitrogen to compounds such as ammonia, which can be assimilated by plants. Denitrifying bacteria return nitrogen gas to the atmosphere.

What happens in between nitrogen fixation and denitrification in terrestrial and aquatic ecosystems is of considerable interest because of its implications for fertility and the dependence of plants and micro-organisms for a supply of nitrogen, be it ammonia, nitrite or

nitrate. The processes of decay and decomposition of organic material yield ammonia by ammonification, some of which is partly recycled by the growth and incorporation of ammonia into the cells of bacteria and fungi. Pools of ammonia generated represent a nutrient source for nitrifying bacteria which can oxidise ammonia to nitrite and nitrate as seen in the aerated lagoons of leachate treatment works. Anaerobic environments encourage the nitrate formed to be reduced to nitrogen gas (N_2) and nitrous oxide (N_2O) during the oxidation of organic compounds by denitrifying bacteria in environments such as the anaerobic digesters of waste water treatment works. In environments like landfills, where ammonia cannot be oxidised to nitrate and there is little denitrification, the ammonia can accumulate to reach toxic concentrations.

6.2.3 The Identification of the Pathways of Nitrate Transformation

The addition of nitrate to refuse cultures (Chapter 5) demonstrated the ability of refuse to denitrify. Nitrate was lost from the systems containing 500 and 1000 mg NO_3^--N l^{-1} within 6 days. It was concluded that the vast majority of the nitrate was reduced to nitrogen gas (N_2) or nitrous oxide (N_2O). Previous work in the laboratory also indicated that landfilled refuse can denitrify. Refuse cultures could be induced to produce nitrous oxide from nitrate by a headspace of 10% acetylene gas (Sinclair, 1994), blocking the reduction of nitrous oxide to nitrogen during the bacterial process of denitrification (Yoshinari & Knowles, 1976). Autoclaving stopped nitrous oxide production identifying a biological process.

In a oxygen free environment there is an increased tendency for the non-biotic reduction of compounds such as nitrate to ammonia. Metals such as zinc are capable of catalysing this reaction, and indeed, the properties of an alloy of zinc, Devarda's Alloy, are used during the analysis of ammonia (see below). The reaction is also carried out biochemically by plants and bacteria as a way of making ammonia available for building proteins.

Some bacteria, typically anaerobes such as *Clostridium*, are capable of releasing ammonia derived from the dissimilatory reduction of nitrate and nitrite (Caskey & Tiedje, 1980; Cole & Brown, 1980). Internally nitrite may be reduced to ammonia without the use of an electron transport chain, electrons are diverted from NADH to nitrite and not to an organic compound. The substrate is further oxidised and the yield via substrate level phosphorylation increased. More ammonia is produced than is required for growth and ammonia is released.

In the cultures supplemented with 500 and 1000 mg NO_3^--N l^{-1} (Chapter 5), increases in ammonia concentrations were observed after 6 days but it was not possible to determine if a proportion of the ammonia originated from the reduction of nitrate. Control cultures prepared without the introduction of nitrate showed a similar increase in ammonia, leading to the conclusion that the increases in ammonia concentrations were not a result of reduction to ammonia but the release of ammonia from the refuse into solution. Introducing ^{15}N nitrate ($^{15}NO_3^-$) should allow the transformation of nitrate to ammonia to be identified by analysis of ^{15}N ammonia ($NH_{3/4}^+$) in the ammonia pool. The proportion of nitrogen derived from the nitrate in the ammonia pool can be quantified exactly, giving a indication of how much will return to the aqueous ammonia pool in refuse should nitrified leachate be recirculated.

6.2.4 The Identification of the Pathways of Ammonia Transformations

In the landfill environment the principal pool of ammonia is bound ammonia, held either as a constituent of proteinaceous material or attached to refuse particles, not freed into solution by mixing and blending. The binding of ammonia is a natural process dependent on the attraction of ammonia to negatively charged refuse and sediment particles. Ammonia in solution typically exists in an ionised state as the ammonium ion (NH_4^+) and will be attracted by the tendency of refuse and sediment particles, including the clay constituent of paper waste, to become negatively charged. Approximately 5-10% of the total ammonia is present in solution in the leachate (Chapter 5). Transformation of ammonia to nitrite and nitrate cannot occur because of the absence of oxygen and the bacteria which carry out these changes (Chapter 6.1). Utilisation of ammonia for growth by anaerobic bacteria during the decomposition of refuse will not generate sufficient demand for ammonia, leading to an accumulation of ammonia as the proteins present in the vegetable and animal matter are broken down. The conditions are set for high leachate ammonia concentrations and with it the risk of the pollution of ground water drinking supplies and surface waters (Figure 2.2).

Ammonia can only be removed from the leachate during bacterial growth and by removing leachate from the landfill and treating it in aerobic lagoons. There is limited opportunity for oxidation or volatilisation (Chapter 2). Incorporation of ammonia into bacterial proteins during the decomposition of waste is an unknown factor but will be small in comparison to aerobic systems where the growth rates of microorganisms are much greater. By adding a traceable amount of ^{15}N ammonia ($^{15}NH_{3/4+}$) it is possible to follow the movement from the ammonia pool in the leachate to the bound state either within bacterial proteins or attached to the refuse particles. The addition of traceable amounts of ammonia ($^{15}NH_{3/4+}$) allows the flux of ammonia to and from the leachate to be calculated and the rate of utilisation of ammonia during refuse decomposition measured. The technique requires only a small proportion (1%) of traceable ammonia to be added, maintaining leachate ammonia concentrations near their natural concentrations.

6.2.5 Materials and Methods

Preparation of Microdiffusion Chambers

Microdiffusion chambers were constructed using 120 ml polypropylene specimen jars (Fisher) containing 24 ml 1 M KCl and approximately 0.2 g of powdered heavy MgO. Whatman GF/D filter disks (6 mm diam.) impregnated with 10 µl of 2.5 M $KHSO_4$ were suspended by a 4:10 stainless steel wire (Goodfellow, Cambridge, UK) from the lid of the container. After addition of prepared samples (see below) the containers were sealed and left for 5-7 days in the laboratory. Ammonia gas was evolved as a result of the high pH of the KCl solution/MgO powder and caught in the acidic $KHSO_4$ impregnated filter disks. The lids containing the filter paper were removed and left upturned in a vacumn dessicator for approximately 1 week to dry (Figure 6.1). The dried filter disks were separated from their wires into tin capsules (Elemental Microanalysis Ltd) and ^{15}N and ^{14}N analysed by direct combustion mass spectrometry. Nitrate was captured in an identical way except the diffusion chamber also contained approximately 0.4 g powdered Devarda's alloy (Aldrich) to reduce nitrate to ammonia. Any nitrite present in the samples was also reduced to ammonia. No nitrite was detected in cultures in samples from similar cultures nor in those containing less than 1000 mg NO_3^--N l^{-1} in the aqueous phase (Chapter 5). The time required for ammonia capture was determined by adding ammonia to replicate micro-diffusion chambers and re-dissolving the

captured ammonia in water for analysis. The capture efficiency was tested by preparing concentrations of ammonia for microdiffusion and direct combustion mass spectrometry.

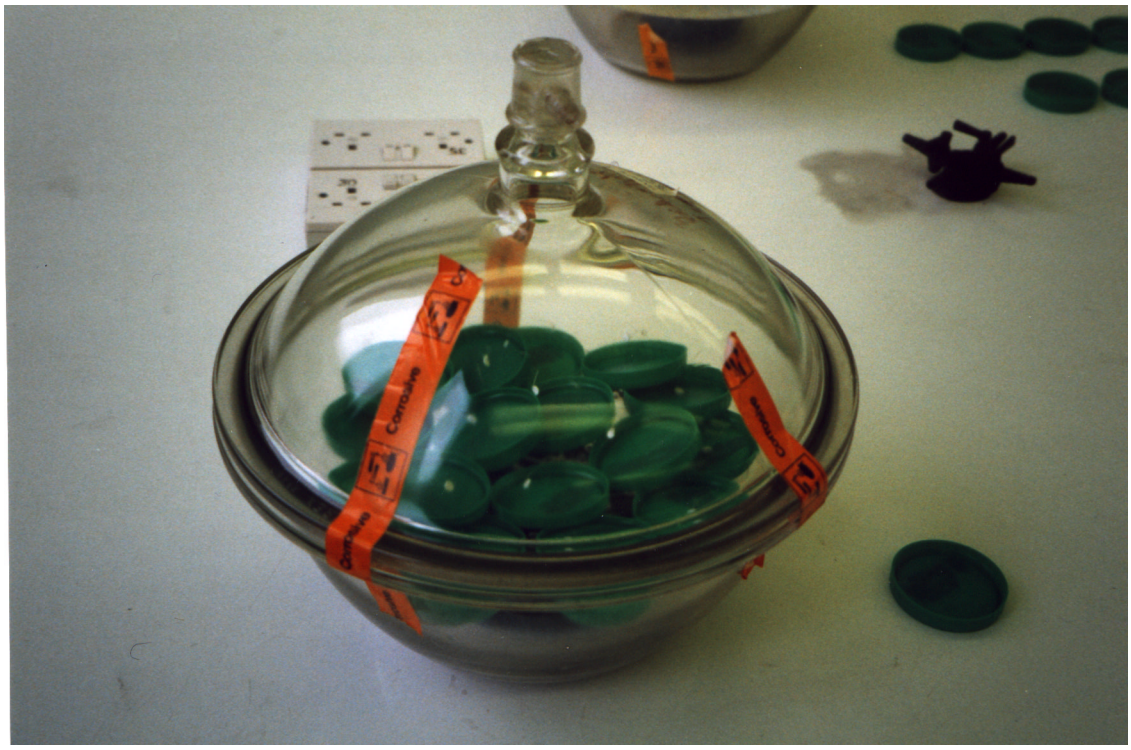


Figure 6.1 Desiccation of filter paper disks containing captured ammonia for continuous flow isotope ratio mass spectrometry.

The complete capture of ammonia in the microdiffusion chambers was confirmed during a dry run of the nitrate experiment. The absence of ammonia within the microdiffusion chambers confirmed by spectrophotometric analysis and a second microdiffusion. Capture of ammonia from 90% efficiency to complete capture was achieved by the addition of 1 M KCl to the diffusion chambers as opposed to distilled water. KCl and Devarda's alloy blanks were prepared by incubating diffusion chambers without liquid samples.

The laboratory at the time of the experiments had a temperature of between 24-25°C which encouraged condensation to form in the microdiffusion chambers. This could remove captured ammonia from the $KHSO_4$ impregnated filter papers if the capture process was either too slow or the chambers left too long before sampling. KCl accelerated the capture process so that the disks could be removed before the condensation appeared.

Advantages of this techniques are that no extraction or distillation method is required for sample preparation. Centrifugation allows the removal of the microbial and non- dissolved suspended sediment, so that only dissolved nitrogen compounds are measured. Alternative systems for the capture of ammonia for direct combustion mass spectrometry are described by Brooks *et al.* (1989).

Analysis of Nitrogen Using Continuous Flow Isotope Ratio Mass Spectrometry

Analyses using direct combustion continuous flow isotope ratio mass spectrometry were carried out at the Scottish Universities Reactor Centre in East Kilbride. The use of the ^{15}N nitrogen isotope allowed specific nitrogen transformations to be followed against the normal background ^{14}N nitrogen. The ^{15}N nitrogen isotope is stable and represented 0.378% (SD 0.0210) of the nitrogen in the experimental systems. It is not radioactive and shares none of the dangers associated with radioactive isotopes. The ^{15}N nitrogen isotope is heavier allowing its separation from ^{14}N nitrogen by mass spectrometry. The sample was burnt in oxygen and then reduced to nitrogen gas and separated into three signal peaks, N_2 gas containing ^{14}N, N_2 gas containing ^{15}N and ^{14}N, and N_2 gas containing ^{15}N. The system is described by Dr. Tom Preston (Preston & Barrie, 1991) who maintains the prototype Europa Scientific Roboprep C/N Analyser system (Figure 6.2) at the Scottish University Reactor Centre.

Addition of ^{15}N Nitrate ($^{15}NO_3^-$) to Methanogenic Refuse

The aim of the nitrate experiment was to identify anaerobic reduction to ammonia and measure the rate of denitrification or loss of nitrate. Separate samples were prepared for nitrate and ammonia for analysis by continuous flow isotope ratio mass spectrometry. The methanogenic refuse cultures (7.6 g dry weight refuse, 57.4% moisture content) were incubated for 3 weeks at 30^oC (Figure 6.3). Triplicate cultures were confined with a rubber Suba-Seal (Merck) and incubated at 30^oC. Cultures were analysed for methane daily and flushed with helium. After 21 days incubation, cultures were adjusted to 50 mg $^{15}NO_3^-$-N l^{-1} with $Na^{15}NO_3$ (Isotech Incorporated, Ohio) in the aqueous phase to a total moisture content of 95% and shaken for 1.5 h. Weighed liquid samples of approximately 7 ml were removed with a syringe following methane analysis, stored in ice in Eppendorf tubes and the refuse cultures flushed with helium. Liquid samples were clarified by centrifugation (MSE Microcentaur centrifuge for 15 min at 17,500 rpm). Aliquots (1 ml) were made to the microdiffusion chambers (see above), incubated and analysed for dissolved nitrogen, ^{15}N and ^{14}N by mass spectrometry. Samples were also analysed for volatile fatty acids. Cultures were compared to water controls, cultures containing either water or 50 mg $^{15}NO_3^-$-N l^{-1} in the aqueous phase (opened for the measurement of pH value and redox potential) and an unsampled control analysed for methane.

Figure 6.3 Refuse cultures prepared at 95% moisture content.

Addition of ^{15}N Nitrate ($^{15}NO_3^-$) to Sterile Gamma Irradiated Refuse

To identify whether the reduction of nitrate to ammonia was mediated by living microbial cells, triplicate refuse cultures (7.6 g dry weight) were degassed with helium for 30 min and gamma irradiated (minimum dose 25 kGy) at the Scottish Universities Reactor Centre. Cultures were supplemented with 1 ml of a filter sterilsed sodium nitrate solution ($Na^{15}NO_3$) to approximately 50 mg $^{15}NO_3^-$-N l^{-1} at 95% moisture content. The refuse remained in the reactor for 41 h, the radiation causing the glass of the bottles to turn black. A liquid sample was taken after shaking for 1.5 h and tested for viable microorganisms by streaking on nutrient agar (Difco) plates and incubation at 30^oC for 5-6 weeks. Micro diffusion chambers were prepared at 1.5 h and 7 days, after which another contamination check was made and the samples analysed by direct combustion continuous flow isotope ratio mass spectrometry. To avoid contamination no gas samples were taken for analysis.

Similar refuse cultures containing 50 mg NO_3^--N l^{-1} were autoclaved at 121^oC, for 20 min at 2 kg cm^{-2} three times, allowing cooling to room temperature before each cycle. The cultures were incubated for 7 days at 30^oC.

Addition of ^{15}N Ammonium ($^{15}NH_4^+$) to Refuse

The aim of the ammonia experiment was to determine the loss of aqueous ammonia (ammonia turnover) to the refuse by differences in the pool sizes of $^{15/14}N$ over time. Loss of ^{15}N to the refuse mass will suggest turnover of ammonia. The total amount of aqueous $NH_{3/4+}$ will increase as the refuse degrades but there may be a decrease in labeled $^{15}NH_{3/4+}$ suggesting the movement of ammonia between the liquid phase and the refuse mass. Cultures were incubated for 160 days, the ammonia was analysed over a 48 day methanogenically active period.

Refuse (53.2 g dry weight) was confined within a 1 l vessel at 95% moisture content with a gas collection apparatus (Figure 6.4). An ammonium chloride (CDN Isotopes Ltd, Canada) solution was added to two replicate refuse cultures at a moisture content of 95% to produce a final $^{15}NH_{3/4}^{+}$ proportion in the ammonia pool of approximately 1%. The refuse cultures were mixed for 1.5 hours. Weighed samples (6 ml) were removed with a syringe and prepared and analysed by microdiffusion and direct combustion continuous flow mass spectrometry. Headspace methane concentrations and volume were measured prior to sampling. The 337 ml headspace in each vessel was degassed with helium at the start of the experiment.

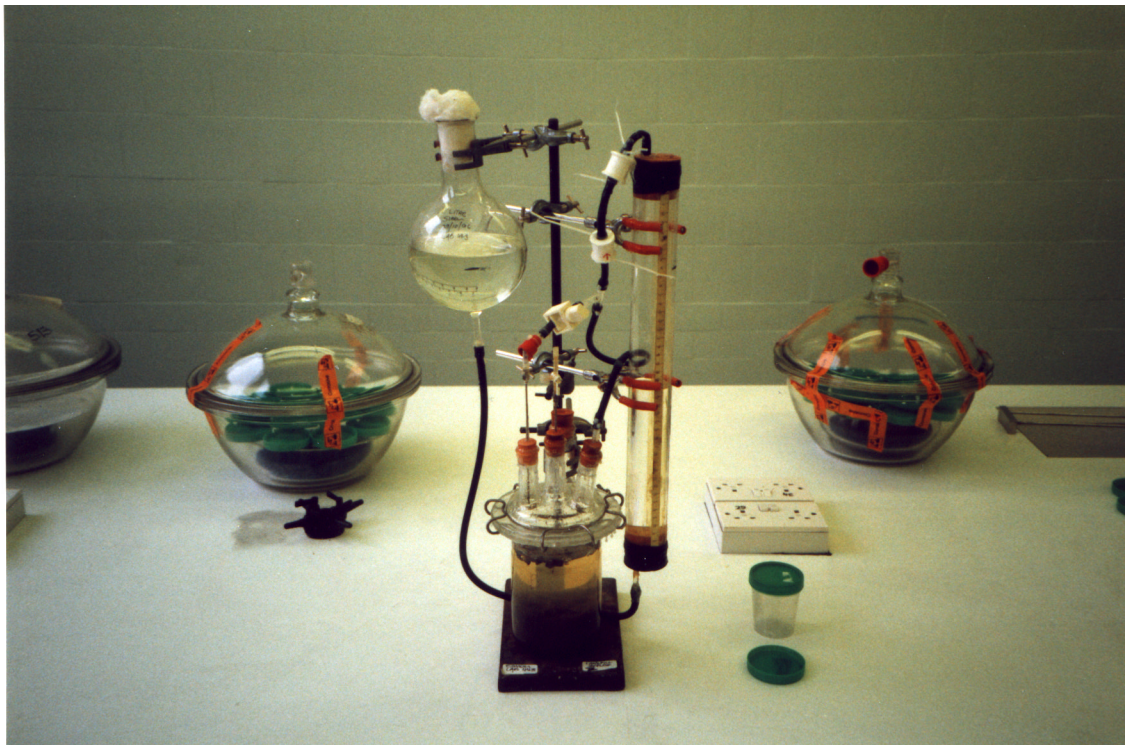


Figure 6.4 A 1 litre vessel containing refuse with gas collection apparatus which was used for experiments with heavy ammonia. To the right is a specimen container used for microdiffusion.

6.2.6 Results

Addition of ^{15}N Nitrate ($^{15}NO_3^-$) to Methanogenic Refuse

There was a rapid decline in the nitrate concentrations in the nitrate adjusted cultures (Figure 6.5). No nitrate was detected after 43.5 h. Ammonia concentrations remained relatively constant at approximately 30 mg $NH_{3/4+}$-N l^{-1} decreasing to a mean concentration of 17 mg $NH_{3/4+}$-N l^{-1} at the final analysis (Figure 6.5). Liquid samples from the water controls contained approximately 20-35 mg $NH_{3/4+}$-N l^{-1} of which 0.378% (SD 0.0210) was ^{15}N

throughout the experiment (Figure 6.6). Methane production resumed after 21 h (Figure 6.7) albeit it at a slightly reduced rate owing to the sampling procedures and the sudden influx of degassed water. Rates of methane production recovered after 71.5 h to rates above that experienced prior or after the analysis period (Figure 6.9). The pH in similar cultures remained relatively constant at about pH 7 (Figure 6.8).

Samples adjusted with 50 mg $^{15}NO_3^-$-N l^{-1} revealed an increase in the ammonia pool of $NH_{3/4+}$-^{15}N such that after 43.5 days 9.5% of the captured ammonia was ^{15}N, derived from the nitrate. The experiment had thus identified a nitrogen transformation pathway from nitrate to ammonia. The heavy nitrate, at its peak, after 43.5 h, yielded approximately 2-3.5 mg $^{15}NH_{3/4+}$-N l^{-1} in the aqueous phase (Figure 6.11). This dissipated slightly after 114.5 h days possibly after interchange with bound $NH_{3/4+}$ (0.378% ^{15}N) and a reduction in the total ammonia pool in the refuse (Figure 6.5) which reduced the amount of ^{15}N analysed. The experiment revealed that should nitrified leachate containing 50 mg NO_3^--N l^{-1} be returned to the landfill 4 -7% of the nitrate added will be reduced to ammonia. The remainder is presumably denitrified to N_2 or N_2O gas. Methane production was inhibited for at least 43 h when nitrate was present in the cultures (Figure 6.7). Methane was detected after 68.5 h and the rates of methane production then increased to reach similar rates as the untreated water control (Figure 6.9). There was some variation in the ability of different cultures to reduce nitrate to ammonia but good replication of analysis by this method (Figure 6.12).

Methane production by cultures containing nitrate, prepared for the analysis of pH and redox potential, was also inhibited over the period when nitrate was being removed from solution indicating inhibition of methane production by nitrate (Figure 6.13).

Opening the cultures for analysis did not appear to affect methane production (Figure 6.10). Cultures which were opened produced a similar response to nitrate as those left unopened and sampled (Figure 6.13). Redox potential and pH fell slightly during analysis (Figure 6.14) but there was no dramatic increase in redox potential or pH value associated with nitrate addition or the process of denitrification and nitrate reduction.

Acetate and propionate concentrations in the cultures after the addition of water remained relatively constant and then increased sharply in each culture after 68.5 h (Figure 6.15). Nitrate additions delayed the increases in acetate and propionate for a further day and there was a slight increase in the acetate concentrations after 19.5h. Butyrate concentrations remained constant during the 114.5 h incubation period.

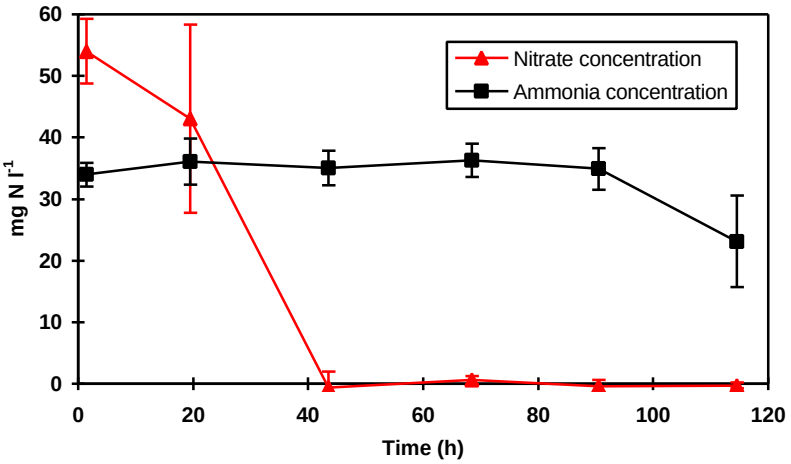


Figure 6.5 The mean concentrations of nitrate (▲) and ammonia (■) present in refuses cultures adjusted with heavy nitrate ($^{15}NO_3^-$) to 95% moisture content. Error bars represent the standard deviation of triplicate cultures.

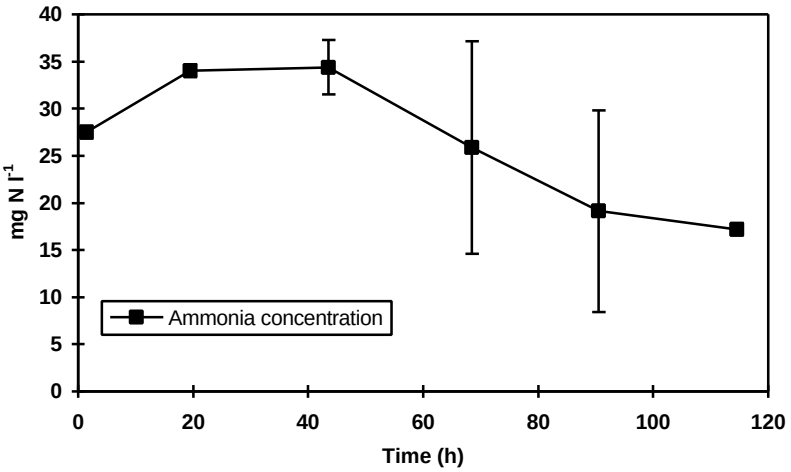


Figure 6.6 The mean concentrations of ammonia (■) present in methanogenic refuse cultures adjusted to 95% moisture content. Error bars represent the standard deviation of triplicate cultures.

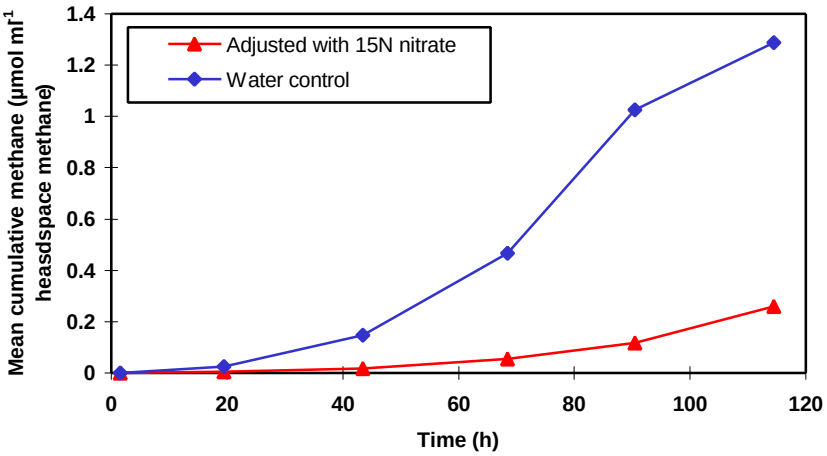


Figure 6.7 The mean cumulative headspace methane concentrations produced by methanogenic refuse cultures treated with heavy nitrate ($^{15}NO_3^-$) (▲) and water (◆) to 95% moisture content.

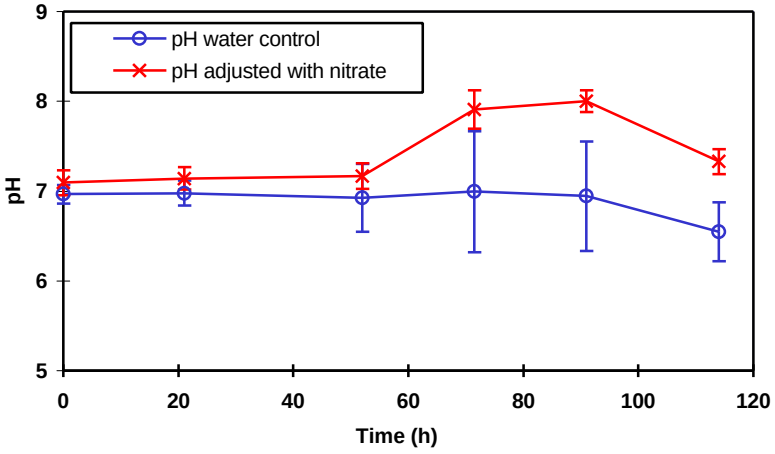


Figure 6.8 The mean pH values of refuse cultures treated with heavy nitrate ($^{15}NO_3^-$) (x) and water (o) to methanogenic refuse cultures at 95% moisture content. Error bars represent the standard deviation of triplicate cultures.

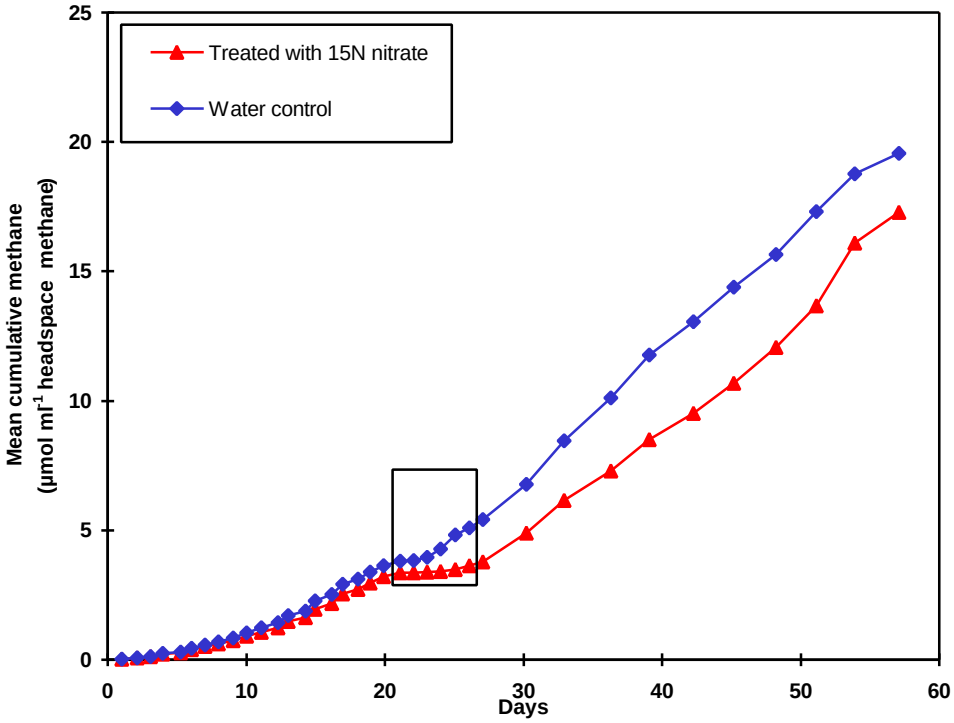


Figure 6.9 The mean headspace concentrations of methane produced by refuse cultures treated with heavy nitrate ($^{15}NO_3^-$) (▲) and water (◆) to 95% moisture content. The box indicates the period over which the ammonia and nitrate concentrations were measured.

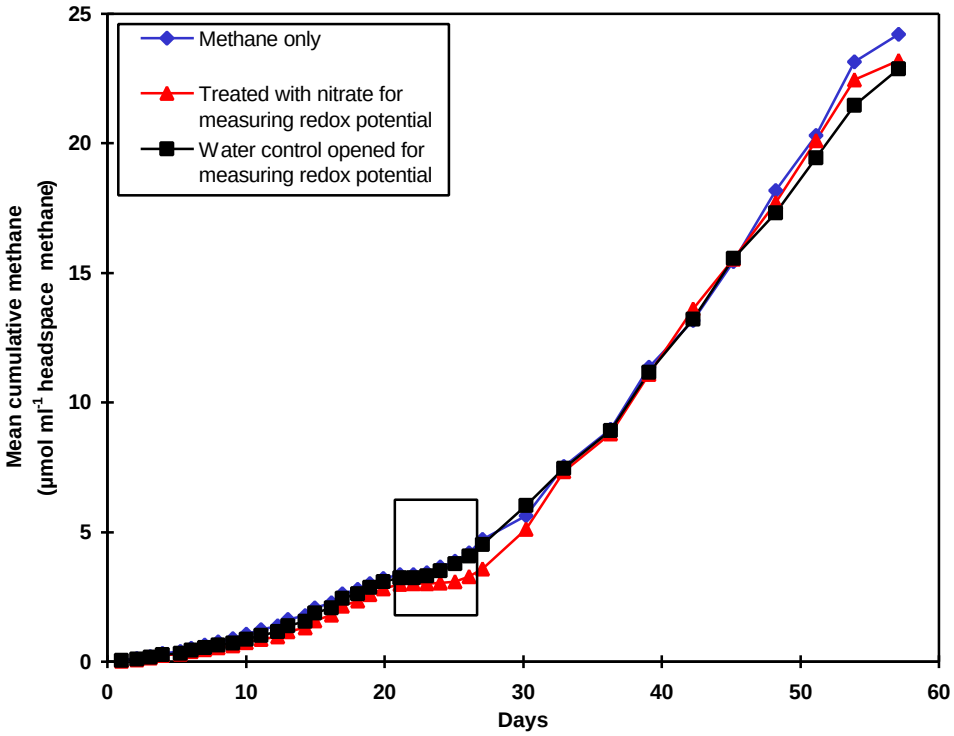


Figure 6.10 The mean cumulative headspace concentrations of methane produced by refuse cultures treated with nitrate (▲) and water opened and measured for redox potential (■), and an unopened culture adjusted with water at 95% moisture content (◆). The box indicates the period over which the redox potential and pH were measured.

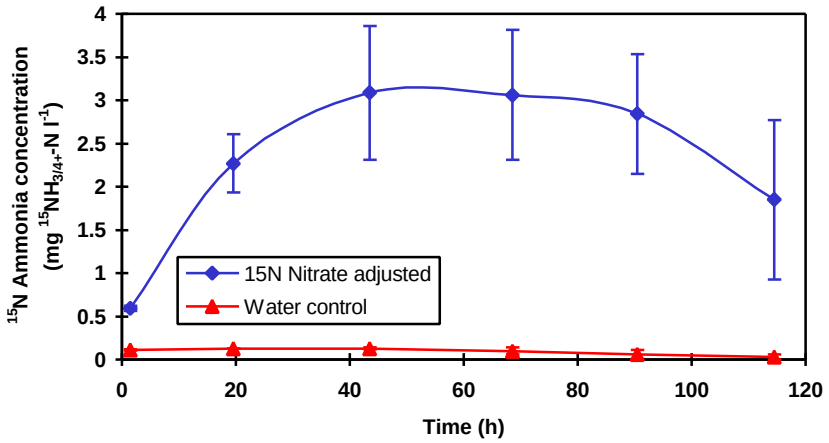


Figure 6.11 The mean concentrations of heavy ammonia (mg $^{15}NH_{3/4+}$-N l^{-1}) present in cultures adjusted with heavy nitrate ($^{15}NO_3^-$)(◆) and water (▲) to 95% moisture content. Error bars represent the standard deviation of triplicate cultures.

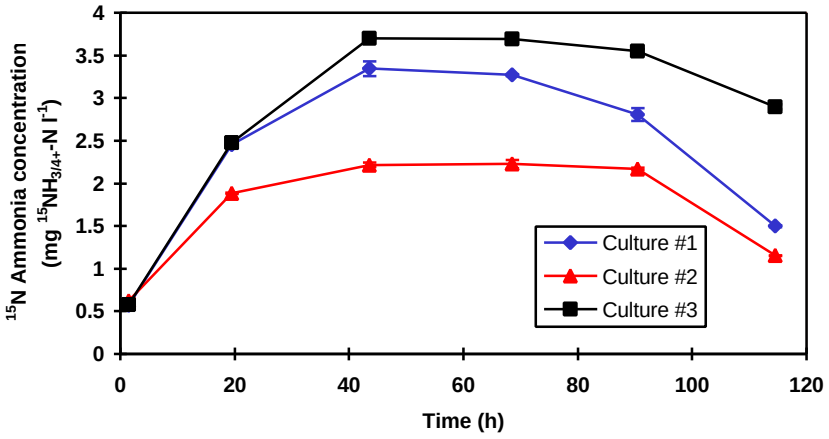


Figure 6.12 The mean concentrations of heavy ammonia (mg $^{15}NH_{3/4+}$-N l^{-1}) present in cultures 1(◆), 2 (▲) and 3 adjusted with heavy nitrate ($^{15}NO_3^-$) to 95% moisture content. Error bars represent the standard deviation of triplicate analyses.

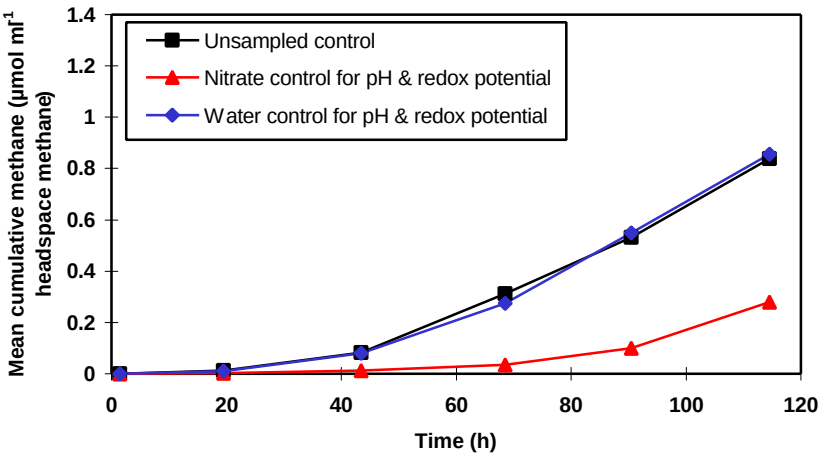


Figure 6.13 The mean cumulative headspace methane concentrations produced by refuse cultures treated with nitrate (◆) and water (▲) opened and measured for redox potential, and an unopened culture (■) adjusted with water at 95% moisture content.

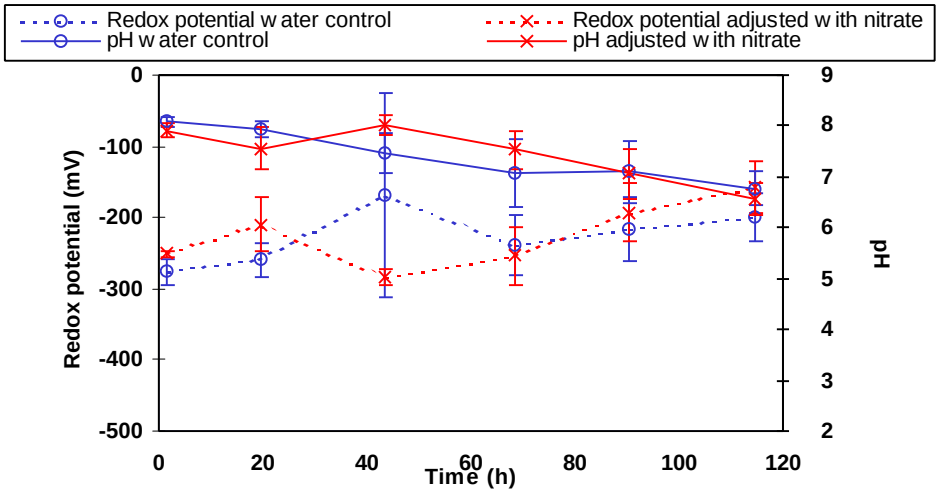


Figure 6.14 The mean pH values and redox potentials of refuse cultures treated with nitrate (o) and water (x) at 95% moisture content opened during measurement. Error bars represent the standard deviation of triplicate cultures.

Addition of ^{15}N Nitrate ($^{15}NO_3^-$) to Sterile Gamma Irradiated Refuse

Analysis of gamma irradiated samples after 1.5 h revealed reduction of nitrate to ammonia to produce 0.133 (SD 0.0303) mg $^{15}NH_{3/4+}$-N l^{-1} in the ammonia pool (Figure 6.16a). Concentrations of heavy nitrate measured were lower than expected (7.87 (SD 0.257) mg NO_3^--^{15}N l^{-1}) after 1.5 h suggesting some loss of heavy nitrate to an unidentifiable pool during this period but most probably inaccuracies introduced during filter sterilisation of heavy nitrate stock solution and calibration of instrumentation. Almost half the heavy nitrate analysed after 1.5 h was present in the ammonia pool after 7 days (2.95 (SD 0.362) mg $^{15}NH_{3/4+}$-N l^{-1}) demonstrating that an increased proportion of nitrate reduction to ammonia occurred in cultures containing non-viable microorganisms (Figure 6.16a). Very small amounts of heavy nitrate were still present after 7 days indicating that the mechanism of nitrate reduction to ammonia and denitrification was not as rapid as had been observed in un-irradiated cultures (Figure 6.16ab). Nitrate concentrations were at the limits of detection and concentrations were too small to be confirmed by chemical analysis. Contamination checks after 7 days revealed small numbers of uniform bacterial colonies, 10-20 colonies 100 μl^{-1} in two cultures and one colony in the remaining culture, appearing after 4-6 weeks incubation indicating growth and recovery by facultatively anaerobic microorganisms. The small numbers, the prolonged incubation time and limited gas pressure in the bottles suggested reduced microbial activity during the incubation of the cultures. The mean pH fell slightly from 7.58 (SD 0.144) to 7.37 (SD 0.112) but the pH in one culture remained constant at pH 7.5. The cultures could not be considered to be entirely sterile since prolonged incubation could resuscitate and identify viable bacteria. For this reason these gamma irradiation experiments share many similarities with the gamma irradiation of soil where complete sterility has often proved to be difficult to achieve with confidence. However, in this experiment no viable colonies could be induced to grow after 1.5 h, nor was significant methane identified after 15 days suggesting that there was a profound decrease in the bacterial population following gamma irradiation.

There was no significant decrease in nitrate concentrations by autoclaved refuse cultures following incubation for 7 days at 30^oC. Contamination checks on nutrient agar plates revealed no bacterial growth.

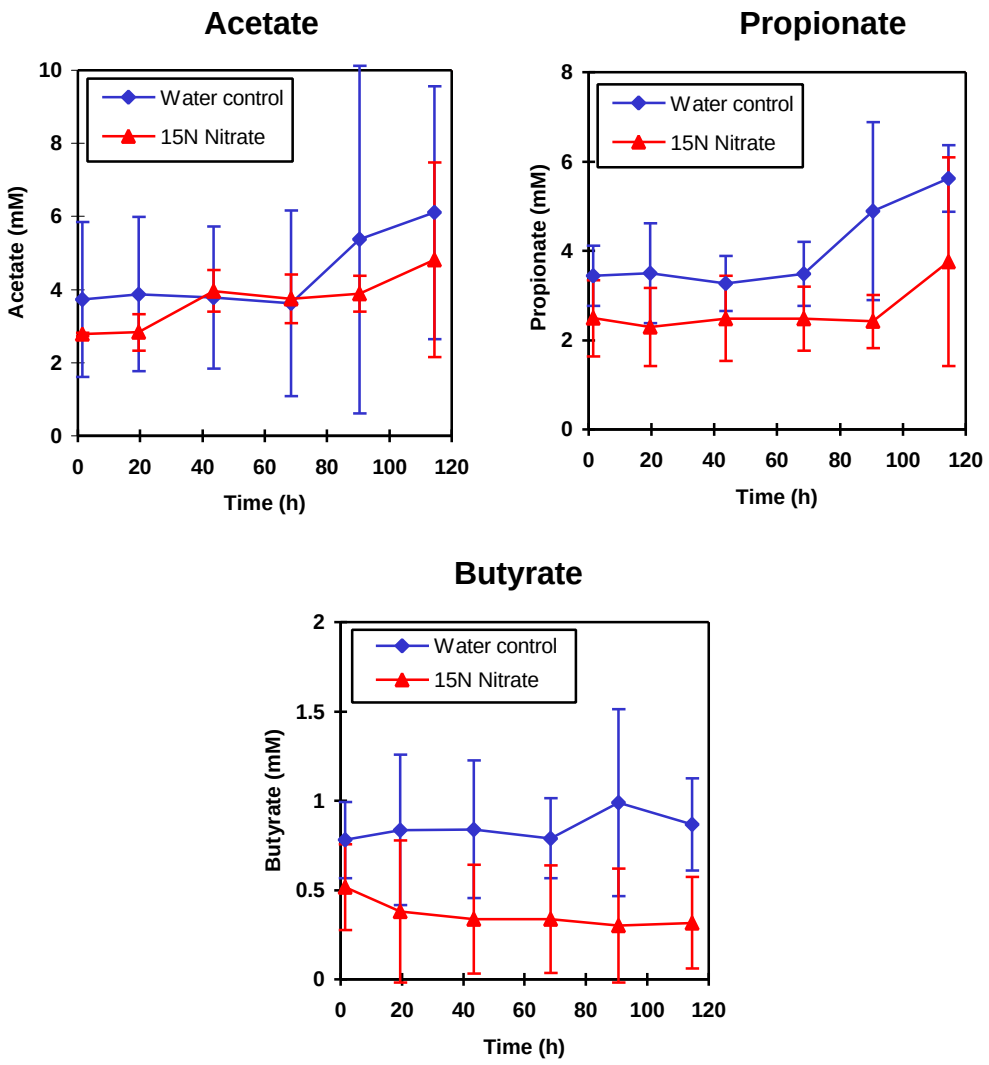


Figure 6.15 The concentrations of acetate, propionate and butyrate in refuse after the addition of heavy nitrate ($^{15}NO_3^-$) at 95% moisture content. Error bars represent the standard deviation of triplicate cultures.

mg $^{15}N\ l^{-1}$ after 1.5h

Heavy ammonia	Heavy nitrate
0.133 (SD 0.0303)	7.87 (SD 0.257)

mg $^{15}N\ l^{-1}$ after 7 days

Heavy ammonia	Heavy nitrate
2.95 (SD 0.362)	0.0887 (SD 0.0340)

Figure 6.16a Dissolved leachate heavy nitrogen concentrations (mg $^{15}N\ l^{-1}$) in gamma irradiated refuse cultures containing heavy nitrate ($^{15}NO_3^-$) after 1.5 h and 7 days incubation.

mg $^{15/14}$N l^{-1} after 1.5h

Ammonia	Nitrate
4.67 (SD 0.694)	9.25 (SD 0.325) (a) 9.16 (SD 0.129) (b)

mg $^{15/14}$N l^{-1} after 7 days

Ammonia	Nitrate
29.5 (SD 9.59)	1.42 (SD1.75) (a) 3.52 (SD 1.54) (b)

a = nitrate calculated by subtracting the ammonia caught one filter paper from the total ammonia and nitrate captured by micro-diffusion chambers containing Devarda's alloy prepared at the same time.

b = nitrate caught on a filter paper after ammonia capture had finished and microdiffusion chamber was incubated again with Devarda's alloy. The sample probably contained a small portion of ammonia which was not diffused during the first capture.

Figure 6.16b Dissolved leachate nitrogen concentrations (mg $^{15/14}$N l^{-1}) of leachate in gamma irradiated refuse cultures containing heavy nitrate ($^{15}NO_3^-$) after 1.5 h and 7 days incubation.

1. Acetate

1.5 h	7 days
3.36 (SD 0.856)	6.60 (SD 1.30)

2. Propionate

1.5 h	7 days
0.529 (SD 0.485)	1.63 (SD 0.946)

3. Butyrate

1.5 h	7 days
0.00883 (SD 0.00981)	0.139 (SD 0.0997)

4. *iso*-Valerate

1.5 h	7 days
0.00183 (SD 0.00317)	0.0987 (SD 0.114)

Figure 6.17 Mean volatile fatty acid concentrations in gamma irradiated refuse cultures after 1.5 h and 7 days incubation.

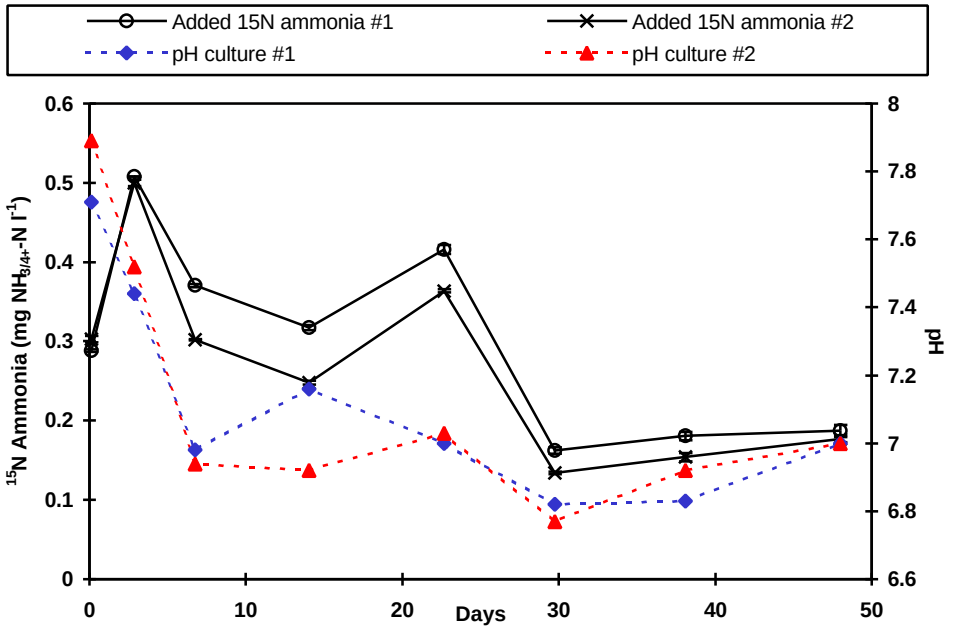


Figure 6.18 The concentrations of added heavy ammonia ($^{15}NH_{3/4+}$) remaining in solution (o)(x), and pH values (◆)(▲) in refuse cultures 1 and 2 respectively, supplemented with water containing $^{15}NH_{3/4+}$ to 95% moisture content. Error bars represent the standard deviation of triplicate analyses.

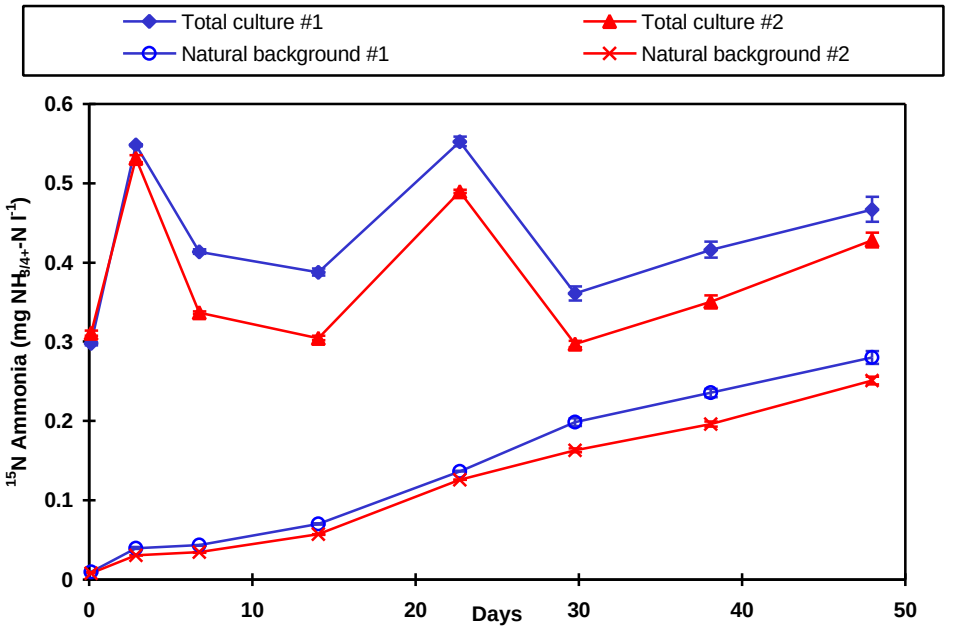


Figure 6.19 The total concentrations of heavy ammonia ($^{15}NH_{3/4+}$) present in the refuse (◆)(▲), and the proportion entering solution (o)(x), in refuse cultures 1 and 2 respectively, supplemented with water containing $^{15}NH_{3/4+}$ to 95% moisture. Error bars represent the standard deviation of triplicate analyses.

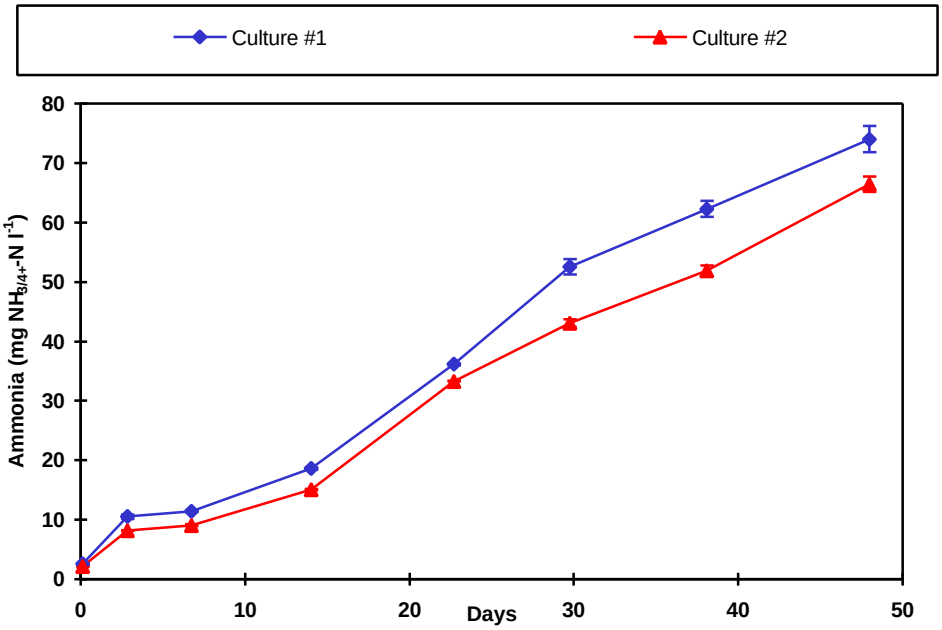


Figure 6.20 The concentrations of ammonia (mg $^{14}NH_{3/4+}$-N l^{-1} and mg $^{15}NH_{3/4+}$-N l^{-1}) in solution in refuse cultures 1 (◆) and 2 (▲) supplemented with water containing $^{15}NH_{3/4+}$ to 95% moisture content. Error bars represent the standard deviation of triplicate analyses.

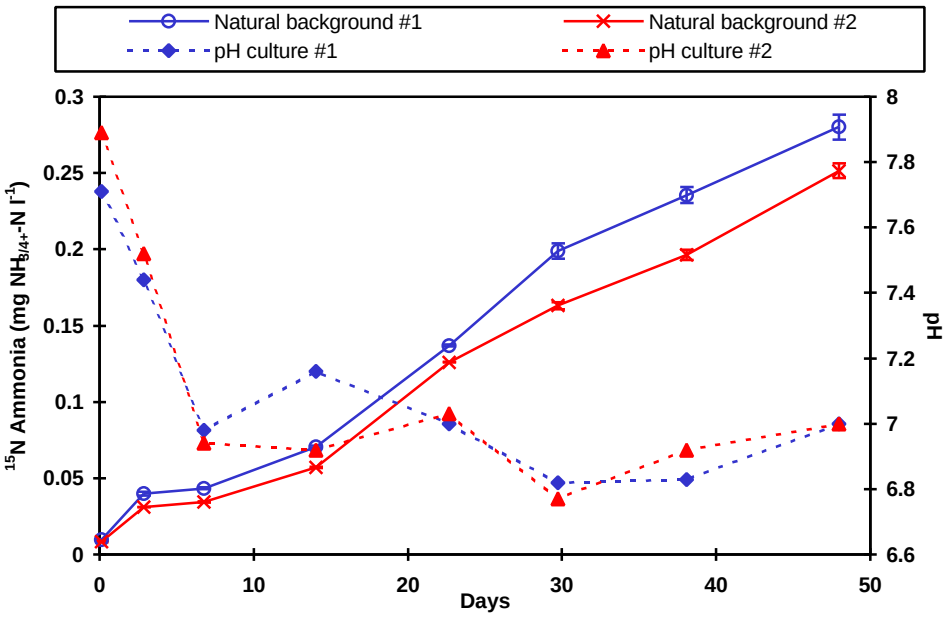


Figure 6.21 The concentrations of heavy ammonia (mg $^{15}NH_{3/4+}$-N l^{-1}) entering solution (o)(x), and pH values (◆)(▲) of refuse cultures, 1 and 2 respectively, supplemented with water to 95% moisture content enriched with $^{15}NH_{3/4+}$. Error bars represent the standard deviation of triplicate analyses.

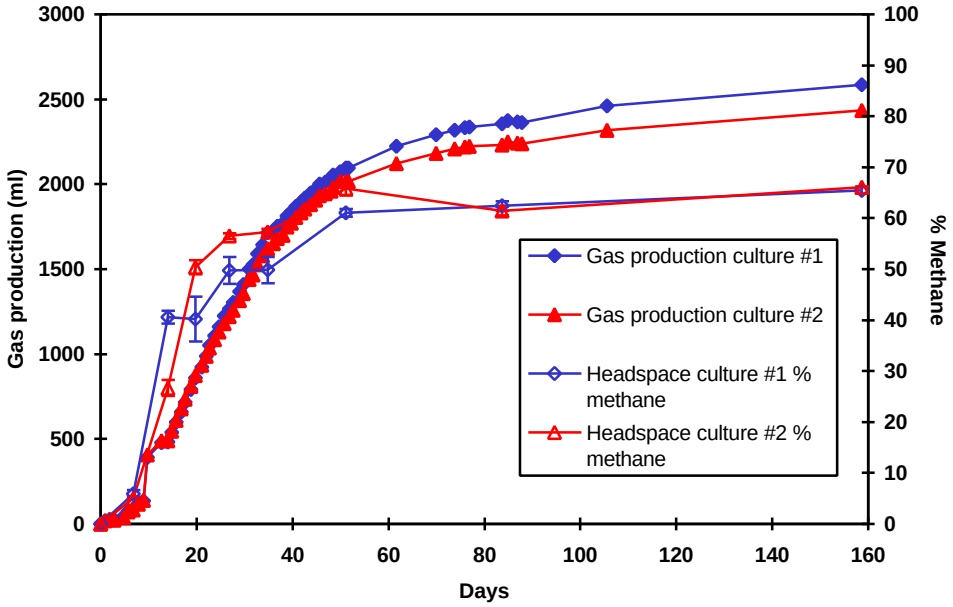


Figure 6.22 Measurement of gas production and headspace % methane concentration from refuse cultures, 1 (◆,◇) and 2 (▲,△) supplemented with water to 95% moisture content enriched with $^{15}NH_{3/4+}$. Error bars represent the standard deviation of triplicate analyses.

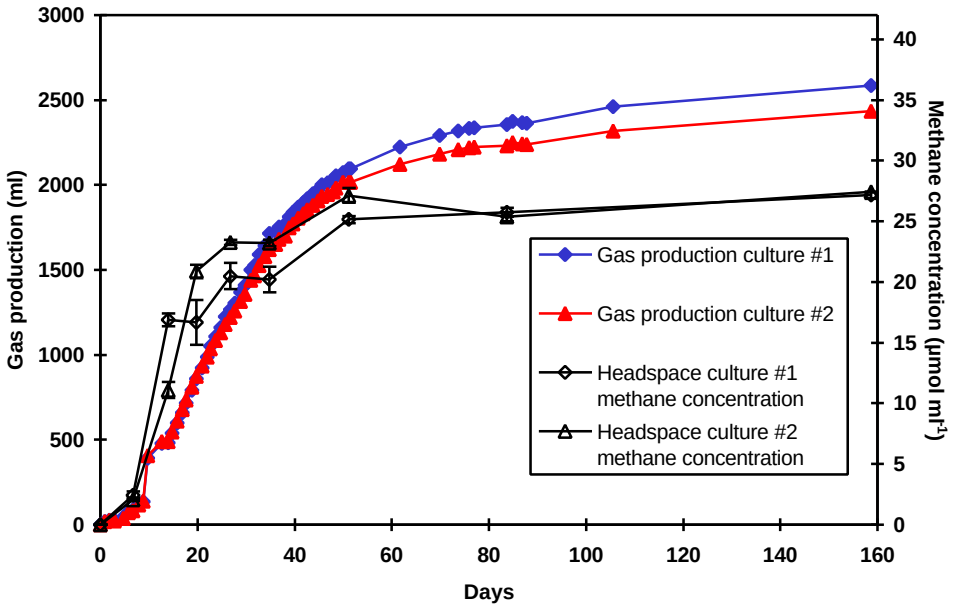


Figure 6.23 Measurement of gas production and headspace methane concentration from refuse cultures, 1 (◆,◇) and 2 (▲,△) supplemented with water to 95% moisture content enriched with $^{15}NH_{3/4+}$. Error bars represent the standard deviation of triplicate analyses.

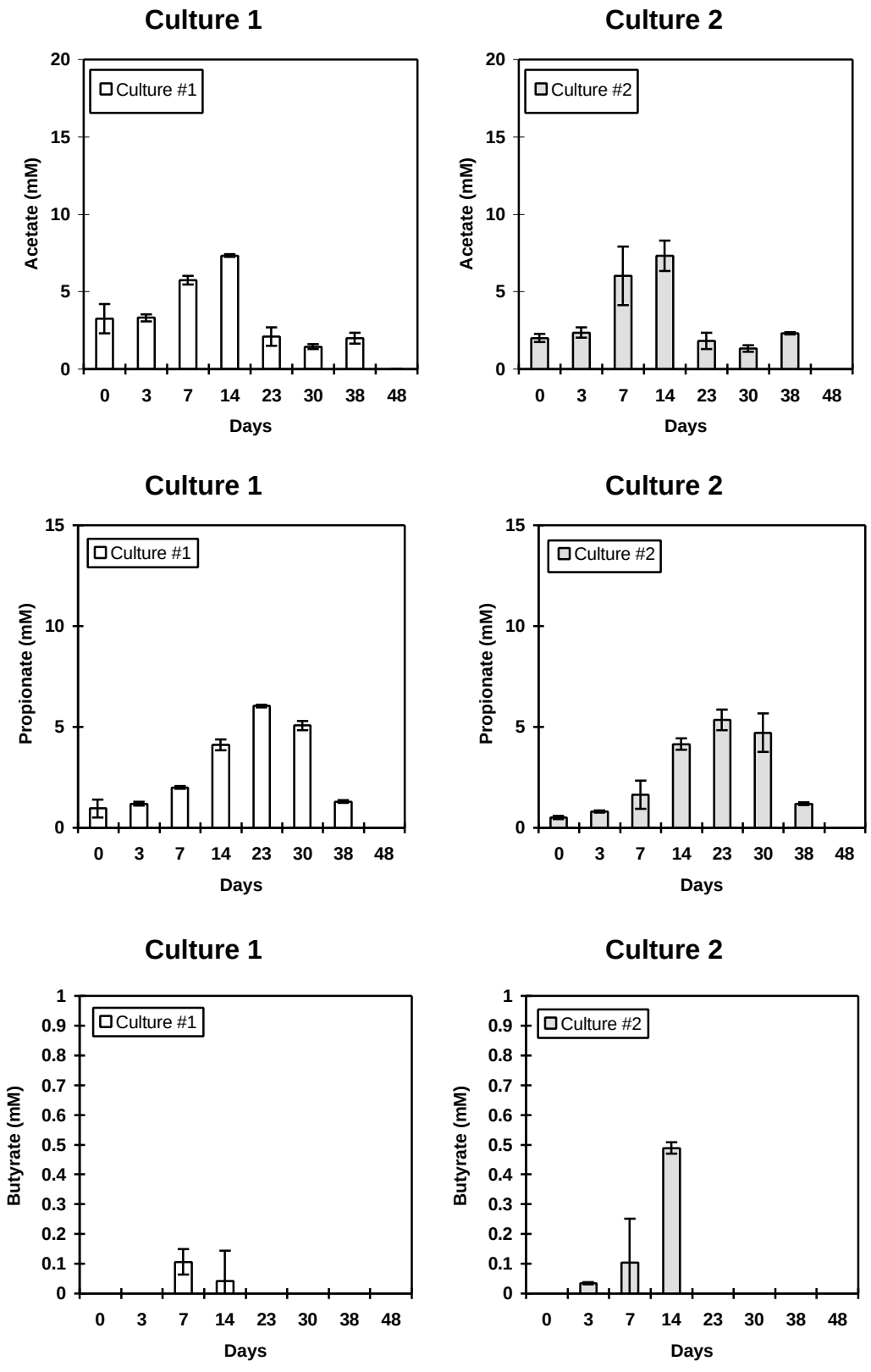


Figure 6.24 Volatile fatty acid production from refuse supplemented with heavy ammonia ($NH_{3/4+}$-^{15}N) at 95% moisture content. Clear and striped shaded areas represent the mean volatile fatty acid concentrations of cultures, 1 and 2 respectively. Error bars represent the standard deviation of duplicate analyses.

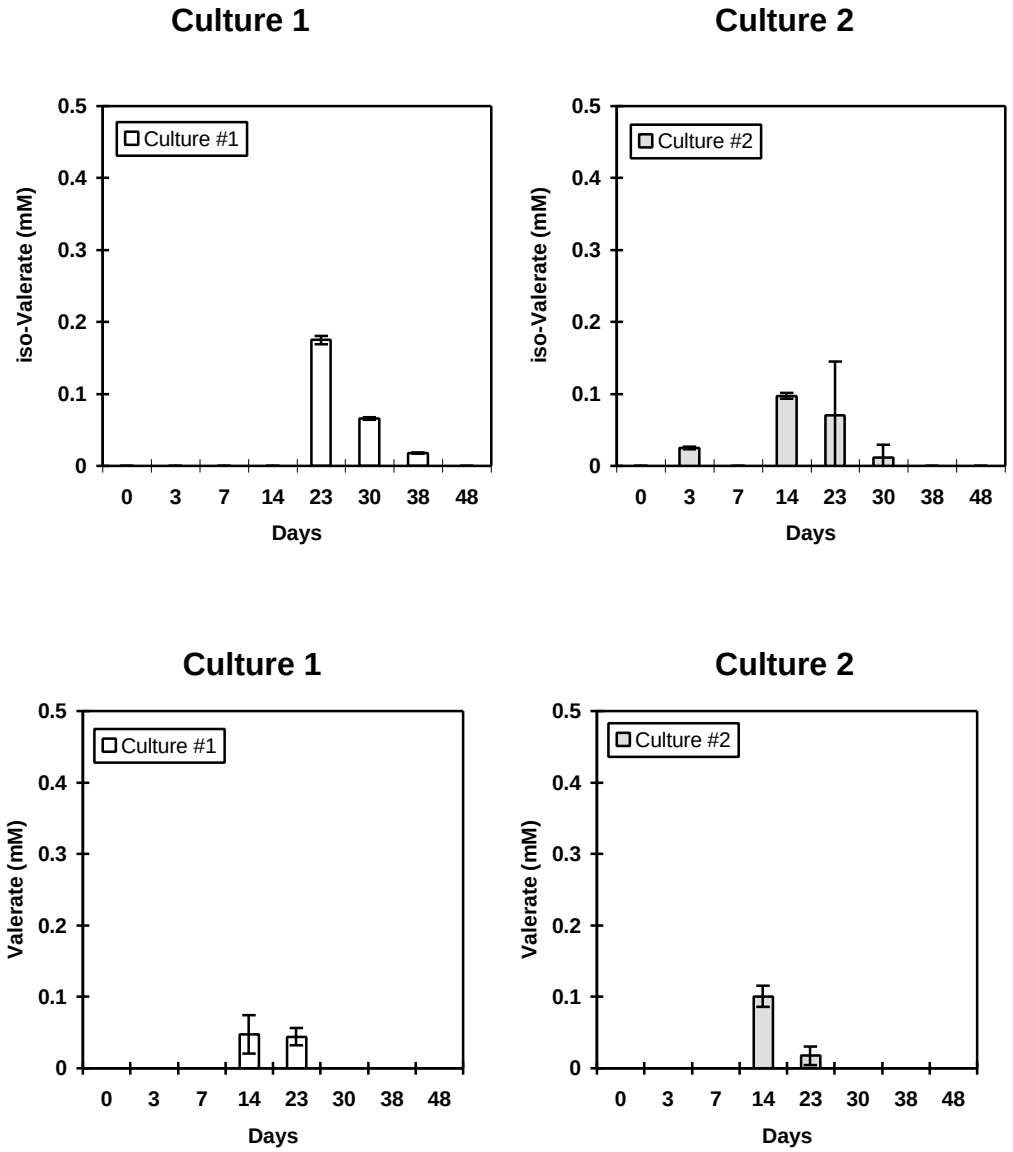


Figure 6.25 Volatile fatty acid production from refuse supplemented with heavy ammonia ($NH_{3/4+}$-^{15}N) at 95% moisture content. Clear and striped shaded areas represent the mean volatile fatty acid concentrations of cultures, 1 and 2 respectively. Error bars represent the standard deviation of duplicate analyses.

Addition of ^{15}N Ammonium ($^{15}NH_4^+$) to Refuse

The supplementation of the ammonia pool with heavy ammonia ($^{15}NH_{3/4+}$) identified the removal of this portion of the ammonia pool to the refuse. The heavy ammonia concentration was calculated from the $\%^{15}N$ analysed within the captured ammonia sample and the natural abundance of ^{15}N entering solution at a natural abundance of 0.378% ^{15}N. There was an initial increase in the concentration of heavy ammonia after 3 days and then a decrease generally associated with changes in pH value (Figure 6.18). Concentrations of ammonia steadily increased during incubation such that the theoretical concentrations of heavy ammonia from the ammonia entering into solution from the refuse became a more significant proportion of the heavy ammonia pool (Figure 6.19). Ammonia (total ^{15}N and ^{14}N ammonia) production

was linear over time (Figure 6.20 and 6.21) reaching concentrations of 60 to 70 mg $^{15}NH_{3/4+}$-N l^{-1} after 48 days (Figure 6.20) after which time most of the methane had been produced (Figure 6.22 and 6.23). Mainly acetate and propionate were produced during anaerobic decomposition (Figure 6.24 and 6.25) with an accumulation of propionate during the later stages of decomposition. Total mean methane production by the two cultures was 68.0 (SD 1.58) mM (approximately 1.5 l methane at STP, 282 ml methane g^{-1} dry weight at STP, 1.09 g methane).

6.2.7 Discussion

The heavy isotope tracer technique revealed a reductive pathway from nitrate to ammonia. Although nitrate was added to batch cultures in previous studies the reduction to ammonia could not be identified because of the small concentrations present and the increase in aqueous ammonia concentrations at that time. At first the transformation appeared to be perhaps due to the reducing environment of landfilled refuse. The lack of oxygen encouraged an abiotic reduction of nitrate to ammonia, probably catalysed on the surface of metals within the refuse. One source of these metals in refuse could be the ash from fires or industrial and commercial waste. The lack of nitrate reduction following autoclaving suggested a dependence on biotic processes. Whilst gamma irradiation may kill the bacterial cells responsible through denaturation of DNA, their enzymes and membranes will remain partially intact and hence carryout nitrogen the transformations observed. Autoclaving has a more deleterious effect, denaturing proteins and hence reducing enzymic activity as well as denaturing DNA. The bacterial cells will also loose their integrity and structure to a much greater extent, limiting the possibility of them facilitating any biochemical transformations.

Live cells within refuse clearly had no part to play in nitrate reduction during the first 1.5 h since gamma irradiated cultures were for all intent and purposes sterile, with no viable facultatively aerobic microorganisms. After this period one must conclude that bacteria or bacterial spores which survived gamma irradiation recovered or germinated to produce bacteria capable of growing into colonies on the plates. The fact that methanogenesis never recovered in the cultures was testament to the destruction of the microbial consortium that controls the degradation of paper waste. The bacteria which survived the gamma irradiation or the dead/non-culturable cells of other bacteria were only capable of reducing half the nitrate to nitrogen and nitrous oxide gas, suggesting that the removal of viable bacteria in the refuse will increase the proportion of nitrate reduced to ammonia. This suggests competition between chemical reduction to ammonia and denitrification. Removing anaerobic samples from a landfill will encourage obligately anaerobic and micro-aerophilic microorganisms to sporulate, and these forms of bacteria can be most resistant to gamma irradiation. The sterilisation by gamma irradiation of environmental samples is in practice difficult to achieve since spores may survive the gamma irradiation process. Sterilisation by autoclaving refuse shares these weaknesses but it was found that complete sterility could be assured by autoclaving refuse three times and allowing a period of cooling to germinate resting spores. This was not initially considered to be an option because of the changes experienced when autoclaving environmental samples. When autoclaved soil samples release phenolic residues and is left with a dark charred appearance. In certain circumstances this can prevent rapid re-colonisation of bacteria following inoculation. Soil represents a relatively small portion of MSW, normally associated with daily cover, garden waste and building waste, so these effects are reduced on refuse when compared to soil. To reach sterility, a regime of autoclaving and incubation was required, germinating spores before sterilisation.

The authors are unaware of any previous attempts at gamma irradiating refuse. The advised dose (25 kGy), devised from soil experiments, was exceeded by deliberately delaying the time at which the samples were to be collected so that the experiment could proceed instantly the cultures were removed from the reactor. A stronger source of gamma irradiation may have been required, although owing to the special nature of landfilled refuse and the length of time required for processing it would be difficult to imagine another facility capable of receiving the refuse. Doubling the advised dose would go some way to giving the refuse the correct amount of gamma radiation for this experiment so that sterility could be guaranteed or activity prevented for the duration of the experiment. Intensifying the radiation source would reduce the treatment time and reduce activity during irradiation but would require a different nuclear installation. The recovery of bacteria in the cultures after gamma irradiation was still surprising considering the time the samples were irradiated and was an indication of the bacterial content and activity of landfilled refuse compared to soil.

Supplementing the refuse with heavy ammonia revealed the interchange of ammonia between the dissolved and the solid phases. Initially there was an increase in the heavy ammonia captured, best explained by the ammonia not mixing fully with the refuse culture and some initial binding (Figure 6.18) but following that the concentrations of heavy ammonia responded to the pH of the culture. Falls in pH value brought about a decrease in heavy ammonia, and any increases allowed some heavy ammonia to return to solution. The greatest changes in the headspace methane composition come after day 7 when rapid methanogenesis caused a stabilisation of the culture pH. After day 14 there was a drop in acetate concentrations (Figure 6.24), presumably due to the activity of acetoclastic methanogens, which encouraged a slight increase in pH and heavy ammonia re-entered solution (Figure 6.18). Acetate concentrations remained stable from day 23 to 38 until on day 48 no volatile fatty acids could be detected.

The response of the heavy ammonia to pH is the first indication that changes in pH values promotes the interchange of the positively charged ammonium ion (NH_4^+) between the solution and the bound state, attached to negatively charged refuse particles. Decreases in pH promote the formation of positively charged ammonium ions (NH_4^+) which have a greater tendency to bind to the refuse mass. Although ammonia entered solution throughout the experiment there still appeared to be interchange between the refuse and the dissolved phase which had the effect of diluting the heavy ammonia pool. Continuation of the experiment would no doubt have seen an equilibrium reached between the dissolved heavy ammonia and the bound refuse ammonia. This would not include ammonia which was present in the protein and bacteria within the refuse which was not mobile and could only be released by the process of ammonification. If the decreases in heavy ammonia were associated with uptake by bacteria for growth and assimilation into proteins one would not expect it to be released by slight increases in pH value. Although ammonia becomes more toxic at higher pH values, bacteria do not store ammonia in their cells to excrete it again when very minor adjustments have to be made in cellular pH value due to changes in environmental conditions. The only other time when bacteria would excrete ammonia is during starvation when bacteria metabolise their own proteins. Conditions at the time of the increases in heavy ammonia concentrations were indicative of rapid microbial activity and volatile fatty acid production. Since it is unlikely that heavy ammonia was being used for growth the experiment revealed limited assimilation of ammonia during anaerobic decomposition of refuse and methane production.

Rapid interchange between the dissolved phase and the bound phase at higher pH values suggests that at higher pH values movement of leachate will encourage the leaching of

ammonia from the refuse. The greatest changes in the movement of the heavy ammonia from the refuse and back into the dissolved phase occurred during increases in pH value. These increases in pH can only be achieved by rapid methanogenesis, indicating that methanogenesis may indirectly contribute to ammonia leaching.

The rate of release of total soluble ammonia from the refuse into the leachate appeared unaffected by pH value and the rate of increase was constant throughout the experiment (Figure 6.20). It is not immediately apparent which mechanisms release ammonia into solution. It may be solubilisation of bound ammonia or ammonification of protein. The interchange of heavy ammonia with the bound phase and its return to the dissolved phase suggests that some binding is related to pH value and that a good proportion of the waste derived ammonia entering solution is coming from other processes such as the ammonification of protein by bacteria.

6.2.8 Conclusions

- A reductive nitrogen transformation pathway exists from nitrate to ammonia capable of reducing 4 -7% of the nitrate added.

- Autoclaving experiments suggested that an enzymatic/biochemical process was involved.

- The reductive pathway from nitrate to ammonia appeared to compete with denitrification of nitrate. Greater proportions could be reduced when the indigenous bacteria were destroyed by gamma irradiation.

- Binding of ammonia to refuse appeared to be controlled by pH value, suggesting greater leaching at higher pH values.

- Utilisation and incorporation into biomass of ammonia produced during the decomposition of landfilled refuse appeared insignificant.

- Ammonia accumulated during anaerobic decomposition.

7. STUDY OF THE RELEASE OF NITROGEN FROM MODEL LANDFILL SYSTEMS

7.1 Experimental Rationale

The release of nitrogenous compounds from landfilled refuse packed in glass columns was measured. The effects of volatile fatty acids and ammonia on the leaching of landfill nitrogenous compounds were tested. Analysis of the total nitrogen content of the refuse before and after percolation was carried out revealing the quantities of nitrogen leached from the refuse.

7.2 Introduction

The modelling of the production of leachate through landfill refuse in the laboratory can be achieved using refuse columns. Refuse columns with standard flow rates have been used on numerous occasions in our laboratory. The high influent flow rate chosen allowed comparison with other models used previously in the laboratory and the use of proven techniques. A considerable amount of information has been gained on the effects of food waste, phenol and *o*-cresol (Watson-Craik, 1987; Watson-Craik & Senior, 1989ab, 1990; Sulisti, 1994; Sulisti & Watson-Craik, 1996) and ammonia release (Sinclair, 1994) from these systems. The high influent flow chosen for these experiments approximated to one bed volume a day which allowed for rapid generation of data from the refuse columns which would take months or years to achieve from a landfill. Refuse columns were constructed to measure the mineralisation (release) of ammonia from refuse of a known initial nitrogen concentration at low medium flow rates.

7.3 Materials and Methods

7.3.1 Refuse Column Preparation

Glass columns containing 675 g of refuse (57. 4 % moisture content) were packed to a volume of 1.1 litres and maintained at an internal temperature of 30^{o}C with a thermal jacket of silicone tubing containing water pumped from a thermo-circulator. Oxygen impermeable butyl rubber tubing (Watson-Marlow) was used throughout the system. To initiate methanogenesis as rapidly as possible the refuse columns were degassed with nitrogen gas for 2 h. The columns were then perfused with influent as summarised in the following table.

Table 7.1 Refuse column influents

Column	Influent
1.	Autoclaved distilled water
2.	Autoclaved distilled water
3.	500 mg $NH_{3/4+}$-N l^{-1} adjusted to pH 7 with NaOH
4.	A high strength carbon substrate modelling acetogenic leachate containing 50 mM sodium acetate, 24.4 mM sodium propionate and 53.8 mM butyric acid adjusted to pH 7 with NaOH.
5.	A high strength carbon substrate modelling acetogenic leachate containing 50 mM sodium acetate, 24.4 mM sodium propionate, 53.8 mM butyric acid and 500 mg $NH_{3/4+}$-N l^{-1} adjusted to pH 7 with NaOH.
6.	A lower strength carbon substrate modelling methanogenic leachate containing 25 mM acetic acid and 500 mg $NH_{3/4+}$-N l^{-1} adjusted to pH 7 with NaOH.

The high and low strength media (10 l) were sterilised by passage through a Whatman 2 µm cellulose nitrate filter held in a Sartorius carrier, supplied from a 20 l pressure container displaced by nitrogen gas. The distilled water in the controls was autoclaved. The ammonia solution was prepared by adding NH_4Cl after autoclaving and adjusting the pH to 7 with a standard amount of 40% w/v NaOH solution. Nitrification in this solution was inhibited by the CS_2 released from the rubber bungs during autoclaving and no nitrate entered the refuse column. The medium was pumped to the top of the column by a Watson-Marlow peristaltic pump and set at a dilution rate of 0.025 h^{-1} (27.5 ml h^{-1}). When the refuse was fully saturated, just over one bed volume passed through the refuse column each day. The leachate was collected with a needle and syringe from a Suba-Seal located on the effluent tubing. The refuse column was isolated form the surrounding air with a weir overflow system. Dissolved methane concentrations in the effluent were measured every 7-21 days using the method of Watson-Craik & Senior (1989c). Headspace methane was collected by gas tight syringe from a Suba-Seal at the top of the column and analysed every 2-14 days by gas chromatography. The pH was determined periodically and a sample prepared for the analysis of volatile fatty acids. A Hanna conductivity meter was used to measure the leaching of mineral ions in the effluent. Ammonia was measured using Reflectoquant test strips and meter (Merck) confirmed by spectrophotometric analysis. To measure the biomass and suspended sediment in the effluent, 500 ml was collected overnight every 1-3 weeks from each column, two 200 ml samples were centrifuged at 10,000 x gravity for 40 min in a refrigerated MSE centrifuge. The supernatant was removed and the pellet resuspended in 10 ml of distilled water and filtered on a dry Whatman GF/C glass filter under vacuum. The filter was dried in a desiccator and the dry weight calculated by difference.

7.3.2 Perfusion of Refuse Columns with Distilled Water

Distilled water was perfused through the columns to measure the leaching of ammonia from the refuse in the leachate. The total Kjeldahl nitrogen was measured before and after incubation to produce a nitrogen budget for the system.

Perfusion of Refuse Columns with Distilled Water Containing Ammonia

Distilled water containing 500 mg $NH_{3/4+}$-N l^{-1} adjusted to pH 7 with NaOH was perfused through one refuse column. The leaching of ammonia from the refuse column was compared to the leaching of ammonia from refuse columns perfused with distilled water to see whether presence of ammonia affected the nitrogen budget of the system and the quantities of ammonia leached. The total Kjeldahl nitrogen was measured before and after incubation to produce a nitrogen budget for the system. The effect of ammonia on the production of methane was quantified by comparison with the water controls.

Perfusion of Refuse Columns with Distilled Water Containing Volatile Fatty Acids and Ammonia

The addition to refuse columns of high strength and the low strength medium modelled the perfusion of younger acetogenic and older methanogenic landfill leachate. Both treatments contained 500 mg $NH_{3/4+}$-N l^{-1}. The concentrations of NaOH were approximately 2.2 and 1.2 g l^{-1} in the high and low strength medium. No additional nutrients were supplied to the refuse making it possible to follow the leaching/mineralisation of $NH_{3/4+}$ with other ions. The refuse was analysed for total Kjeldahl nitrogen before and after perfusion to determine the net loss of nitrogen from the system.

A refuse column was also prepared and percolated with high strength leachate without 500 mg $NH_{3/4+}$-N l^{-1} to see if ammonia influences the methanogenic decomposition of volatile fatty acids.

The concentrations of volatile fatty acids and their representative ratios to each other were chosen using published data on acetogenic leachate by Rees (1980), Harmsen (1983) and Robinson and Maris (1985). A total concentration of 50 mM acetate was chosen, close to the mean concentrations of acetate reported in these publications (86 mM). The mean ratios of acetate to propionate and acetate to butyrate were used to calculate the concentrations of propionate and butyrate supplied in the influent.

To measure the effect of increasing the concentration of ammonia the refuse column supplied with low strength leachate containing ammonia was subjected to increased ammonia concentrations of 1000 mg $NH_{3/4+}$-N l^{-1} for 47 days and 3000 mg $NH_{3/4+}$-N l^{-1} for 1 day.

7.4 Results

7.4.1 Perfusion of Refuse Columns with Distilled Water and Distilled Water Containing Ammonia

No nitrate was found in the effluent. Methane was detected in all the treatment regimes after one day. Headspace methane concentrations in columns 1 and 2 supplied with distilled water and the column supplied with 500 mg $NH_{3/4+}$-N l^{-1} increased gradually (Figure 7.1). The column supplied with ammonia reached its maximum headspace methane concentration later than the two controls. The headspace methane concentration fell at approximately the same

rate as the water controls although in the majority of cases the ammonia treated column had a headspace methane concentration below that of the two controls. The soluble methane concentrations in the effluent revealed that the column receiving ammonia was releasing less methane (Figure 7.3). The pH of the effluent appeared buffered by the ammonia in the influent remaining at approximately 0.2 pH units above that of the controls (Figure 7.5).

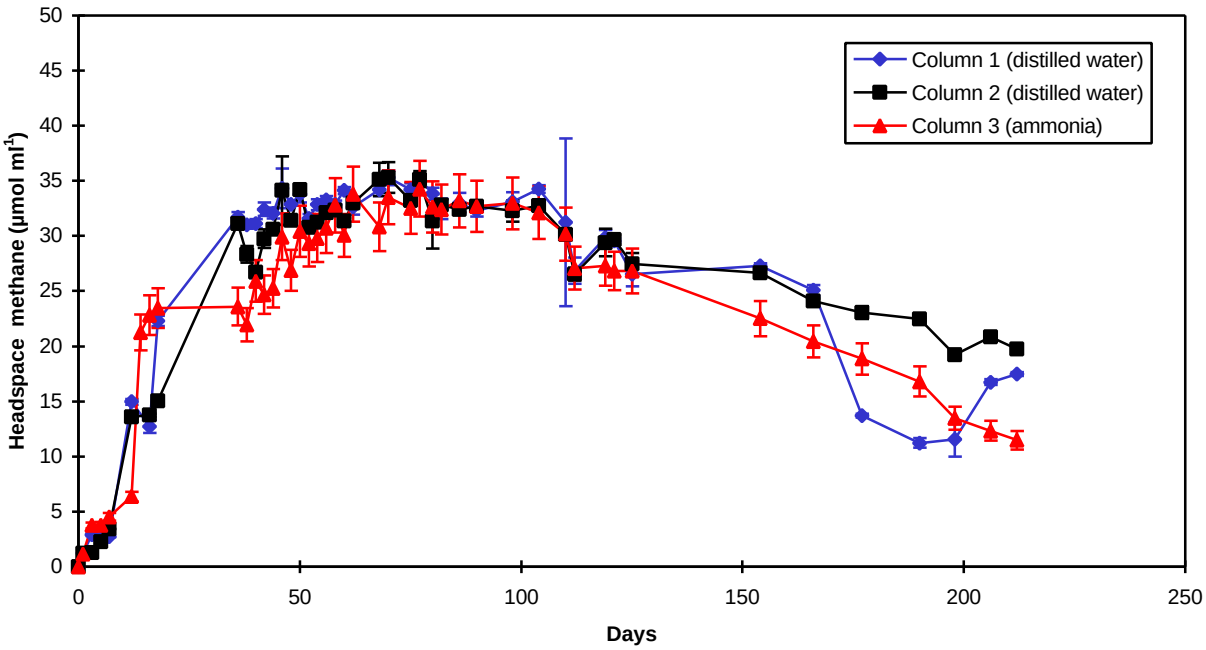


Figure 7.1 Headspace methane concentrations in column 1 (distilled water, ◆), column 2 (distilled water, ■) and column 3 (500 mg $NH_{3/4+}$-N l^{-1}, ▲). Error bars represent the standard deviation of the mean. (42 µmol ml^{-1} ? pure methane)

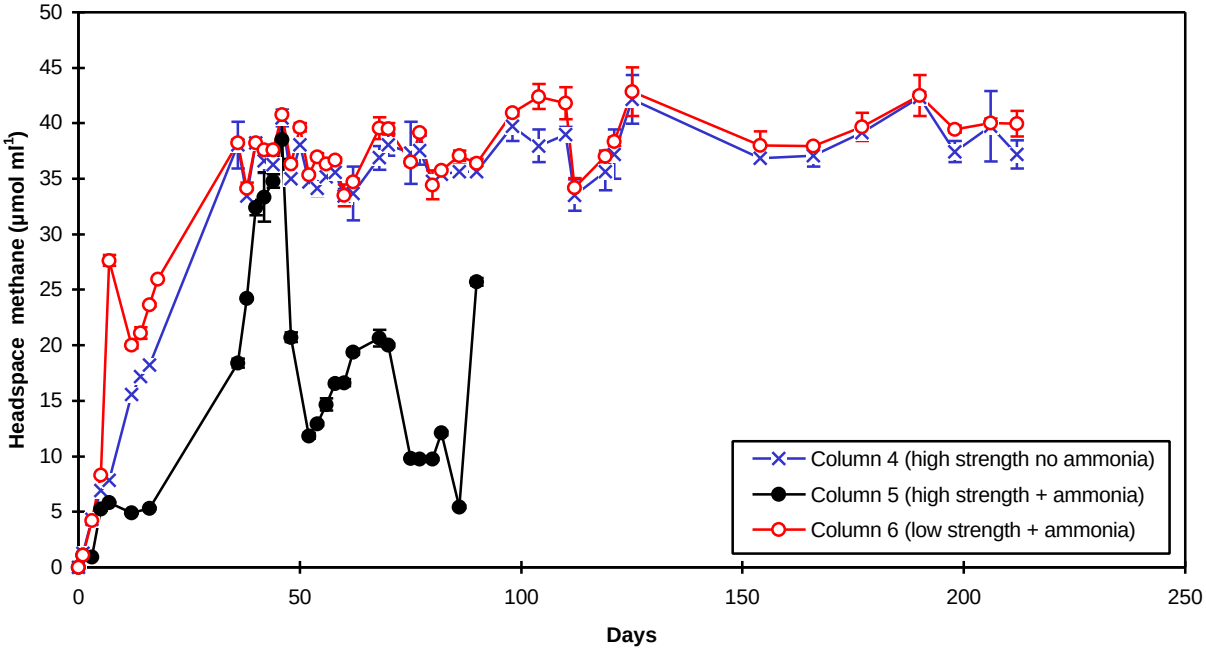


Figure 7.2 Headspace methane concentrations in column 4 (high strength leachate, ×), column 5 (high strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}, ●) and column 6 (low strength leachate + 500 mg $NH_{3/4+}$-N l^{-1},o). Error bars represent the standard deviation of the mean. (42 µmol ml^{-1} ? pure methane)

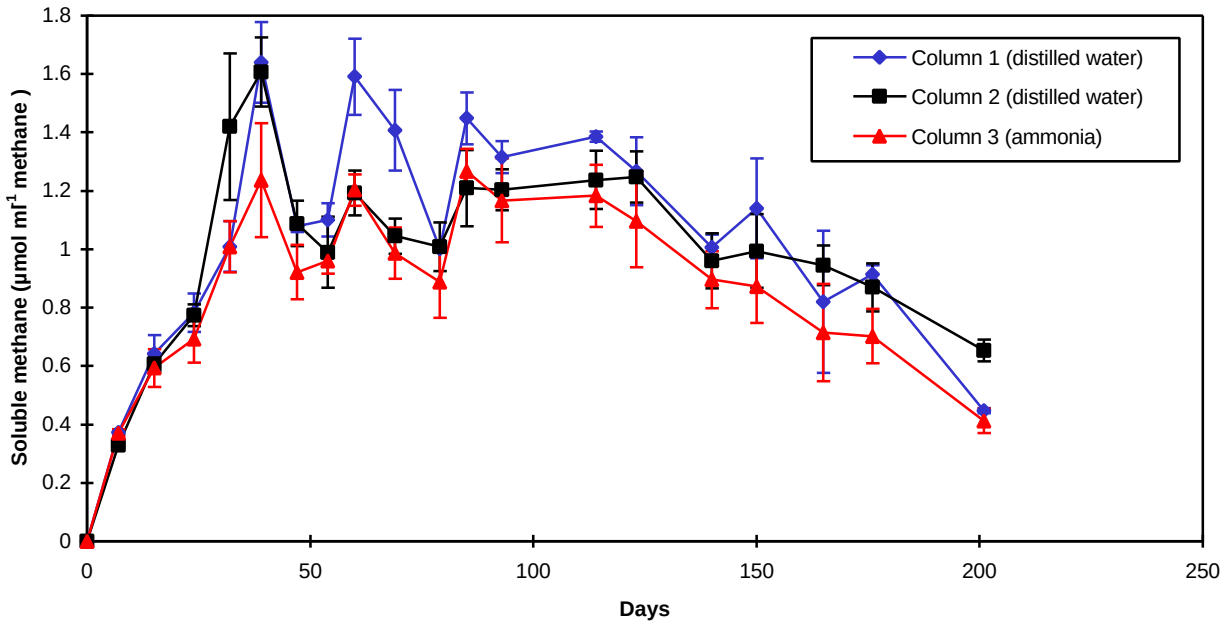


Figure 7.3 Soluble methane concentrations in column 1 (distilled water, ◆), column 2 (distilled water, ■) and column 3 (500 mg $NH_{3/4+}$-N l^{-1}, ▲). Error bars represent the standard deviation of the mean.

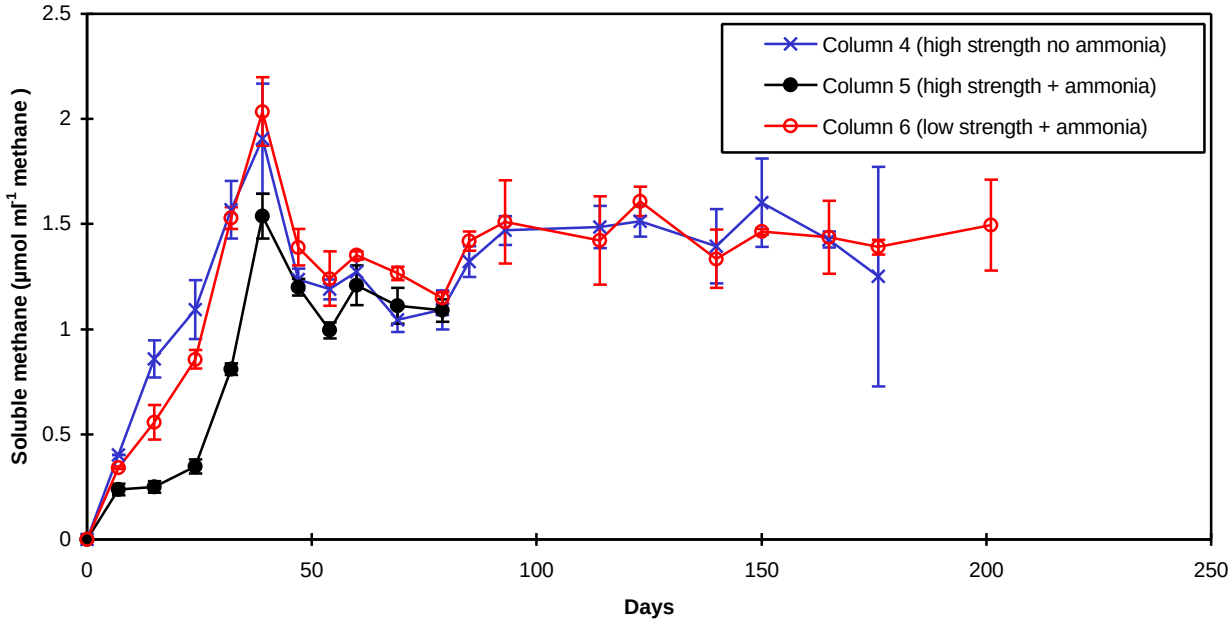


Figure 7.4 Soluble methane concentrations in column 4 (high strength leachate, ×), column 5 (high strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}, ●) and column 6 (low strength leachate + 500 mg $NH_{3/4+}$-N l^{-1},o). Error bars represent the standard deviation of the mean.

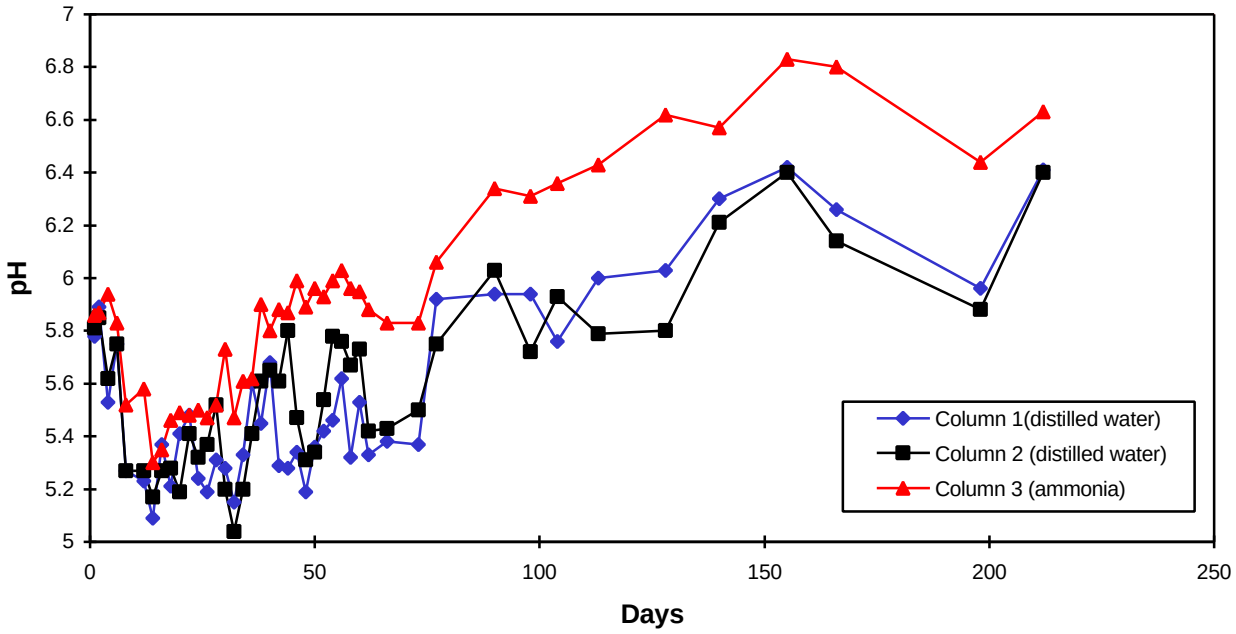


Figure 7.5 The pH values of the effluent from column 1 (distilled water, ◆), column 2 (distilled water, ■) and column 3 (500 mg $NH_{3/4+}$-N l^{-1}, ▲).

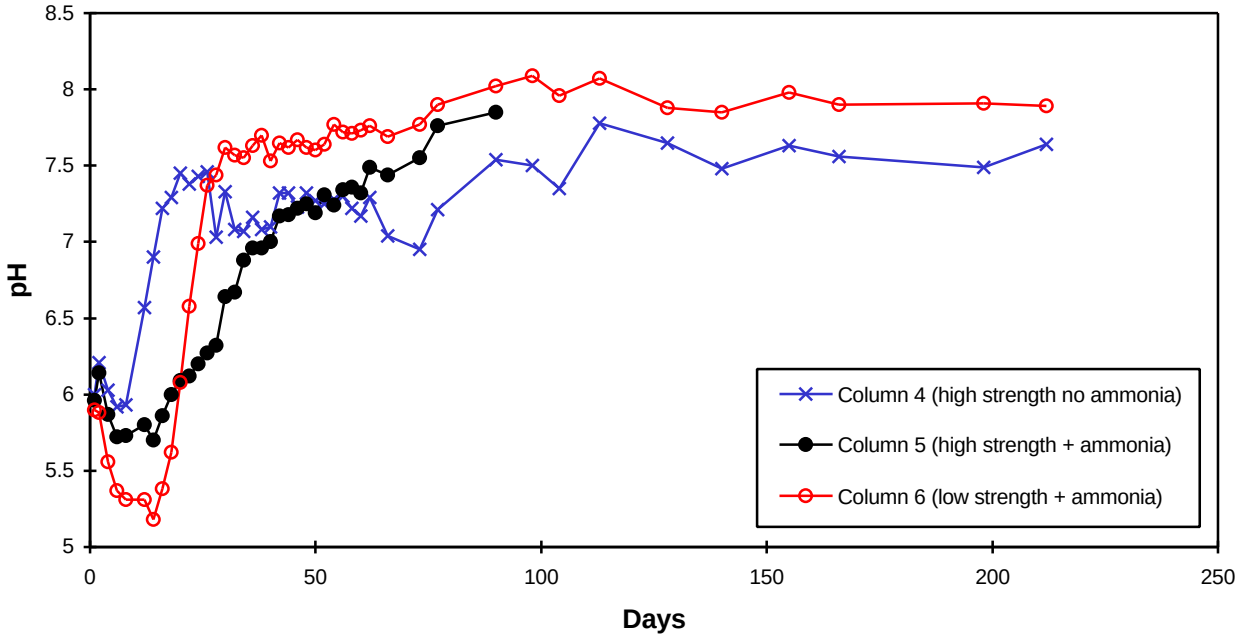


Figure 7.6 The pH values of the effluent from column 4 (high strength leachate, ×), column 5 (high strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}, ●) and column 6 (low strength leachate + 500 mg $NH_{3/4+}$-N l^{-1},o).

The settlement of the refuse within the columns was similar for the two water controls and the ammonia treated column with little more than a 5% difference in the loss of refuse volume associated with refuse settlement (Figure 7.7). Also the suspended sediment gradually decreased in the effluent with little difference between treatments as the finer particles were rinsed from the column (Figure 7.8). After the first day the suspended sediment in all the columns was approximately 1g l^{-1} dry weight consisting of coarse material such as sand and grit which collected in the weir over flow and proved too difficult to measure with any accuracy. A reasonable proportion of the weight loss associated with the perfusion of leachate could have been due to this rapid loss of suspended sediment. The sediment contained bacteria as well as refuse particles. At the end of the measurements, after 72 days, only suspended sediment in the leachate was measured and no material sank to the bottom of the collection containers. Approximately 50 g dry weight was lost from the refuse columns in comparison to the amount packed in the columns at the start of the experiment (Figure 7.9). Compared to the distilled water controls the total nitrogen content of the refuse column supplied with leachate containing 500 mg $NH_{3/4+}$-N l^{-1} had the greatest nitrogen content, similar to that of the refuse prior to packing into the columns (Figure 7.10). The ammonia nitrogen content increased as a result of perfusion indicating that some the ammonia may had become bound to the refuse (Figure 7.10). The refuse in this column when sampled had approximately 468 ml of water. Assuming that this liquid was the influent and contained 500 mg $NH_{3/4+}$-N l^{-1}, then only 0.0991% N could be accounted for by the ammonia present in the leachate when the columns were dismantled. The majority of the increase observed, when compared to the controls, must have come from the presence of bound ammonia in the refuse.

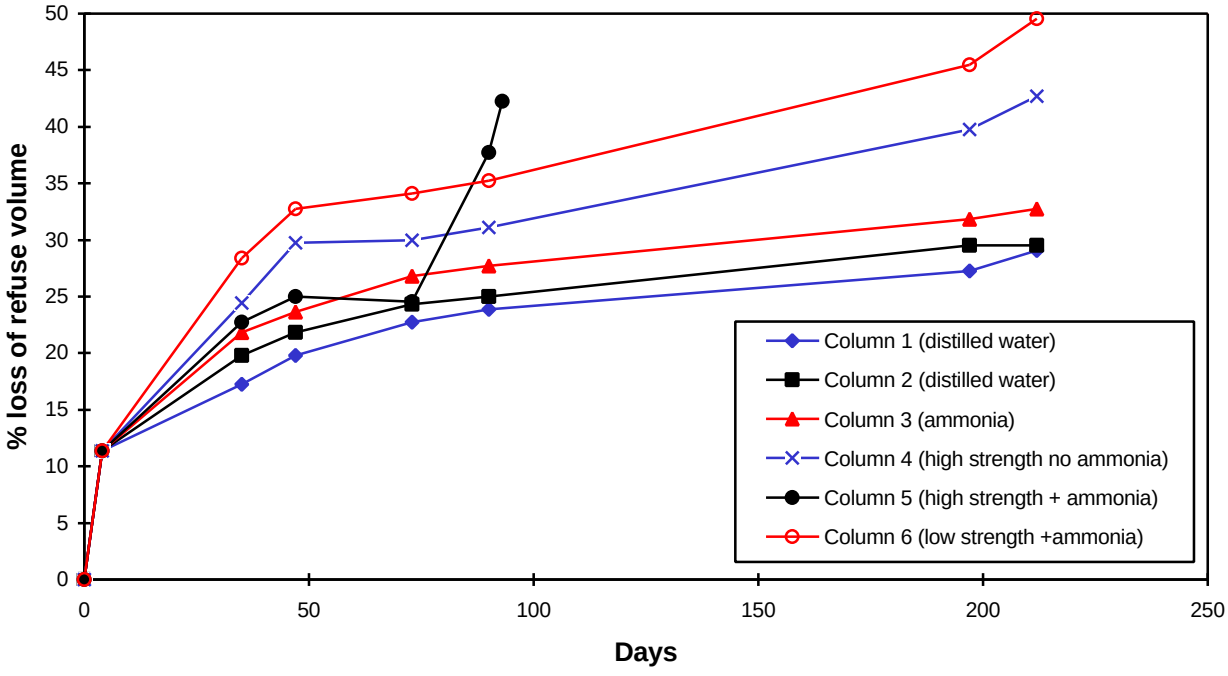


Figure 7.7 Settlement of refuse by column 1 (distilled water, ◆), column 2 (distilled water, ■), column 3 (500 mg $NH_{3/4+}$-N l^{-1}, ▲), column 4 (high strength leachate, ×), column 5 (high strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}, ●) and column 6 (low strength leachate + 500 mg $NH_{3/4+}$-N l^{-1},o).

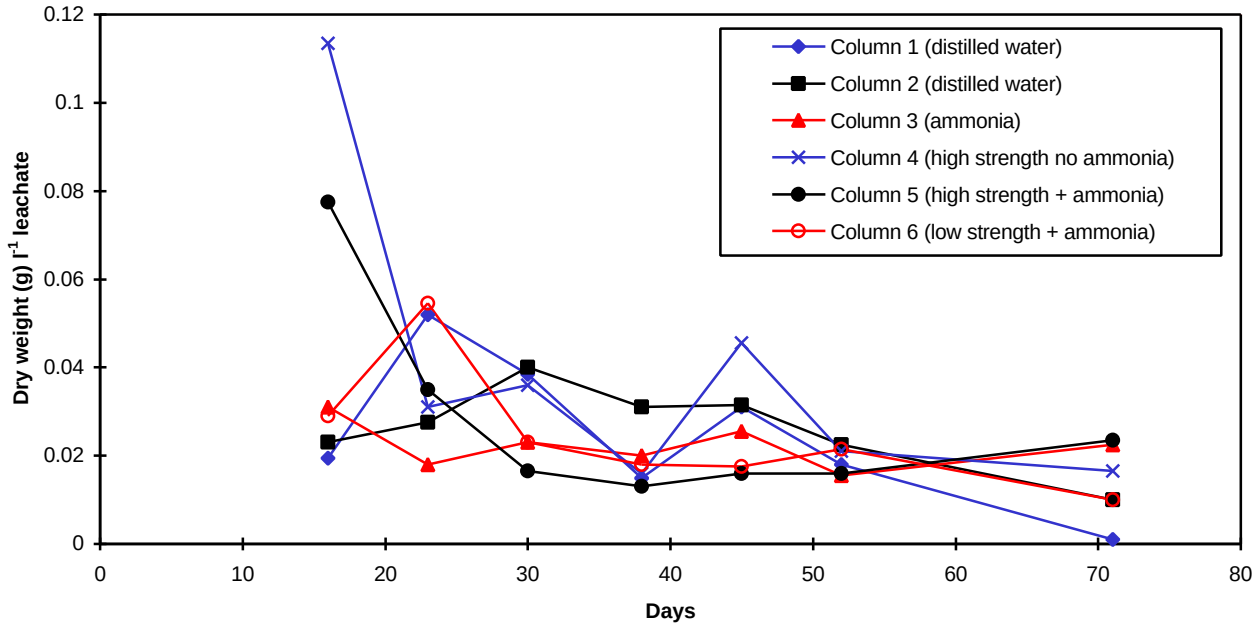


Figure 7.8 Suspended sediment in the effluent from column 1 (distilled water, ◆), column 2 (distilled water, ■), column 3 (500 mg $NH_{3/4+}$-N l^{-1}, ▲), column 4 (high strength leachate, ×), column 5 (high strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}, ●) and column 6 (low strength leachate +500 mg $NH_{3/4+}$-N l^{-1},o) over 80 days.

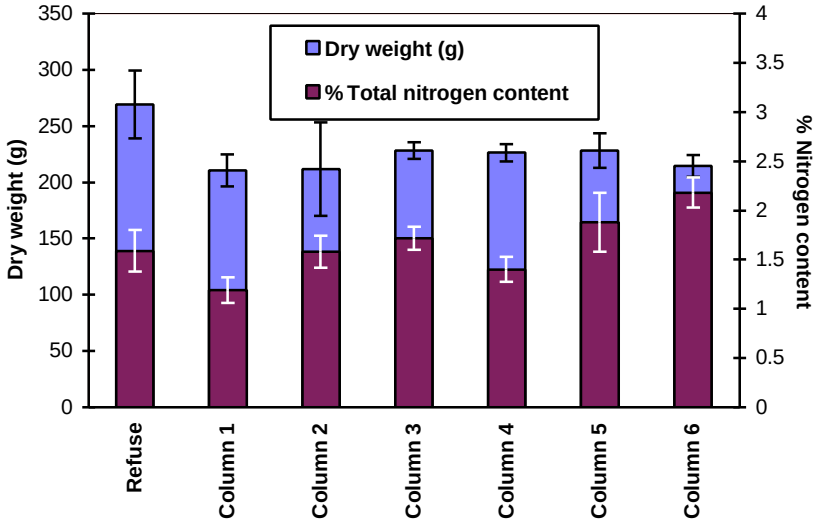


Figure 7.9 The dry weight and the % total nitrogen content (dry weight) of the refuse packed into the columns and that left in the refuse columns after perfusion with leachate. Error bars represent the standard deviation of the mean.

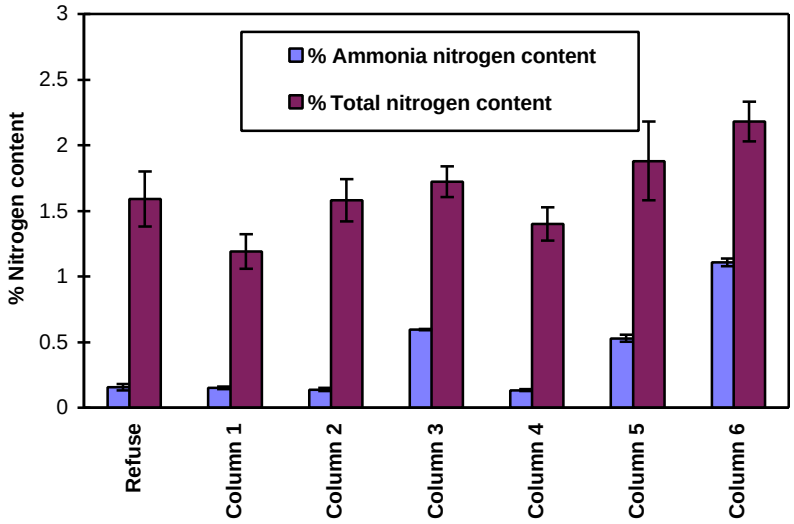


Figure 7.10 The % ammonia nitrogen content (dry weight) and the % total nitrogen content (dry weight) of the refuse packed into the columns and that left in the refuse columns after perfusion with leachate. Error bars represent the standard deviation of the mean.

The loss of ammonia from the distilled water controls was observed over the first 50 days of perfusion after which no ammonia could be detected in the effluent (Figure 7.11). There was a steady loss over the first 8 days from concentrations of 57.5 (SD 4.33) and 59.0 (SD 1.10) mg $NH_{3/4+}$-N l^{-1} from columns 1 and 2 to 0.466 (SD 0.659) and 2.06 (SD 0.110) respectively. There was a slight recovery in ammonia production after 8 days by both the control columns, presumably due to a mobilisation of ammonia in the waste either through the breakdown of proteins or a change in the physical conditions of the refuse. There was 1.59 (SD 0.210)% nitrogen dry weight in the refuse before perfusion equivalent to 4.22 (SD 0.565) g N. The total amount of ammonia leached in the effluent from columns 1 and 2 was 0.186 (SD 0.0231) g N over the first 50 days, 4.41 (SD 0.547) % of the total nitrogen dry weight. The perfusion of 500 mg $NH_{3/4+}$-N l^{-1} through column 3 could be measured in the effluent (Figure 7.12). Analysis of the influent during the study gave approximately 500 mg $NH_{3/4+}$-N l^{-1} after preparation. A total of 0.130 g $NH_{3/4+}$-N was analysed in the leachate approximately 3.08 % of the nitrogen present in the column before perfusion.

There was a gradual reduction in the conductivity of the effluents from the water controls and a slight rise after 12 days and then a gradual decrease such that after 200 days the conductivity was close to zero, signifying distilled water (Figure 7.14). The second control column maintained its effluent conductivity in comparison to the first. This could not be attributed to the release of ammonia which were similar from both refuse columns (Figure 7.11). The conductivity of the effluent from column 3 was higher and related to the concentration of ammonia.

There was a sharp rise in the concentrations of volatile fatty acids in the effluent after the first day as they were rinsed from the columns (Figure 7.16). There was continued acetate

production from the refuse columns for 50 days at low levels with little difference between the treatments.

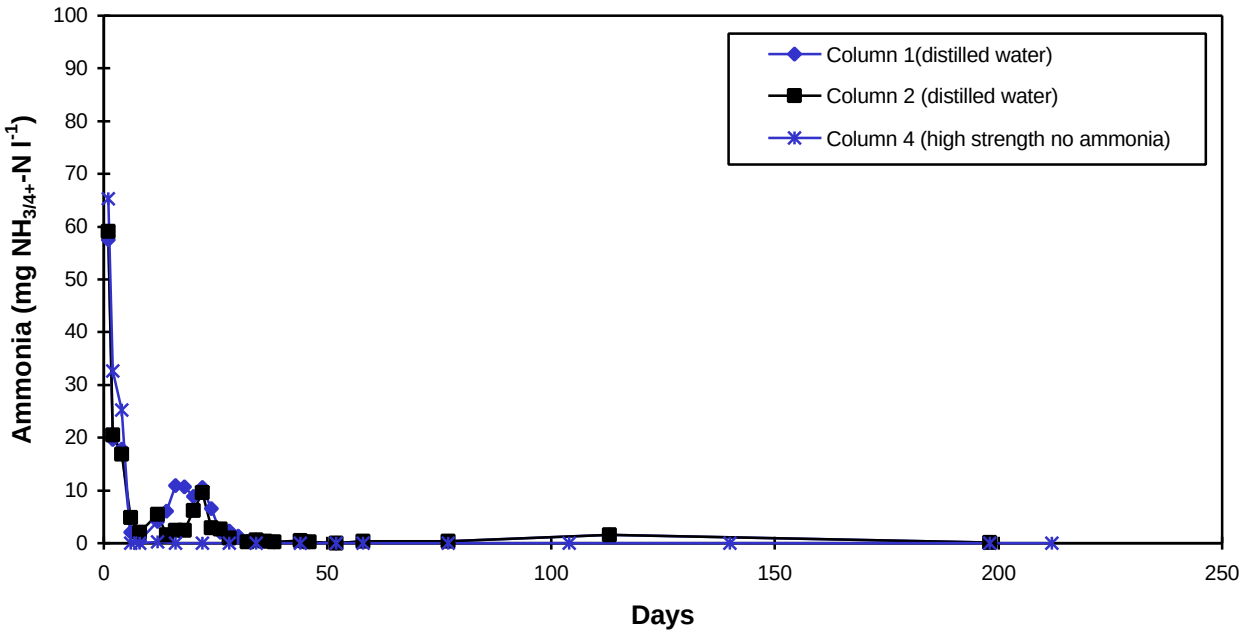


Figure 7.11 The ammonia concentration of the effluent from column 1 (distilled water, ◆), column 2 (distilled water, ■) and column 4 (high strength leachate, ×). Only the first 50 days are shown when ammonia could be detected in the effluent. Error bars represent the standard deviation of the mean.

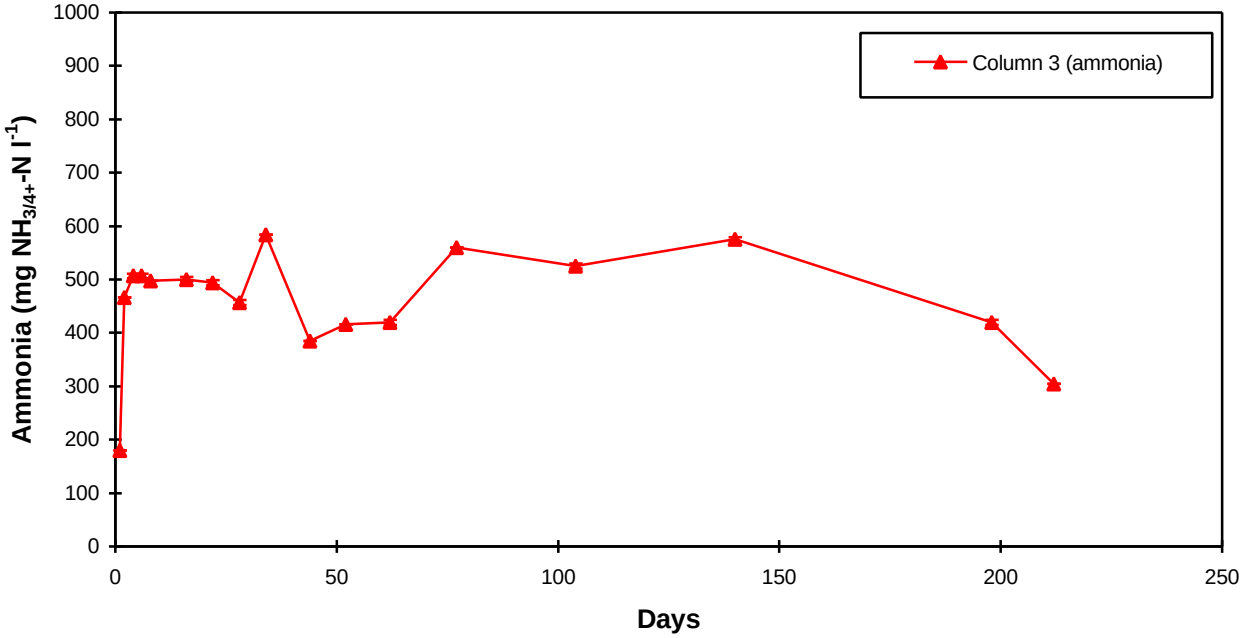


Figure 7.12 The ammonia concentration of the effluent from column 3 (500 mg NH3/4+-N l-1, ▲). Error bars represent the standard deviation of the mean.

7.4.2 Perfusion of Refuse Columns with Distilled Water Containing Volatile Fatty Acids and Ammonia

No nitrate was found in the effluent. Methane was detected in all the treatment regimes after one day. The refuse column supplied with high strength medium containing ammonia became blocked after 90 days and was disassembled and sampled. Headspace methane concentrations from the refuse columns perfused with high strength leachate with and without ammonia and the low strength leachate rose rapidly to reach a peak after 50 days at concentrations close to pure methane (Figure 7.2). Lags in the development of headspace methane were not replicated by the soluble methane concentrations which were dependent on the concentration of leachate and the presence of ammonia (Figure 7.4). These were similar to the development of neutral to pH values observed as methanogenesis increased the pH of the effluent (Figure 7.6).

The settlement of the refuse in columns supplied with high and low strength leachate containing ammonia was greater than the two water controls and the ammonia treated column, but overall the settlement of the refuse was similar in all the treatments (Figure 7.7). The settlement of the column supplied with high strength leachate containing ammonia was accelerated by blocking. Suspended sediment in the leachate was also similar for all the treatments (Figure 7.8).

There was little difference in the dry weight of the refuse after perfusion (Figure 7.9) but the refuse from column 5 and 6 where high and low strength ammonia containing leachate had been perfused had slightly higher total nitrogen contents suggesting accumulation of ammonia in the refuse. Large differences in the total nitrogen content were not observed and there was little evidence for significant accumulation of nitrogen into the biomass. Column six had been perfused with leachate containing 3000 mg $NH_{3/4+}$-N l^{-1} in the day prior to disassembling the refuse column which would have contributed to approximately 0.454% increase in the nitrogen content if all the water liberated on drying was assumed to be influent leachate. Taking this factor into account there was probably not a significant increase in the nitrogen content of columns 3, 5 and 6 revealing conservation of nitrogen in the refuse during perfusion. Both columns 3 and 5 would have contained approximately 0.1% extra nitrogen due to the presence of the influent (see above). Because of the small differences observed, a large number of replicate columns would have been required to demonstrate conclusively differences in the total nitrogen content of the refuse compared to that originally packed. Evidence of the contribution of the supplied leachate to column 6 was revealed by the high ammonia content of the refuse (Figure 7.10). Both columns 5 and 6 smelt heavily of ammonia suggesting that volatilisation of ammonia had occurred in these columns.

The leaching of ammonia from column 4 was similar to that of the water controls but no ammonia could be detected in the leachate after 12 days. This was probably due to the difficulties of measuring extremely low concentrations of ammonia in leachate containing very high concentrations of volatile fatty acids since interferences in the detection of very low concentrations of ammonia could not be alleviated by dilution (Figure 7.11). Concentrations of ammonia in the effluent from columns 5 and 6 showed the wash out of ammonia during the first 5 days (Figure 7.13). The conductivity of the leachate was dependent on the strength of the influent, higher conductivity measurements were made during the washing out of mineral ions from the refuse at the start of perfusion (Figure 7.15).

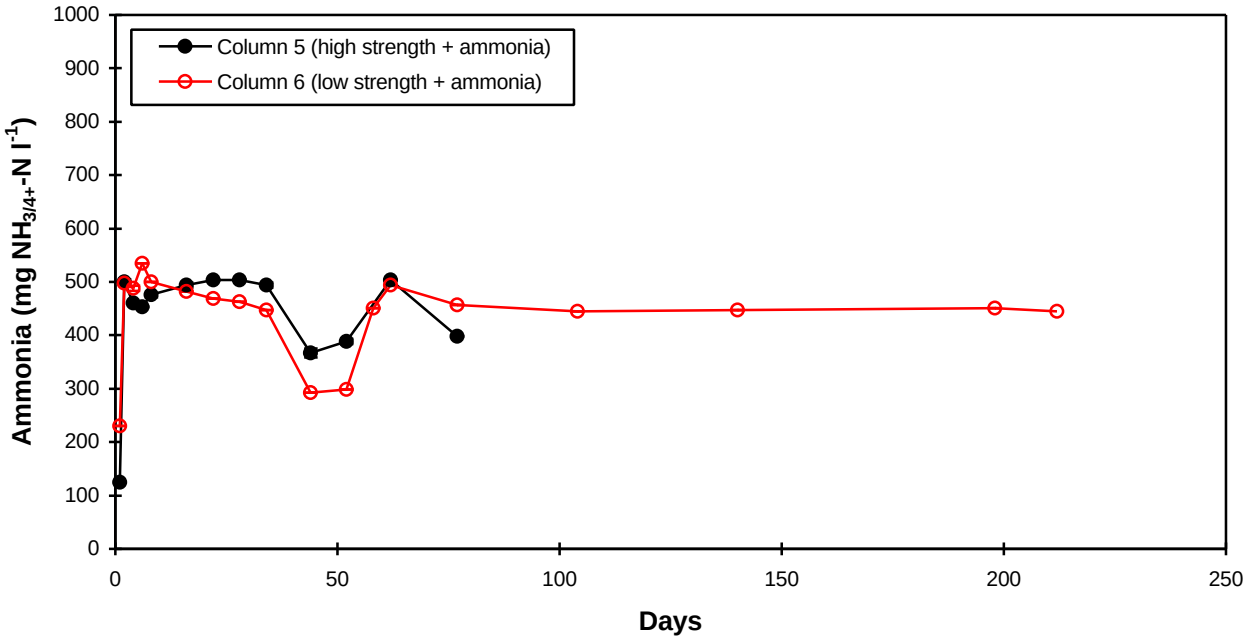


Figure 7.13 The ammonia concentration of the effluent from column 5 (high strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}, ●) and column 6 (low strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}, o). Error bars represent the standard deviation of the mean.

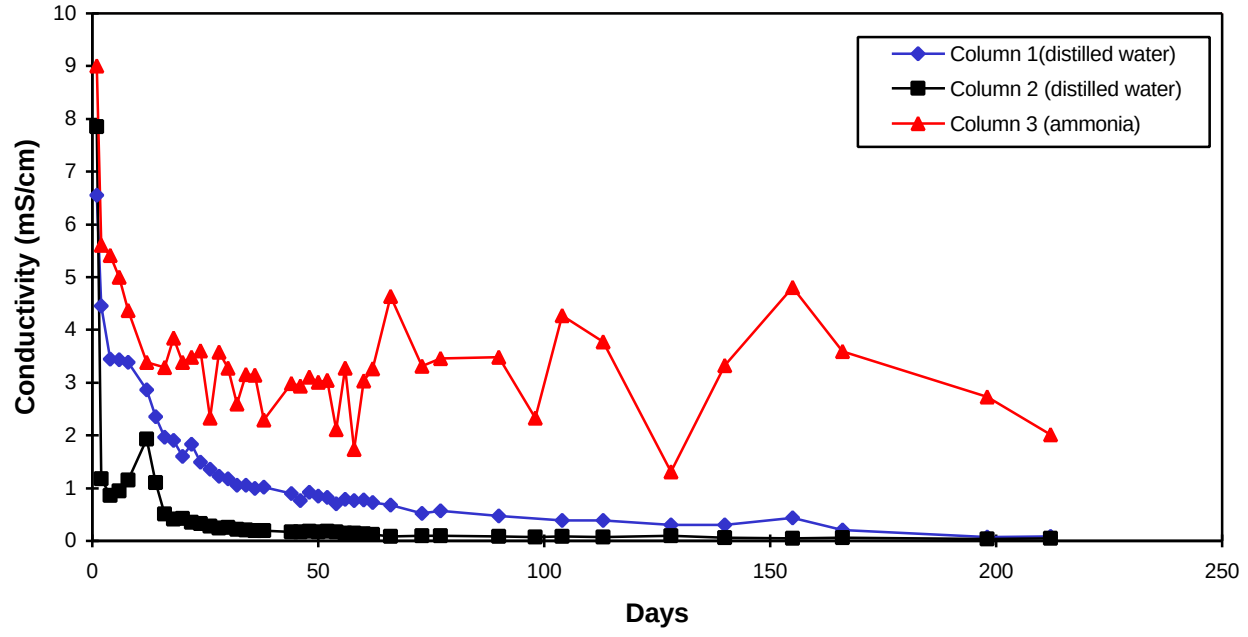


Figure 7.14 The conductivity of the effluent from column 1 (distilled water, ◆), column 2 (distilled water, ■) and column 3 (500 mg $NH_{3/4+}$-N l^{-1}, ▲).

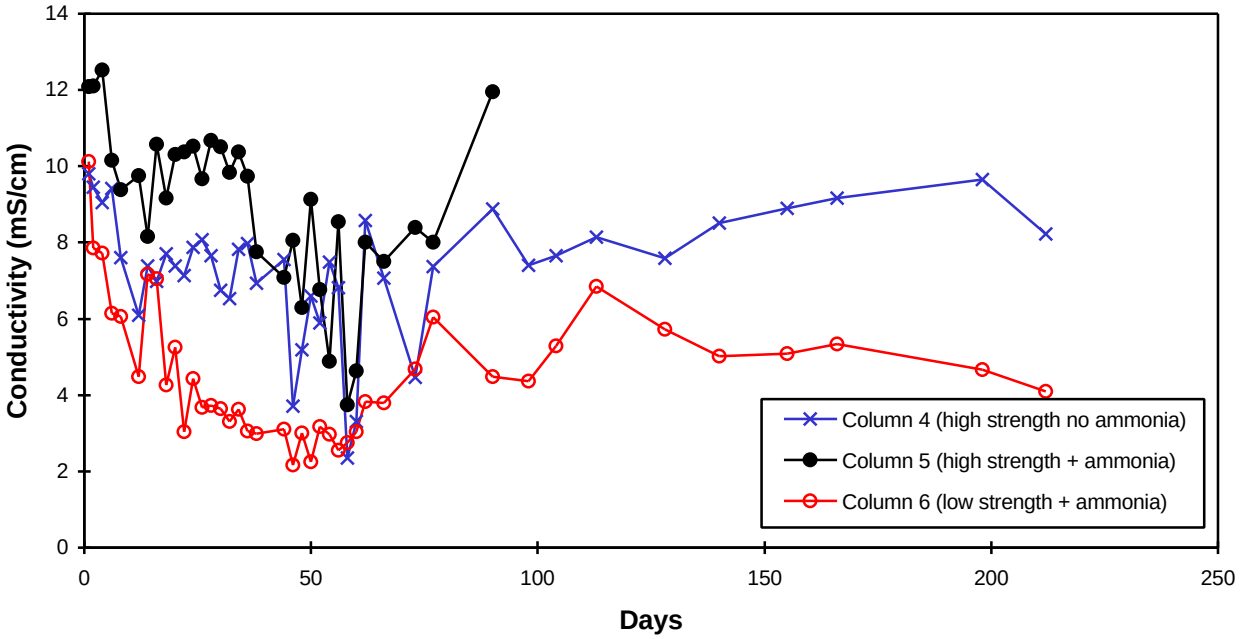


Figure 7.15 The conductivity of the effluent from column 4 (high strength leachate, ×), column 5 (high strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}, ●) and column 6 (low strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}, o).

The volatile fatty acids supplied to columns 4, 5 and 6 were identified in the leachate in addition to those washed from the refuse (Figure 7.17). The effluent from column 6 which was supplied with 25 mM acetate and ammonia contained approximately two thirds of the influent acetate concentrations. Propionate and butyrate were leached in a similar way to the water controls and the ammonia perfused refuse column. The appearance of almost pure methane in the headspaces of the columns supplied with volatile fatty acids and the identification of volatile fatty acids in the effluent suggested that the rate limiting step for volatile fatty acid breakdown was acetogenesis. Nearly all the carbon dioxide normally produced during anaerobic decomposition was reduced to methane.

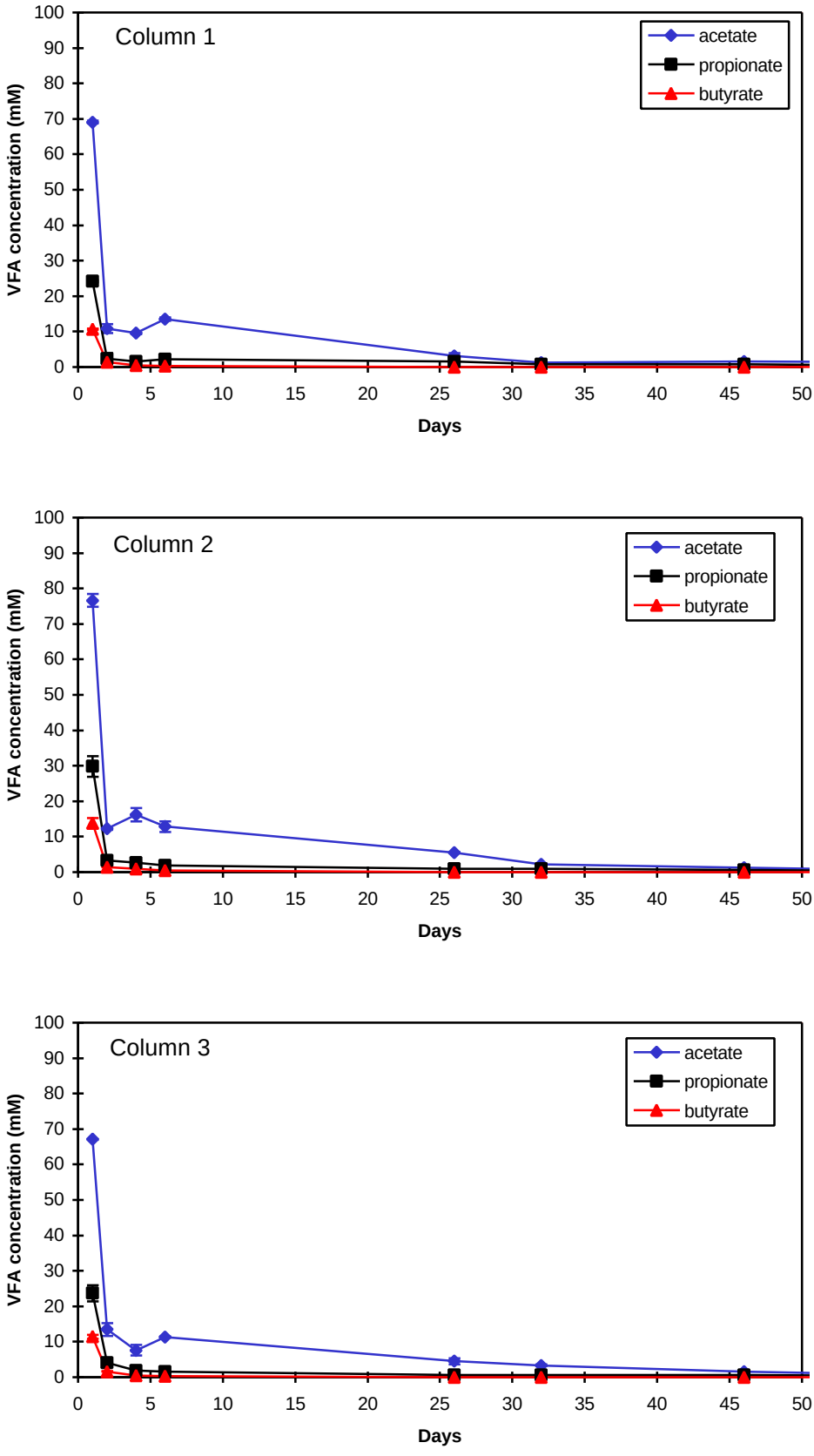


Figure 7.16 The concentrations of acetate (◆), propionate (■) and buytrate (▲) in the effluent of column 1 (distilled water), column 2 (distilled water) and column 3 (500 mg $NH_{3/4+}$-N l^{-1}). No volatile fatty acids were present in the effluent after 50 days. Error bars represent the standard deviation of the mean.

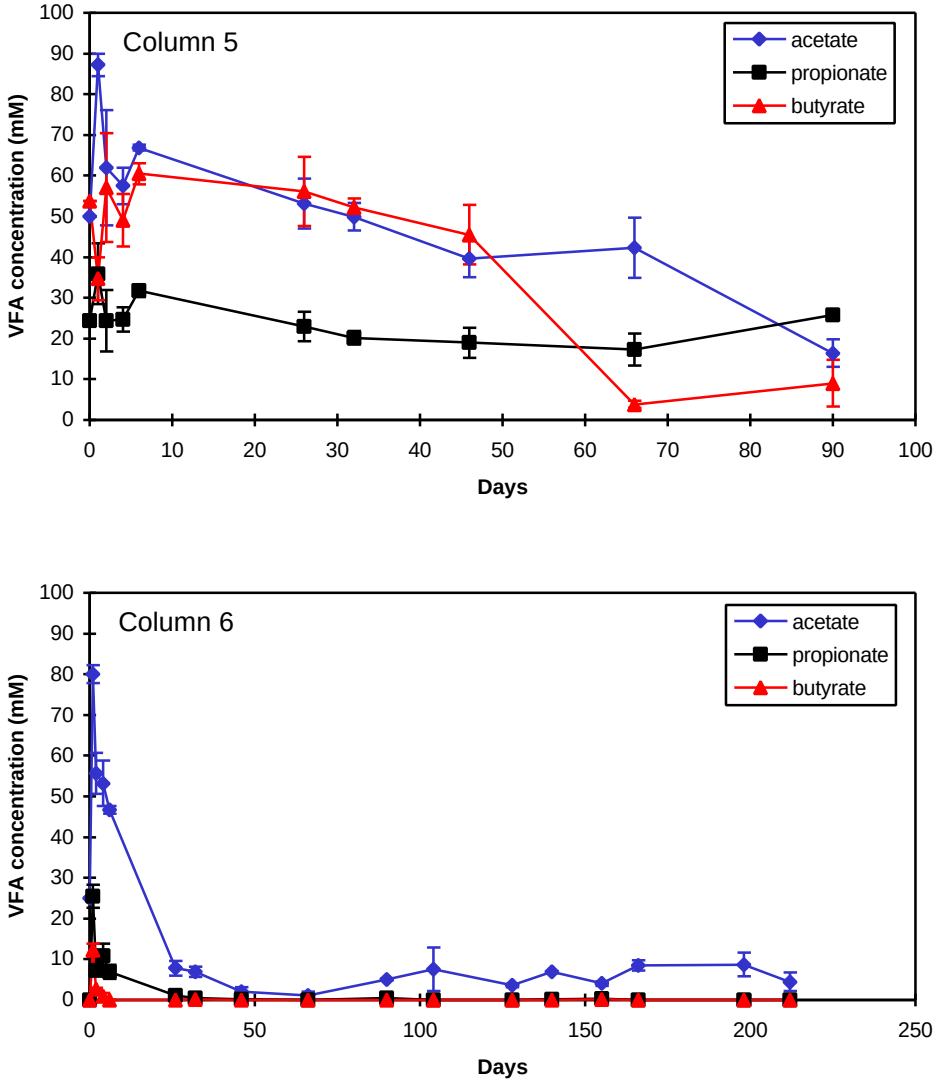


Figure 7.17 The concentrations of acetate (◆), propionate (■) and buytrate (▲) in the effluent of column 4 (high strength leachate no ammonia), column 5 (high strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}) and column 6 (low strength leachate + 500 mg $NH_{3/4+}$-N l^{-1}). Column 5 was only measured for 90 days. Error bars represent the standard deviation of the mean.

7.4.3 Effect of Ammonia on Methane Production from a Column Supplied with 25 mM Acetate

The effect of ammonia toxicity on methane production by the column supplied with 25 mM acetate and 500 mg $NH_{3/4+}$-N l^{-1} was tested by increasing the concentrations of ammonia present in the influent to 1000 mg $NH_{3/4+}$-N l^{-1} for 47 days and 3000 mg $NH_{3/4+}$-N l^{-1} for 1 day. There was no change in the headspace methane or soluble methane concentrations tested after the ammonia concentrations in the influent were increased from 500 mg $NH_{3/4+}$-N l^{-1} to 1000 mg $NH_{3/4+}$-N l^{-1} (Figure 7.18). There was no effect on the production of methane by 1000 mg $NH_{3/4+}$-N l^{-1}, although ammonia concentrations and conductivity in the effluent doubled after the change in the influent concentrations. Increasing the concentrations from

1000 to 3000 mg $NH_{3/4+}$-N l^{-1} lead to a sharp reduction in the headspace methane concentration and the column became blocked and was dismantled and sampled. The increase in ammonia concentration may have resulted in a loss of gas pressure in the headspace and the eventual blocking of the column but there was little evidence to support this other than a decrease in headspace methane concentrations.

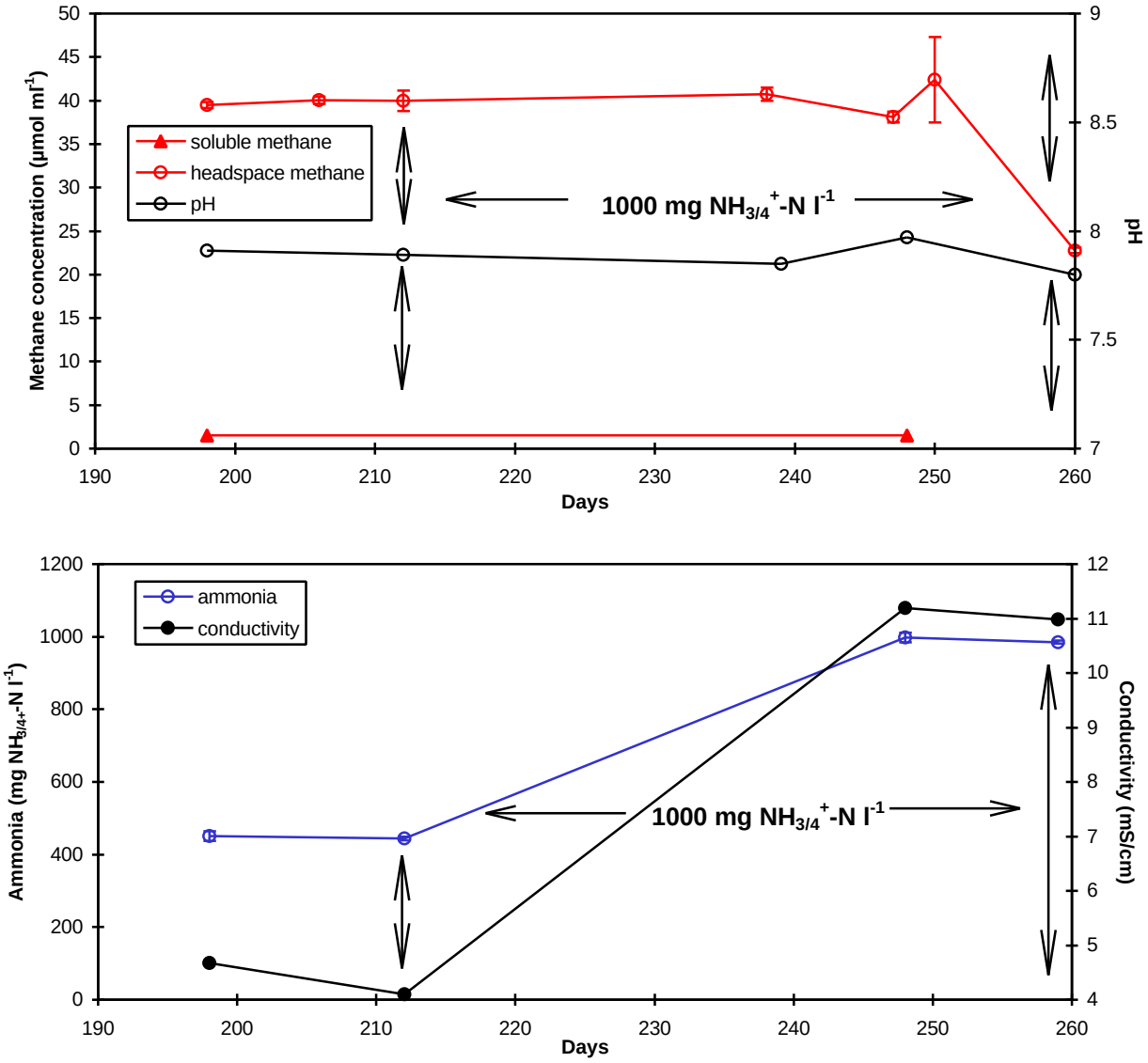


Figure 7.18 Column 6 effluent acetate (◆), headspace methane (o), soluble methane (▲), pH value (o), ammonia concentrations (o) and conductivity (●) after increases in ammonia influent concentrations. Vertical arrows represent the change in the influent from 500 to 1000 and 1000 to 3000 mg $NH_{3/4+}$-N l^{-1}. Error bars represent the standard error of the mean.

7.5 Discussion

The leaching of ammonia from all the refuse columns was restricted to the first 10 to 50 days perfusion. Results were consistent with the general perceived view within the industry that approximately 10 bed volumes will be required to flush the ammonia from the refuse during perfusion (Robinson, 1995, Burton and Watson-Craik, 1996). Increases in the total nitrogen concentrations were found in the refuse in the columns perfused with ammonia. There was little to suggest that ammonia was being consumed during the anaerobic decomposition of refuse and incorporated into the biomass since most of the increases could be explained by the ammonia present in the refuse when the columns were dismantled and analysed. Large increases in the biomass (calculated from the % total nitrogen - ammonia nitrogen) in the refuse would signify bacterial proteins and therefore bacterial growth. The absence of these proteins suggested that there was little bacterial growth in the refuse columns despite the

supply in some instances of high concentrations of volatile fatty acids on which the anaerobic bacteria could grow. Bacteria contain approximately 15% nitrogen by weight and so if significant bacterial growth occurred, the total nitrogen concentrations and biomass concentrations would increase. The lack of accumulation or utilisation of ammonia into biomass is further evidence for the lack of bacterial growth in refuse during decomposition. It also suggests the reason for high ammonia concentrations in leachate despite the relatively modest carbon to nitrogen ratios in refuse of around 50:1 (Senior and Balba, 1987). Anaerobic bacteria are active, producing methane, carbon dioxide, volatile fatty acids and ammonia in landfill refuse, but this does not translate into vigorous growth because of the limited energy gained from these processes. Bacteria will metabolise or use the protein in refuse for energy, liberating ammonia in the process, but very little of this ammonia will be used for growth. Growth occurs but it is slow, probably too slow to be measured accurately by analysing the nitrogen concentrations in an undefined substrate such as refuse.

In the landfill, ammonia will be produced through the anaerobic decomposition of proteins in the refuse but little will be consumed by bacteria and incorporated into bacterial biomass. The nitrogen in readily degradable protein will be released as ammonia. The proteinaceous nitrogen present in skin, hair, wool, wood and nails are trapped within densely bound secondary structure and do not degrade quickly. These refuse components were observed intact after incubation in columns and refuse cultures. Meat and plant tissues will contain readily available protein for rapid conversion into ammonia particularly the proteins dissolved within the liquids contained within them. Industrial sludges will also contain a high proportion of dissolved protein which will be released during decomposition but much of the bacterial structural proteins will probably persist in the landfill for longer. The speed at which ammonia will enter the leachate will depend on the permeability and moisture characteristics of the refuse. The moisture content will also determine the speed at which it decomposes since wetter refuse will degrade more quickly.

Although not identified experimentally the binding of ammonia to refuse will be important in determining the release of ammonia into leachate. At acidic pH values one would expect some leaching of ammonia to occur because of the interchange between the H^+ ions (protons) present in acidic conditions and ammonia in the form of NH_4^+ (ammonium) bound to negatively charged refuse particles. Ammonia will naturally bind to negatively charged surfaces and pH value will determine whether it will remain tightly bound or leach.

Although refuse column experiments were not carried out in this study on the recirculation of leachate it may be possible to identify the binding of leachate ammonia from the acetogenic to the methanogenic stage (Chapter 1, Figure 1.2). A burst of ammonia will be released during the acetogenic phase at low pH due to the effect of the interchange of H^+ and NH_4^+ and the sudden release of ammonia from protein degradation and solublisation. A rise in pH associated with methanogenesis may encourage binding and so after a recirculation event the effluent of a refuse column may contain slightly less ammonia than during the acetogenic stage. The proportion of this binding will be dependent on the pH value of the leachate during acetogenesis and refuse during methanogenesis. Binding would be revealed from increases in the ammonia nitrogen content of the refuse analysed by distillation. Higher pH values of above 7.5 will enable the formation of NH_3 from NH_4^+ and may encourage volatilisation of ammonia gas or even leaching of ammonia from the refuse columns. Significant volatilisation was not observed as similar influent and effluent ammonia concentrations were observed from the refuse columns supplied with ammonia. Whether the pH value of the refuse and leachate exert an effect on the release of ammonia needs further study.

The very high concentrations of methane observed in the headspace of the refuse columns supplied with volatile fatty acids were not typical of landfill gas. Concentrations of methane in landfill generally approximate to 60% methane and 40% carbon dioxide with a minor proportion of trace gases. The high methane concentrations were probably a feature in part of the presence of volatile fatty acids in the influents since the refuse columns perfused with water had headspace methane concentrations closer to that of landfill gas. The high acetate concentrations and therefore substrate concentrations may have promoted methanogenesis by acetoclastic methanogens. The neutral to moderately alkaline pH may have contributed to the carbon dioxide normally evolved dissolving or remaining dissolved in the leachate to be lost from the refuse column as bicarbonate in the rapidly flowing effluent.

The total nitrogen content of the refuse packed into the columns at 1.59 (SD 0.210) % nitrogen dry weight was lower than that observed in previously analysed refuse (Chapter 5). However, the proportion of ammonia leached from columns 1 and 2 at 4.41 (SD 0.547) % of the total nitrogen content over the first 50 days was similar to that observed in newly prepared batch cultures where approximately 5 % of the total nitrogen was ammonia. Slightly lower amounts of ammonia were leached from the column 4, perfused with volatile fatty acids, but this may have been due to the difficulties in analysing high strength leachate for very low concentrations of ammonia.

The limited effect of ammonia concentrations on column 6 receiving 25 mM acetate suggest that ammonia was not inhibiting methanogenesis between 500 to 1000 mg $NH_{3/4+}$-N l^{-1}. The anaerobic decomposition of acetate did not appear to be ammonia inhibited at these concentrations. The methanogenic association may have become acclimatised to the high concentrations of ammonia. The blocking of the column after the increase in ammonia concentrations from 1000 to 3000 mg $NH_{3/4+}$-N l^{-1} may have been due to inhibition of methanogenesis and a reduction in gas pressure but without the correct operation of the refuse column it is not possible to explain this effect.

7.6 Conclusions

- Ammonia leached from refuse columns during perfusion of distilled water with significant concentrations appearing in the leachate the first 1-10 bed volumes. Significant proportions of bound ammonia which could be analysed by direct distillation remained after perfusion in the refuse.

- The supply of ammonia to refuse columns containing distilled water appeared to slightly delay the onset of methanogenesis and the concentrations of soluble methane in the effluent.

- Increases in the concentration of ammonia from 500 to 1000 mg $NH_{3/4+}$-N l^{-1} did not affect the soluble or headspace methane concentrations of a refuse column receiving 25 mM acetate, suggesting limited effects of ammonia on methanogenesis at these concentrations.

- Ammonia did not accumulate significantly during the growth of anaerobic bacteria in refuse columns supplied with combinations of ammonia and volatile fatty acids. Utilisation of ammonia during bacterial growth is probably not an important route for the nitrogenous components in decomposing landfill refuse.

8. STUDY OF THE COMPONENT GROUPS INVOLVED IN LANDFILL REFUSE DEGRADATION AND THEIR RESPONSE TO THE ADDITION OF NITRATE

8.1 Experimental Rationale

A multi-stage continuous flow model system containing the different groups of microorganisms associated with refuse degradation was constructed in the laboratory. The effect of nitrate was tested on an anaerobic microbial consortium degrading the polymer cellobiose within the system. The effect of nitrate on the release of volatile fatty acids and methane production was quantified.

8.2 Introduction

The anaerobic fermentation of paper wastes is a highly complicated process performed by a number of very different microorganisms. The microorganisms form a consortium (Chapter 1) in which the byproducts from one group are used by others leading typically to the production of end products such as carbon dioxide and methane. Studies on such consortia represent a challenge because of the intimate nature in which the functional groups of bacteria are linked to each other during anaerobic decomposition. In practice, the best way of looking at the anaerobic breakdown of any compound by such consortia is to separate the functional groups spatially and look at the performance of the individual groups to experimental perturbations. Spatial separations can be achieved by applying knowledge on the growth rates of the different functional groups of bacteria involved. It is possible to separate the functional groups of bacteria in vessels according to their growth rates in liquid media. A continuous culture chemostat system of inter-linked vessels of various sizes and therefore dilution rates is used to maintain the separation. By separating functional groups to one or more vessels it is possible to follow the performance of each group by their product formation or substrate utilisation.

Previous studies have looked at the breakdown of the volatile fatty acid hexanoic acid ($CH_3CH_2CH_2CH_2CH_2COOH$) by a microbial consortia in continuous culture chemostat systems (Coutts *et al.*, 1987; Watson-Craik & Senior, 1989d; Sinclair, 1994). The systems were also used during experiments on interspecies interactions, Elucidation of Refuse Interspecies Interaction by Use of Laboratory Models, ETSU B/B2/00148/REP (James & Watson-Craik, 1993). A weaknesses of these systems is that they are liquid models and contain none of the physical characteristics of landfill refuse, and the effect of introducing surfaces to these systems was explored in collaboration with another DoE/DTI funded project in the laboratory, Assessment of the Role of Surfaces in Refuse Degradation (Jones & Watson-Craik, 1994). Advantages are that there are no barriers to the rapid diffusion of dissolved compounds within the system vessels. The medium within each vessel is relatively uniform. The surface area open for attachment by bacteria is severely limited resulting in diffuse growth in the medium. Wall growth and sedimentation limits the flushing out of bacteria from one vessel to another but these effects can be reduced by regular mixing and the cleaning of the sides of the vessels and the associated pipe-work. Long periods of acclimatisation to a specific flow rate optimises the system making for stable substrate (nutrient) levels within each of the vessels, the flushing out of slower growing bacteria to lower vessels and the creation of what can be considered as a steady state.
A culture was enriched and grown on the glucose dimer, cellobiose, in a four stage continuous culture system. Cellobiose was chosen for its similarity to residues of cellulose found in paper

waste and cotton, and since it was soluble, to enable continuous perfusion. Cellobiose consists of two glucose molecules held by a β(1-4) glucosidic linkage. Cellulose also contains these bonds between its glucose molecules, but the chains of glucose are considerably larger and interwoven with several thousand other chains to create an insoluble fibril. The secondary structure makes the cellulose more resistant to rapid degradation by bacterial enzymes. Hydrolysis (cleavage in water) of cellulose yields mostly glucose and cellobiose.

The cellobiose degrading culture was acclimatised within the four stage continuous culture system and subjected to perturbations containing nitrate to test the effect of nitrate on acetogenesis, sulphate reduction and methanogenesis during the decomposition of cellobiose. A second system was used as an unadjusted control to which no nitrate was added. Medium supplied to the top vessel then percolated to the next vessel. Bacteria can only remain in a specific vessel if their growth rate in the liquid culture is sufficiently rapid so they do not become washed out. Acetogens and sulphate reducing bacteria possess a more rapid growth rate and were maintained in the upper vessels whereas methanogens, with lower growth rates in comparison, dominated in the lower vessels. Adding nitrate to the vessels allows denitrifying bacteria to proliferate. The growth rates of these bacteria were expected to be rapid owing to the increased energy yield liberated by the process of denitrification in comparison with the other functional groups. Although denitrification is a separate biological process with a different biochemical pathway many bacteria are capable of denitrifying and will switch from the fermentation of cellobiose in the culture vessels to the denitrification of cellobiose when given a nitrate supply. The ability to denitrify is conserved even though nitrate is not a specified component of the influent medium nor a component of landfill leachate (Chapter 4). Insignificant amounts of nitrate will still be present in the influent medium as impurities within the analytical grade chemicals used. Analysis of solutions of 500 mg $NH_{3/4+}$-N l^{-1} gave less than 2.25 mg NO_3^--N l^{-1}. The manufacturing limits specified would allow approximately 2 µg NO_3^--N l^{-1} in a solution of 500 mg $NH_{3/4+}$-N l^{-1}. The rapid denitrification by cultures would be an indication of the fact that denitrifying bacteria were present in the cultures as a matter of course despite the absence of significant concentrations of nitrate in the influent.

The aim of the experiments with cultures held in the four stage fermenter system was to measure the effects of nitrate addition to the microbial communities which breakdown paper waste in a landfill. As a result no additions of nitrate were made in the influent feed during acclimatisation period of 4 months because of the absence of nitrate in landfills. Experiments were intended to model previous experiments which had all received shock concentrations of nitrate without the chance of acclimatisation.

8.3 Materials and Methods

8.3.1 Preparation of an Inoculu m for the Multi -stage System

Refuse (250 g: approximately 100 g dry weight) and 1l distilled water containing 5 mM cellobiose was confined within a fermentation vessel and gas collection apparatus and incubated at 30^{o}C (Figure 6.4). Anaerobic decompositon led to the formation of methane and carbon dioxide which was periodically released during the incubation. Every 3-4 weeks the culture was inoculated with a cellobiose suspension/solution (approximately 10-20 ml) to bring the total concentration of cellobiose in the culture to 5 mM. This was repeated seven times after which the period over which gas was produced had fallen to 6-7 days. The cellobiose was then added every 6-7 days for a further six times enriching the cellobiose

degrading association. An inoculumn (100 ml) was removed 2 days after the addition of cellobiose to the enrichment and injected into the uppermost vessel of the two four stage systems. Mean gas production from the final four enrichments was 836 ml (SD 49.1) containing 60% v/v methane.

8.3.2 Preparation of the Multi -stage System for Nitrate Perturbations

Four stage continuous culture systems were constructed in two arrays consisting of four vessels of 203 ml, 317 ml, 704 ml and 1152 ml culture volume (Figure 8.1 and 8.2). Oxygen impermeable butyl rubber tubing (Watson-Marlow) was used for gas lines and vessel influent and effluent. Autoclavable marprene (Watson-Marlow) was used for the influent supply to vessel 1 in each of the two systems because of it small bore size and flexibility. This was autoclaved with the drip feed at the top of the system and changed when new medium was prepared to limit bacterial growth in the these tubes. A magnetic aquarium scraper (VNK aquarist supplies) was used to remove the surface growth on the vessel walls every 4-7 days. When not in use the magnetic scraper was left in the neck of the lower vessels or introduced before scraping in the uppermost vessels when the influent medium was changed. The system was permanently overgassed with oxygen free nitrogen gas to prevent aerobic growth by microorganisms. Gas leaving each vessel was passed through a 1% zinc acetate trap to indicate the presence of H_2S gas and sulphate reduction. A barium hydroxide (18g l^{-1}) trap gave an indication of the presence of carbon dioxide gas. The clear aqueous solutions formed a white precipitate in the presence of the appropriate gases.

Cultures were grown in a mineral medium (appendix 2) poised at pH 7.0. The influent flow rate was gradually increased from 3.7 ml h^{-1} to 28 ml h^{-1} over 5 months to give dilution rates of 0.138, 0.088, 0.040 and 0.024 h^{-1} from the uppermost vessel to the bottom vessels respectively. Culture volume changes, equivalent to a bed volume, took place after 7.25 h, 11.32 h, 25.14 h and 41.14 h respectively.

Liquid samples from each of the vessels were taken weekly for the analysis of volatile fatty acid, pH value, redox potential, ammonia and sulphate. Methanogenesis from each vessels was measured by measuring the gas flow rate with a column of water and reservoir whilst taking a gas sample for analysis by gas chromatography.

Both multi-stage arrays were left at this dilution rate for 4 months before nitrate perturbations were attempted. Since the two multi-stage arrays were each built to reproduce each other, a 100 ml sample from the uppermost vessel was swapped after 7 months with a 100 ml syringe with that from the opposing multi-stage array to ensure that the bacterial populations in the systems remained similar. Steady state conditions in all the parameters tested became apparent after 5 months, the two multi-stage systems remained similar in most respects allowing the perturbation experiments to proceed (Figure 8.3 and 8.4). No sulphate was detected in any of the vessels. Sulphate reduction was confined to the uppermost vessel (vessel 1) during acclimatisation, as indicated by the zinc acetate trap. Ammonia concentrations remained constant in all the vessels. As indicated by the barium hydroxide trap, the bulk of carbon dioxide was produced in the uppermost vessels where acetogenesis was the dominant process and the concentrations of volatile fatty acids were the greatest. Methane production was greatest from vessels 3 and 4, although small amounts of methane could still be measured from vessels 1 and 2 despite regular cleaning of the sides and shaking of the vessels. The rate of methane production in the uppermost vessel, vessel 1, was variable and influenced by cleaning and shaking. Bacterial growth in vessels 1 and 2 was considerable. Layers of this

biomass became visible after two days at the bottom of the vessels after the emptying of vessels 1 and 2 on two separate occasions prior to acclimatisation. This would have been the most likely habitat for the methanogens and methanogenic associations in these vessels. The complete removal of methanogenesis from the upper vessels may have been achieved by continuous stirring and the use of a less energy rich substrate in the influent.

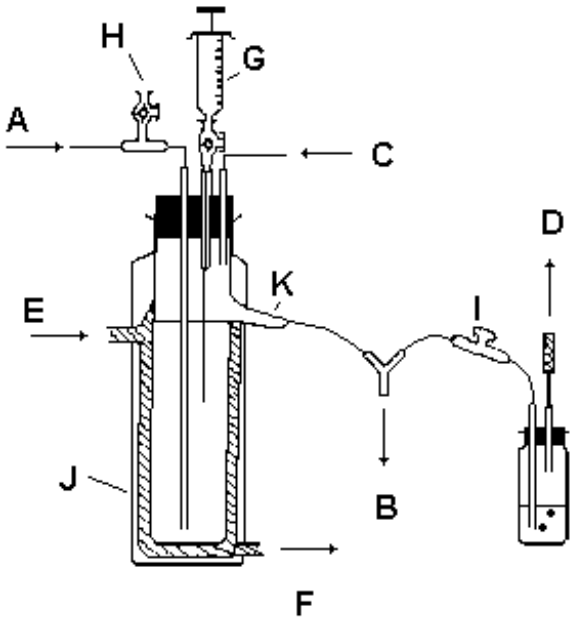


Figure 8.1 One vessel from the four stage continuous culture system. (A) Vessel influent; (B) Vessel effluent to subsequent vessel or effluent reservoir; (C) Gas inlet; (D) Gas outlet from 1% w/v zinc acetate trap; (E) Water jacket inlet; (F) Water jacket outlet; (G) Syringe sample port; (H) Tubing sample port; (I) Suba-Seal gas sampling port; (J) Vessel wall and cover; (K) Weir overflow.

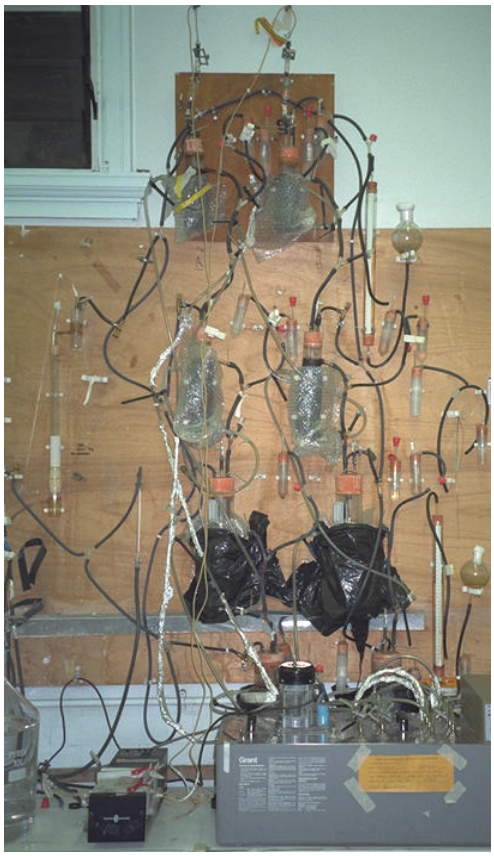


Figure 8.2 Two four stage continuous culture systems, a control array and an array used for perturbation assessment.

8.3.3 Assessment of Nitrate Perturbations on the Methanogenic Decomposition of Cellobiose

To measure the effect of nitrate on the methanogenic degradation of cellobiose nitrate perturbations, both pulsed and continuous, were made to one of the multi-stage arrays and compared to the unadjusted array.

Pulsed Nitrate Perturbations

Nitrate perturbations were made to the bottom vessel first and then up the multi-stage array from bottom to top. An addition of nitrate (1 ml) dissolved in cellobiose and sulphate free medium was made by syringe to bring the concentration of nitrate in the vessel to 4 mM (approximately 57 mg NO_3^--N l^{-1}). The addition was made to each of the vessels in turn, starting from the bottom up. The vessel was shaken prior to the addition of nitrate to remove the bubbles of gas collected on the sides of the vessel. The methane production rate was measured before and after addition and the volatile fatty acid concentrations, pH value and redox potential measured. To the control array an identical amount of nitrate-free, cellobiose-free and sulphate-free medium was made, reproducing the syringe inoculation.

The Effect of Nitrate Addition to the Multi-stage Cultures

Since the denitrification of nitrate to nitrogen gas is an extremely rapid process it was considered that in addition to introducing to vessels pulses of nitrate, which would be denitrified and diluted from the vessels in a number of hours, a continuous feed of nitrate should be made to the influent (thus maintaining the concentration of nitrate in the vessel) but at a limited rate so as not to increase the dilution rate significantly. A steady increase in the amount of nitrate entering each vessel would give an indication of the effects of nitrate on the cultures in each vessel and an indication of the carbon substrates used during denitrification.

The nitrate dissolved in cellobiose and sulphate free mineral medium was added to the vessel by a separate pump whilst the influent from the preceding vessel or the medium reservoir remained constant. To compensate for increases in the rate of denitrification in the vessels over time the nitrate concentration in the feed was increased whilst the overall dilution rate remained the same. The amount of nitrate added to the vessel was steadily increased thus subjecting the culture to increased nitrate availability. To the corresponding vessel in the multi-stage array not receiving nitrate was pumped cellobiose and sulphate free mineral medium at the same rate (<1 ml h^{-1}). By monitoring the concentration of nitrate in the vessels using a Kent nitrate electrode the appropriate influent nitrate concentrations could be prepared and the rate of denitrification by the bacterial populations in the vessel calculated.

Array 1 (used for nitrate perturbations)	Array 2 (control)
Vessel 1 (top)	Vessel 1 (top)
pH 5.39 (0.215) redox potential -222 (16.7) mV 7.34 (1.21) µmol min^{-1} methane	pH 5.41 (0.212) redox potential -221 (24.7) mV 7.45 (3.12) µmol min^{-1} methane
Vessel 2	Vessel 2
pH 5.74 (0.120) redox potential -189 (31.7) mV 5.11 (0.793) µmol min^{-1} methane	pH 5.84 (0.113) redox potential -179 (35.3) mV 8.25 (4.39) µmol min^{-1} methane
Vessel 3	Vessel 3
pH 6.37 (0.095) redox potential -181 (30.5) mV 12.39 (4.72) µmol min^{-1}methane	pH 6.50 (0.117) redox potential -186 (41.1) mV 12.35 (5.28) µmol min^{-1}methane
Vessel 4 (bottom)	Vessel 4 (bottom)
pH 6.86 (0.118) redox potential -202 (24.4) mV 9.80 (1.24) µmol min^{-1} methane	pH 6.93 (0.132) redox potential -205 (38.2) mV 9.10 (1.26) µmol min^{-1} methane

Figure 8.3 The mean pH value, redox potential and methane production rate of the acclimatised culture vessels at steady state. Values enclosed by parentheses represent the standard deviation of the mean.

Array 1 (used for nitrate perturbations)	Array 2 (control)
Vessel 1 (top)	Vessel 1 (top)
12.24 (1.85) mM acetate 1.03 (0.228) mM propionate 2.89 (0.433) mM butyrate	12.11 (2.09) mM acetate 1.00 (0.209) mM propionate 3.45 (0.560) mM butyrate
Vessel 2	Vessel 2
10.74 (0.842) mM acetate 0.945 (0.0980) mM propionate 2.43 (0.374) mM butyrate	10.44 (1.76) mM acetate 0.818 (0.250) mM propionate 2.63 (0.905) mM butyrate
Vessel 3	Vessel 3
6.52 (0.727) mM acetate 0.852 (0.146) mM propionate 0.409 (0.267) mM butyrate	6.29 (1.629) mM acetate 0.779 (0.111) mM propionate 0.347 (0.173) mM butyrate
Vessel 4 (bottom)	Vessel 4 (bottom)
3.01 (0.763) mM acetate 0.676 (0.155) mM propionate 0.0273 (0.0236) mM butyrate	2.55 (0.674) mM acetate 0.600 (0.0878) mM propionate 0.0100 (0.00782) mM butyrate

Figure 8.4 The mean volatile fatty acid concentrations of the acclimatised vessels at steady state. Values enclosed by parentheses represent the standard deviation of the mean.

8.4 Results

8.4.1 The Effect of Pulsed Nitrate Perturbations on Me thanogenesis

The analysed parameters in the control array remained constant throughout the addition of the mineral medium allowing comparisons with the nitrate adjusted array.

Methanogenesis was severely inhibited by concentrations of 4 mM (57 mg NO_3^--N l^{-1}) in each of the culture vessels. Methanogenesis was completely inhibited after the addition of nitrate although the measured redox potential did not significantly change. No sulphate was detected in any of the vessels after 3 h. Methanogenesis had recovered in all the adjusted vessels when tested after one day. Sulphate reduction was confined to the uppermost vessels during nitrate

perturbations.

8.4.2 The Effect of Continuous Nitrate Addition to the Multi -stage Cultures

Initially, additions of nitrate to 0.4 mM (5.7 mg NO_3^--N l^{-1}) were only slightly inhibitory to methanogenesis. The cultures in vessels 1 and 2 showed relatively little response to nitrate additions and redox potential and methane production remained relatively constant (Figure 8.5, 8.6, 8.9 and 8.10). Nitrate was assumed to be removed by the process of denitrification (Figure 8.8 and 8.12). There was little change in the volatile fatty acid concentrations during the addition of nitrate (Figure 8.7 and 8.11). Continued supply of nitrate to the lower culture vessels 3 and 4 and maintenance of the measured concentration at 0.4 mM initiated denitrification which led to an increase in the pH values and redox potential (Figure 8.13, 8.17). Methanogenesis was gradually inhibited (Figure 8.14 and 8.18). Propionate concentrations within vessel 4 became exhausted, probably due to denitrification (Figure 8.19). This was repeated in culture vessel 3 after a reduction in butyrate concentrations which may have indicated the preferential denitrification of longer chain volatile fatty acids by denitrifying bacteria in the culture (Figure 8.15).

The effect of the addition of 0.4 mM (5.7 mg NO_3^--N l^{-1}) nitrate to culture vessel 2 was limited in comparison to the cultures in vessels 3 and 4. Limited denitrification was observed (Figure 8.8) but methane production was significantly lower than in vessels 3 and 4 and fell sharply as a result of the addition of nitrate. The pH of the culture consequently decreased from 5.5 to 5.3 despite denitrification in the culture. Methanogenesis was almost completely inhibited after half a culture volume change (5.66 h). Without significant methanogenesis and denitrification, the two processes that can lead to a net increase in pH value, the culture became more acidic and the pH value fell slightly owing to acetogenesis carried out in the culture and the preceding culture vessel. There was little increase in the rate of denitrification whilst nitrate additions were made to the culture in vessel 2 and it appeared that without methanogenesis the culture had ‘soured’, the acidic pH value contributing to the limited denitrification. Longer periods may have led to increases in denitrification and the pH value of the culture. The concentrations of volatile fatty acids were high (Figure 8.11), comparable to those from the culture in vessel 1 (Figure 8.7). This, and the acidic pH values measured, would have made the culture highly buffered and resistant to increases in the pH value by alkaline producing processes such as methanogenesis and denitrification. The acidic pH values of the nitrate adjusted culture were close to the pKa values for the volatile fatty acids analysed and their greatest buffering potential. The lower pH values and the inhibitory effect of the volatile fatty acids may have contributed to slow overall growth and activity as the bacterial cells attempted to regulate their internal pH values .

Faster rates of denitrification were observed in the culture in vessel 1 whose pH value increased after four culture volume changes (Figure 8.5). Net concentrations of volatile fatty acids did not change (Figure 8.7). Since cellobiose is entering vessel 1 it would be the most likely candidate for a carbon substrate for denitrification, owing to the additional energy the compound would provide for growth. The process of acetogenesis was unaffected by the presence of 0.4 mM (5.7 mg NO_3^--N l^{-1}) in vessels 1 and 2. Sulphate reduction remained localised in the vessel 1 culture, and no sulphate was detected prior to or after the addition of nitrate. The process of sulphate reduction was unaffected by the presence of 0.4 mM (5.7 mg NO_3^--N l^{-1}) in the vessel 1 culture.

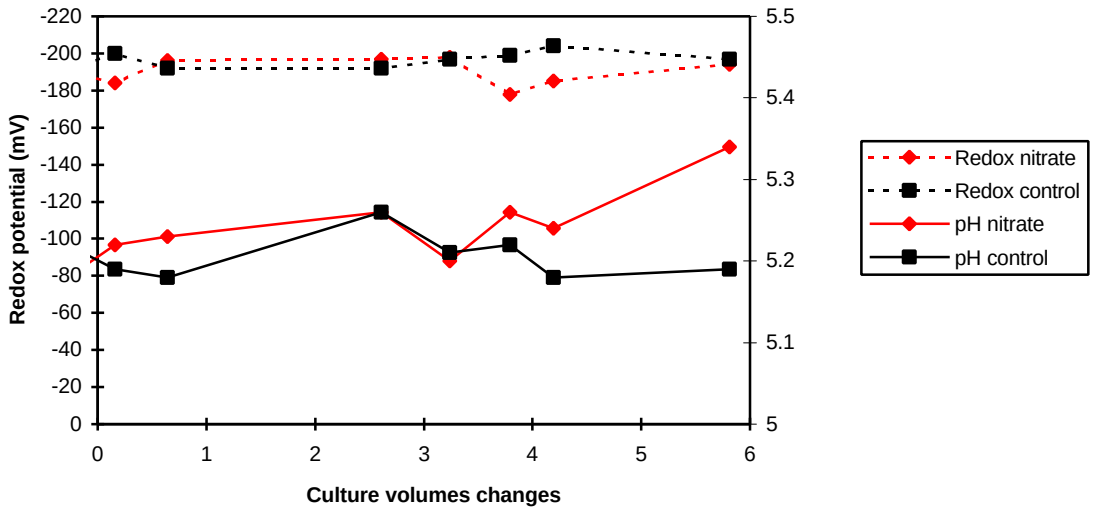


Figure 8.5 The redox potential and pH value of the cultures within vessel 1 of the nitrate treated (◆) and control (■) four stage arrays.

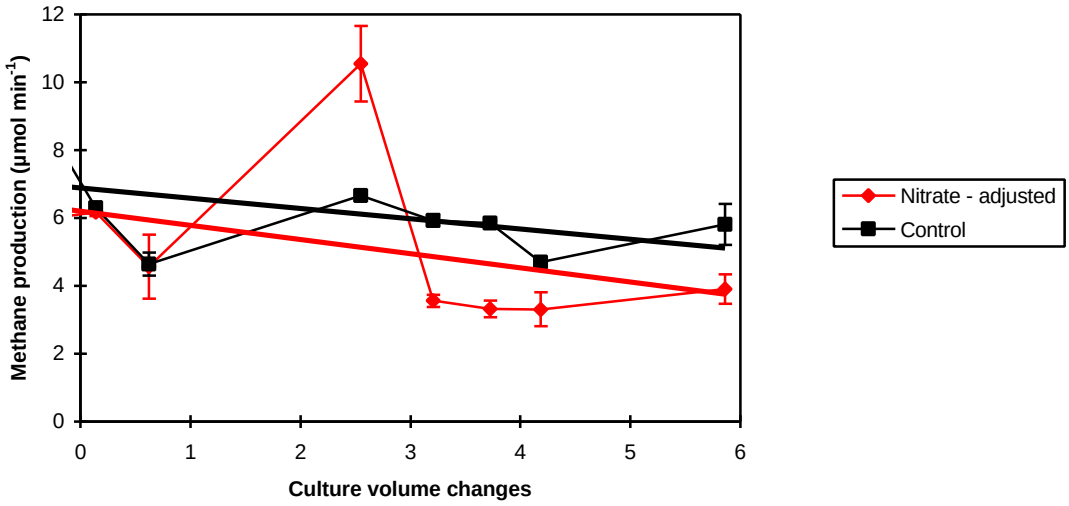


Figure 8.6 The methane production by cultures within vessel 1 of the nitrate treated (◆) and control (■) four stage arrays. Error bars represent the standard deviation of the mean. Linear regression performed by the Microsoft Excel 95 ™ computer programme.

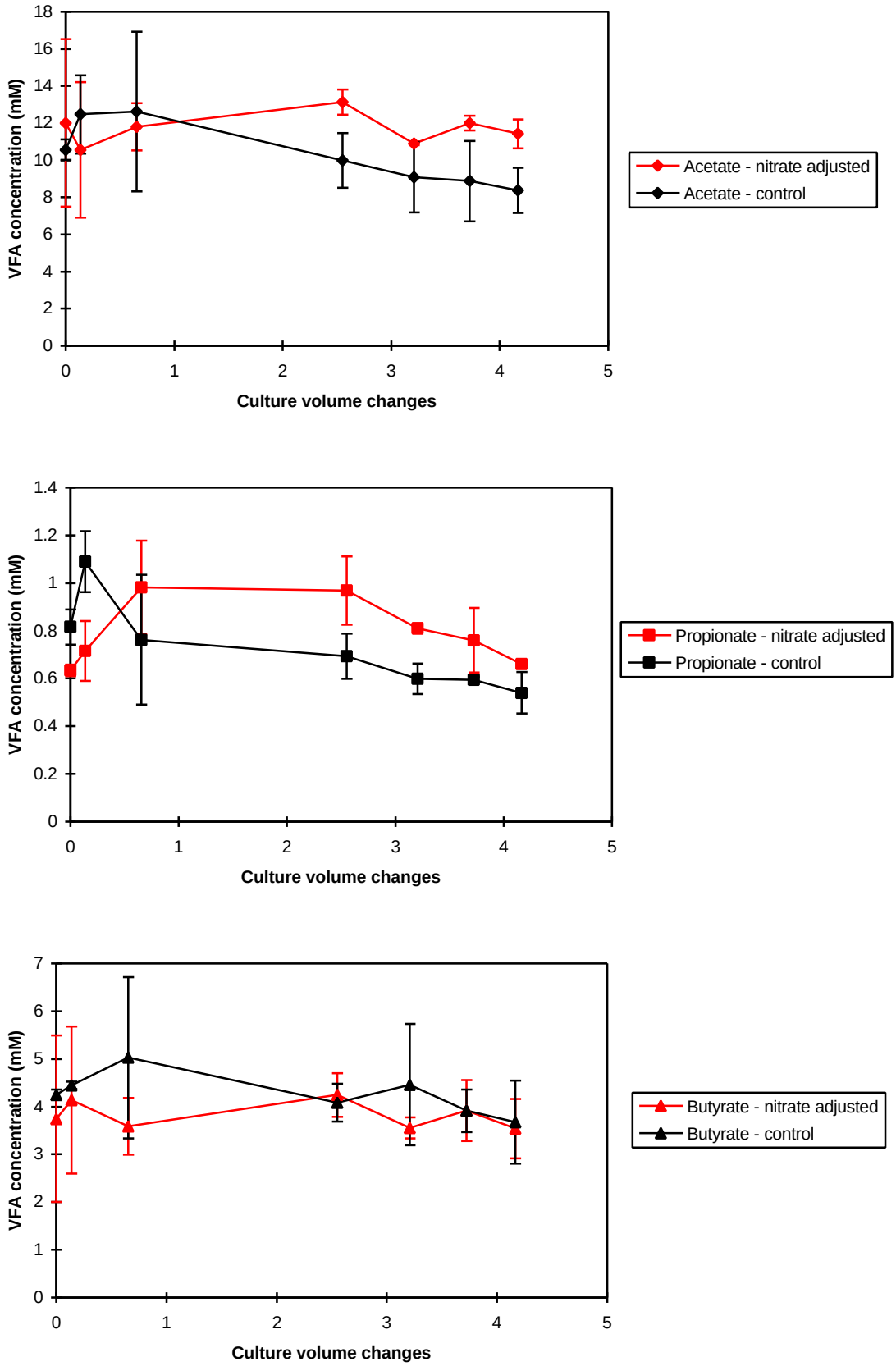


Figure 8.7 The acetate, propionate and butyrate concentrations within vessel 1 of the nitrate treated (◆■▲) and control (◆■▲) four stage arrays.

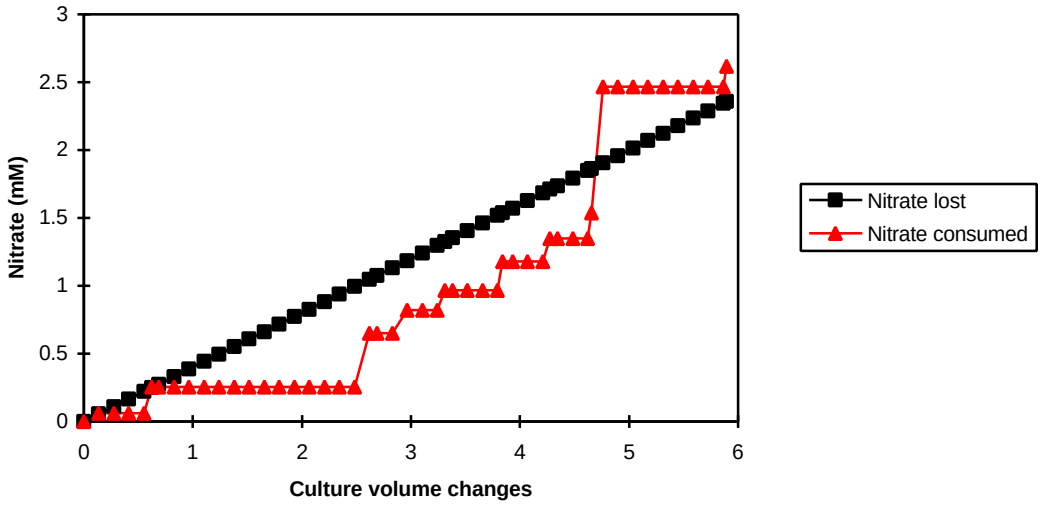


Figure 8.8 Nitrate consumption (▲) by the culture within vessel 1 of the nitrate treated four stage array and the loss of nitrate in the effluent (■) from the culture.

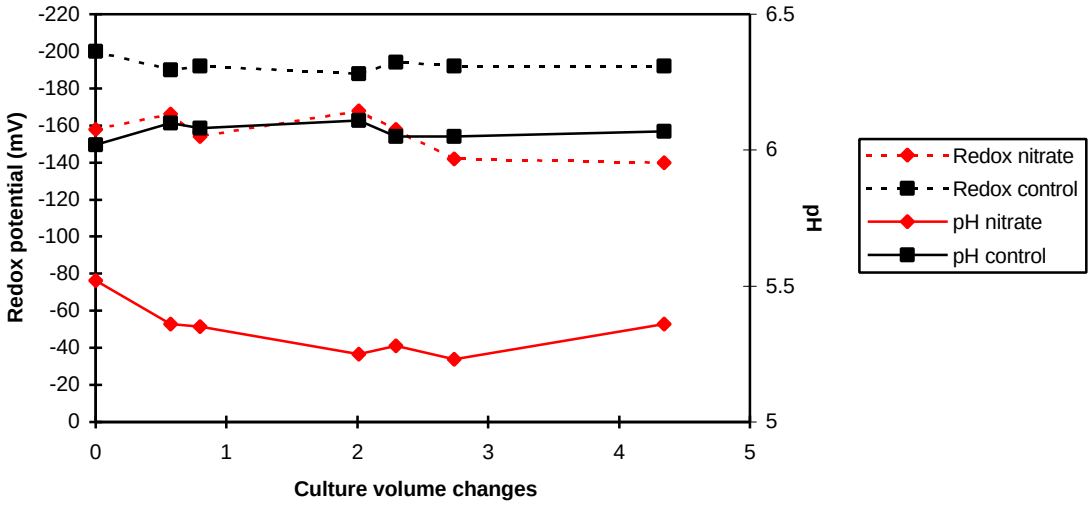


Figure 8.9 The redox potential and pH value of the cultures within vessel 2 of the nitrate treated (◆) and control (■) four stage arrays.

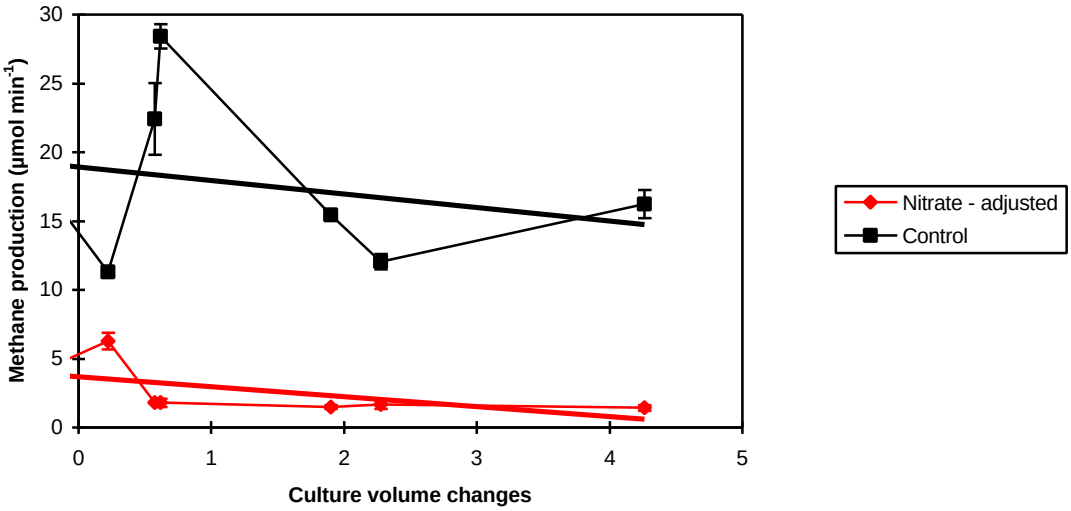


Figure 8.10 The methane production by cultures within vessel 2 of the nitrate treated (◆) and control (■) four stage arrays. Error bars represent the standard deviation of the mean. Linear regression performed by the Microsoft Excel 95 ™ computer programme.

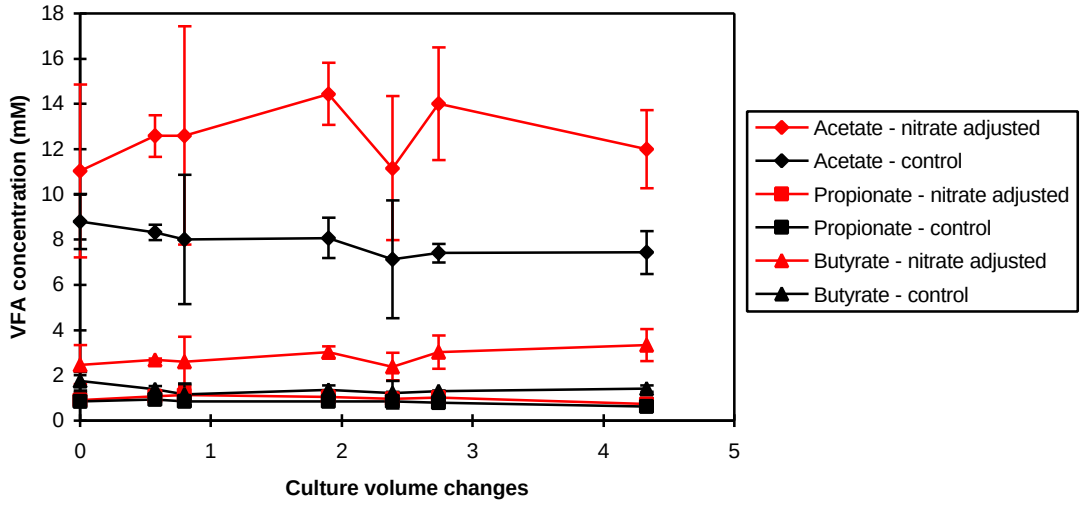


Figure 8.11 The acetate, propionate and butyrate concentrations within vessel 2 of the nitrate treated (◆■▲) and control (◆■▲) four stage arrays.

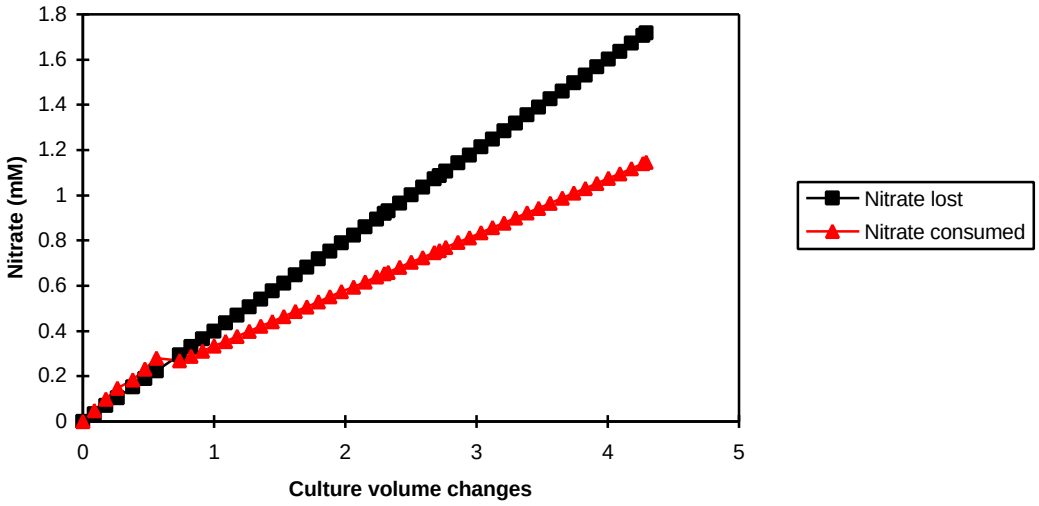


Figure 8.12 Nitrate consumption (▲) by the culture within vessel 2 of the nitrate treated four stage array and the loss of nitrate in the effluent (■) from the culture.

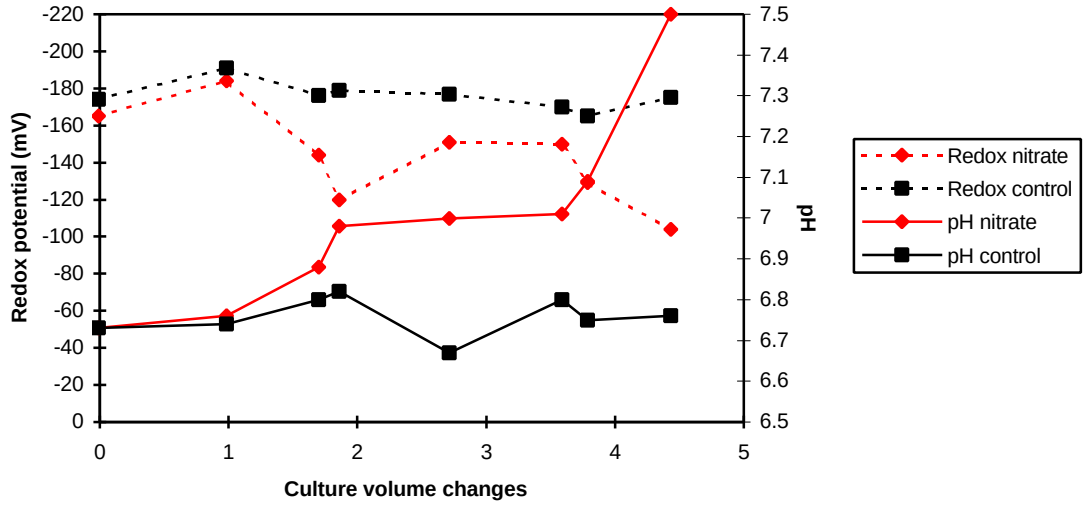


Figure 8.13 The redox potential and pH value of the cultures within vessel 3 of the nitrate treated (◆) and control (■) four stage arrays.

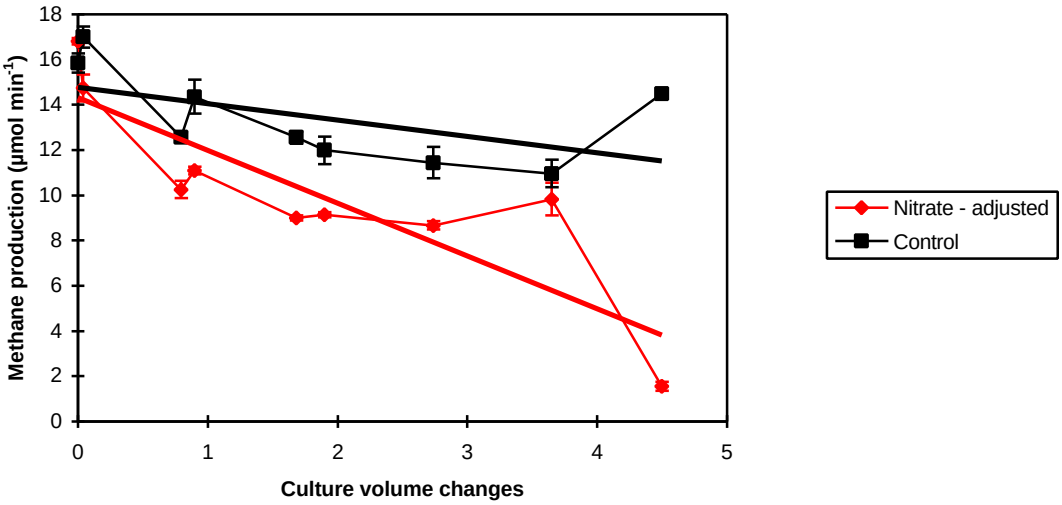


Figure 8.14 The methane production by cultures within vessel 3 of the nitrate treated (◆) and control (■) four stage arrays. Error bars represent the standard deviation of the mean. Linear regression performed by the Microsoft Excel 95 ™ computer programme.

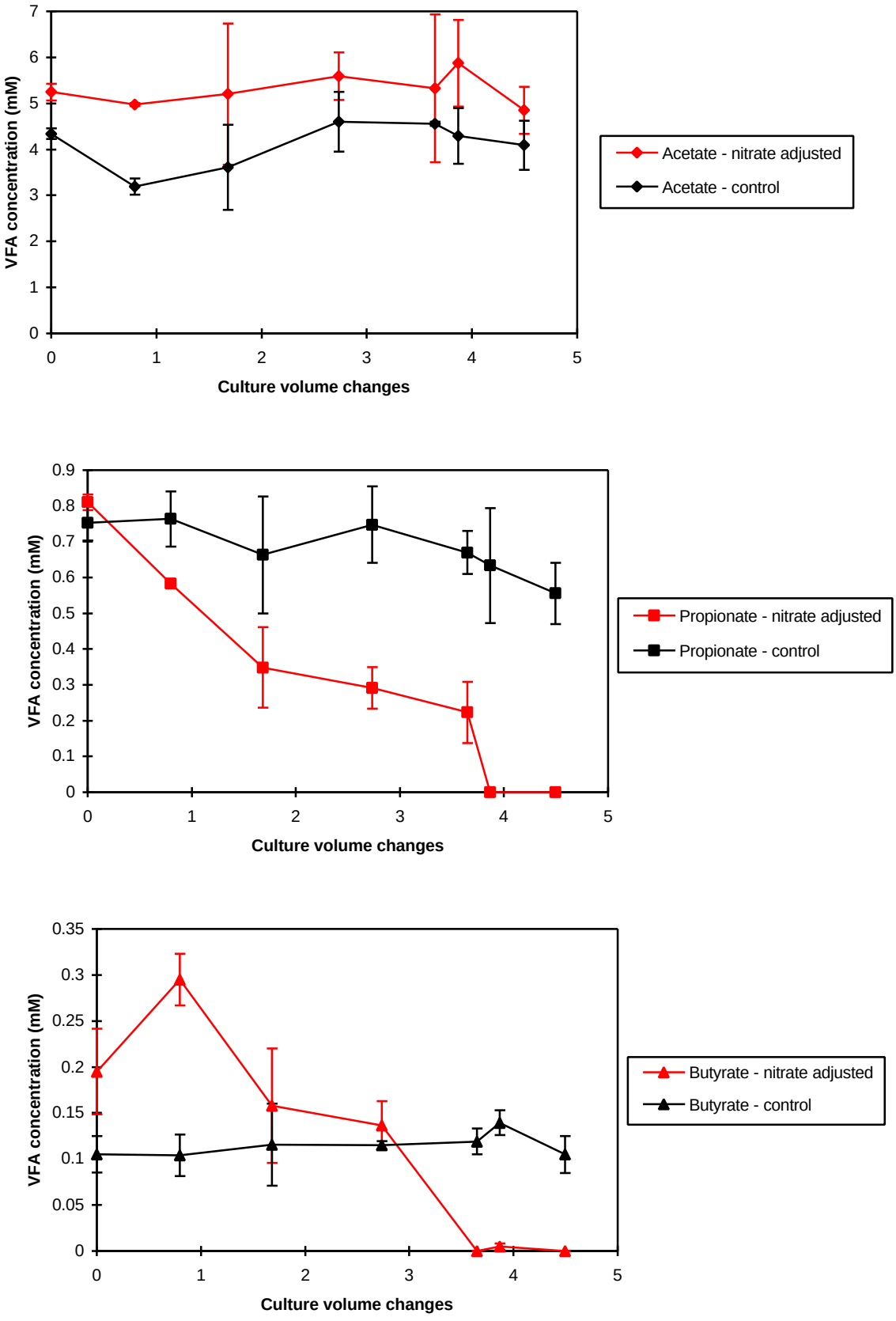


Figure 8.15 The acetate, propionate and butyrate concentrations within vessel 3 of the nitrate treated (◆■▲) and control (◆■▲) four stage arrays.

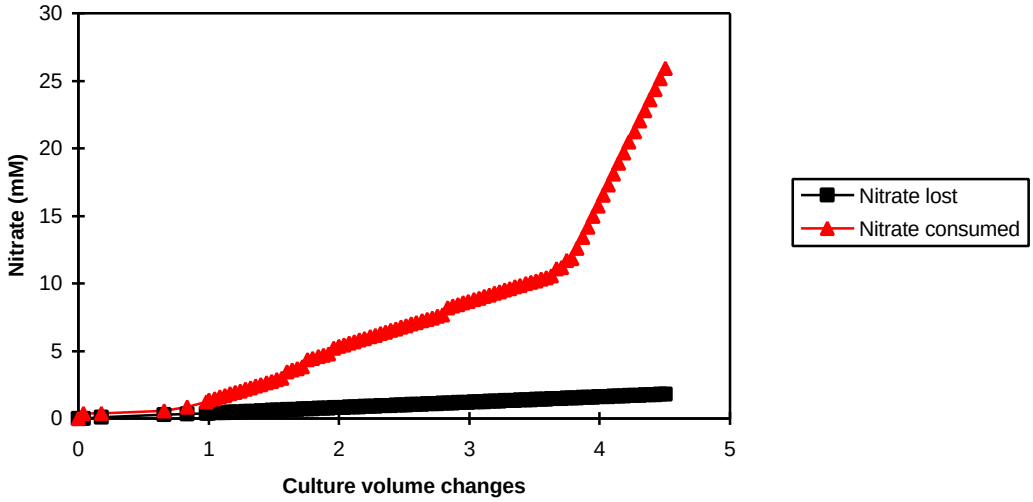


Figure 8.16 Nitrate consumption (▲) by the culture within vessel 3 of the nitrate treated four stage array and the loss of nitrate in the effluent (■) from the culture.

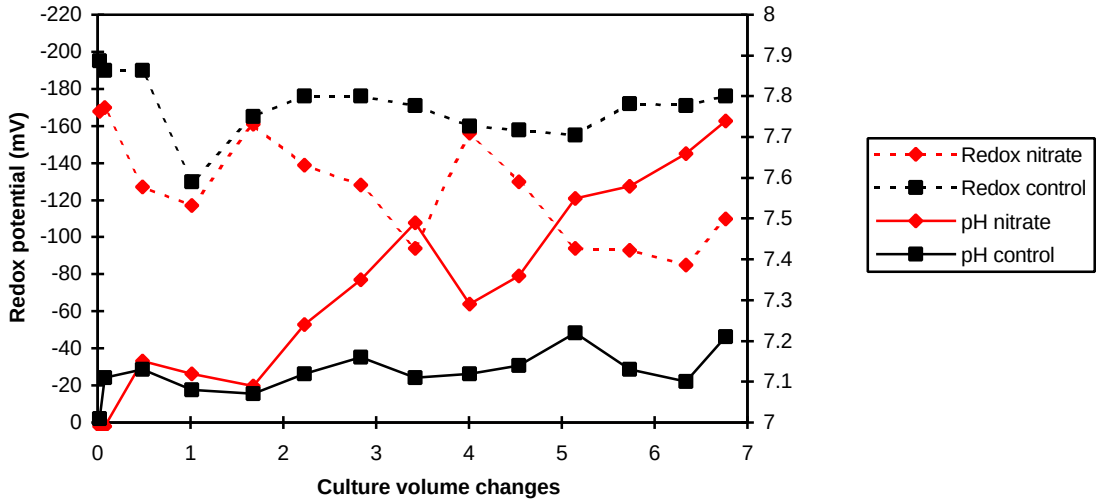


Figure 8.17 The redox potential and pH value of the cultures within vessel 4 of the nitrate treated (◆) and control (■) four stage arrays.

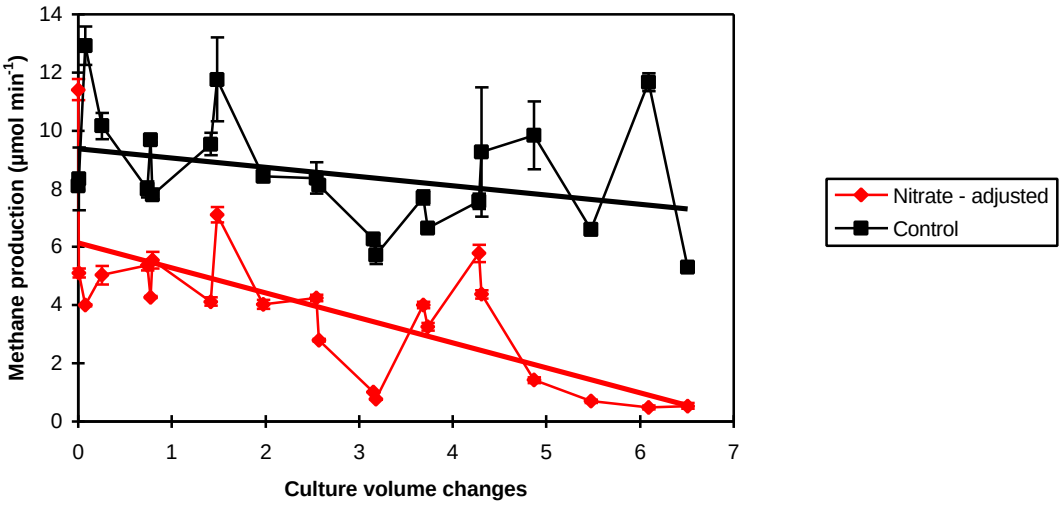


Figure 8.18 The methane production by cultures within vessel 4 of the nitrate treated (◆) and control (■) four stage arrays. Error bars represent the standard deviation of the mean. Linear regression performed by the Microsoft Excel 95 ™ computer programme.

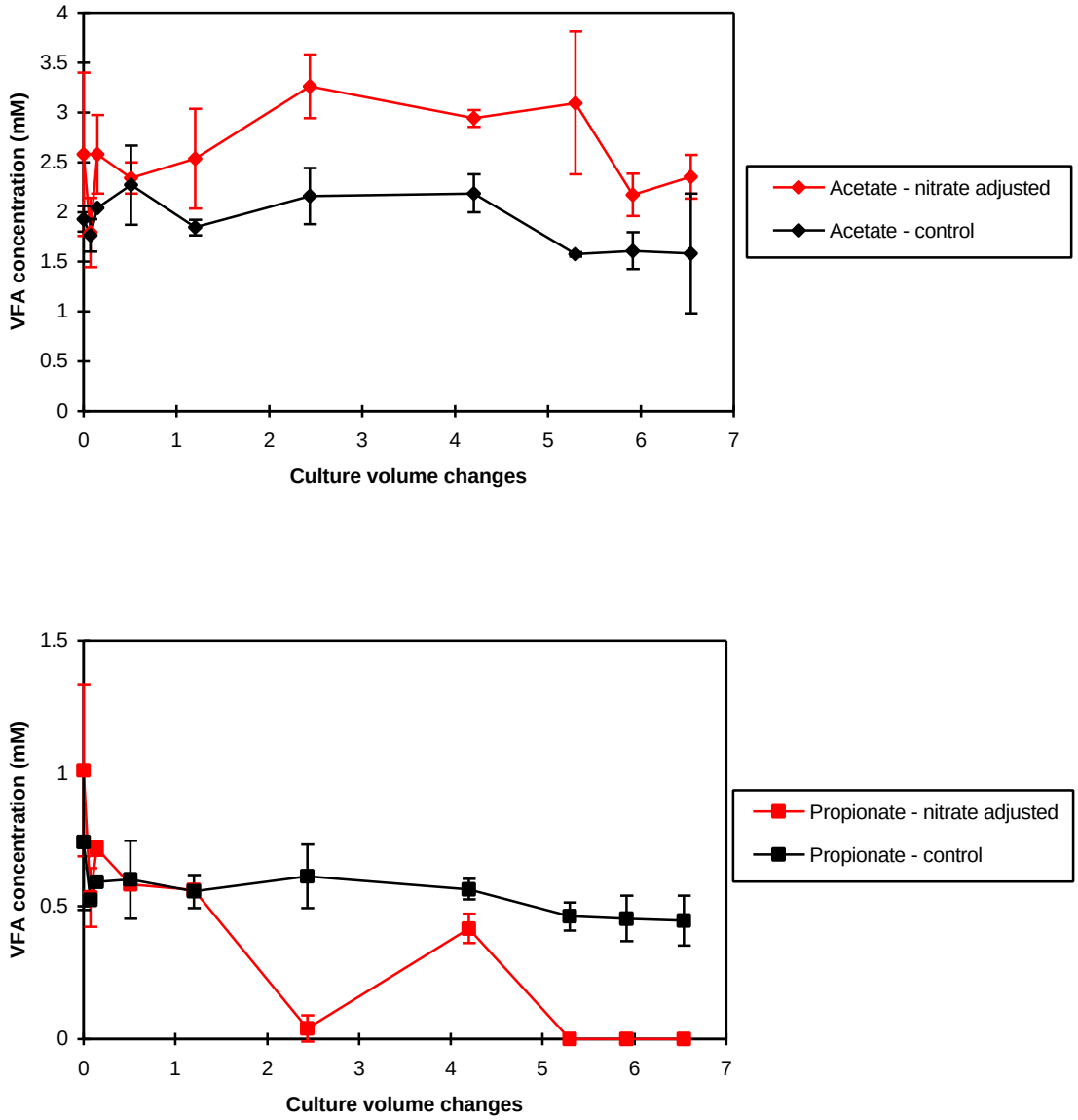


Figure 8.19 The acetate and propionate concentrations within vessel 4 of the nitrate treated (◆■) and control (◆■) four stage arrays.

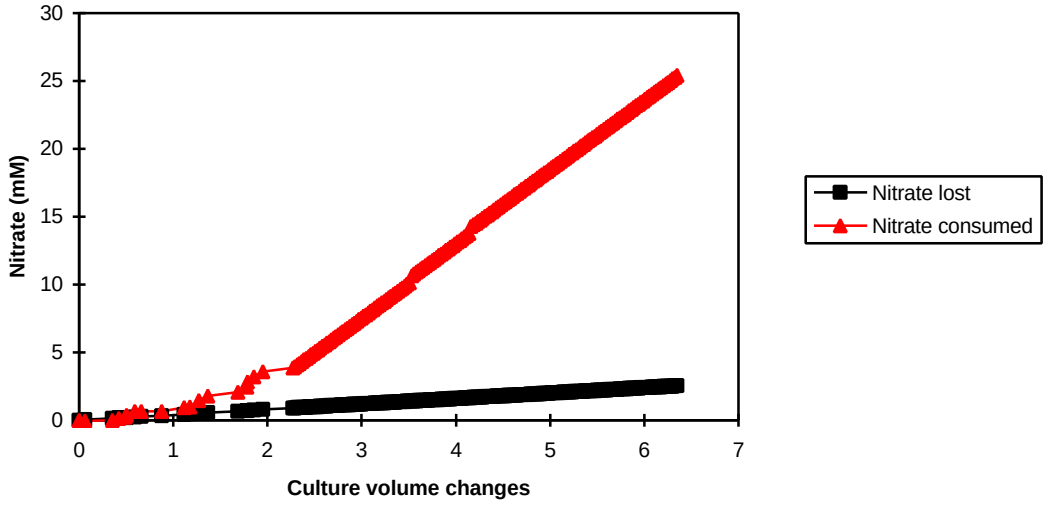


Figure 8.20 Nitrate consumption (▲) by the culture within vessel 4 of the nitrate treated four stage array and the loss of nitrate in the effluent (■) from the culture.

8.5 Discussion

The pulsed addition of 4 mM nitrate (57 mg NO_3^--N l^{-1}) to the cultures inhibited methanogenesis in all of the vessels tested. The inhibition was caused by the properties of nitrate and not by changes in the redox potential or the consumption of acetate since these remained constant immediately after the addition. Methanogenesis recovered in the cultures quite quickly, since a day after the additions were made the cultures were producing methane at similar levels to the unadjusted controls and at rates identical to those prior to the addition of nitrate. Should nitrate be added during the recirculation of nitrified leachate then additions at approximately these concentrations would perhaps allow denitrification in the landfill without long-term inhibition of methanogenesis. Indeed batch cultures provided with 50 mg NO_3^--N l^{-1} started producing methane a day after water controls and achieved complete removal of nitrate in only 3 days (Figure 6.7). From experiments (Chapter 5) when additions of nitrate 500 to 1000 mg NO_3^--N l^{-1} were made during methanogenesis it was possible to say that these concentrations were toxic to the methanogens and methanogenic associations in the batch cultures whereas a concentration of 57mg NO_3^--N l^{-1} was inhibitory to the four stage cultures.

The ability of cultures in the four stage array to remain methanogenic in the presence of 0.4 mM (5.7 mg NO_3^--N l^{-1}) was unexpected because of the liquid environment and the diffuse growth of the bacteria. Methanogens in the vessel cultures were resistant to nitrate at these concentrations. Wall growth and the protection from nitrate by bacterial slime layers and surfaces would have played a part in this resistance but the cultures were regularly mixed and the sides scraped clear of bacterial biofilms so it seems likely that diffusely growing methanogens can maintain their activity in these conditions. In the upper culture vessels (1 and 2) growth in the collecting sediments must have been the only habitat for the methanogens or methanogenic associations since the dilution rates were high enough to

displace the slow growing methanogens and methanogenic associations to the lower vessels (Sinclair, 1994).

A search of the literature regarding anaerobic digester systems revealed relatively little work on combining methanogenesis and denitrification. Some workers suggest that it is not just a change in redox potential that inhibit methanogenesis but that nitrate itself may cause inhibition (Chen & Lin, 1993). Direct inhibition by nitrogen oxides was shown in pure cultures of methanogens and salt marsh sediments by Balderston & Payne (1976) but without an investigation of the effect of redox potential. In sediments, inhibition was achieved with 1 and 10 mM nitrate concentrations. Methanogenesis recovered after 4 days after the addition of 1 mM nitrate but was inhibited for a further 6 days after addition of 10 mM nitrate only to recover and produce significantly lower concentrations of methane. Inhibition was also observed in a number of pure cultures of methanogens.

One reason of the lack of interest shown in combining methanogenesis and denitrification is that it has been taken for granted that methanogenesis will not proceed in a denitrifying reactor because the process of denitrification proceeds at much higher redox potentials in the presence of nitrate than are tolerated by methanogens. The extra energy afforded to bacteria by the process of denitrification will also enable them to out compete other bacteria for carbon substrates in a nitrate rich environment. These bacteria will include acetoclastic methanogens which use acetate and acetogenic bacteria which breakdown volatile fatty acids and supply hydrogen to methanogens. In the highly reduced environment of a landfill the redox potentials are significantly lower and it is possible that both methanogenesis and denitrification could proceed at once provided that the carbon and hydrogen requirements of methanogenesis and the methanogenic associations are not removed by the denitrification process nor inhibitory nitrate concentrations of approximately 4 mM (57 mg NO_3^--N l^{-1}) nitrate allowed to accumulate. A decrease in the carbon sources available to methanogenesis in the lower vessels was observed after nitrate was continually supplied to the vessels 3 and 4. A nitrate limiting environment would therefore suit combined denitrification and methanogenesis provided the aqueous concentrations of nitrate were never supplied at or above 4 mM (57 mg NO_3^--N l^{-1}) when methanogenesis would be almost completely inhibited by nitrate. Since landfill refuse is relatively impermeable compared to the four stage systems it seems likely that the processes could segregate allowing zones of methanogenesis and zones of denitrification. The lower the concentrations of nitrate added the more rapidly a denitrifying zone would return to a methanogenic zone.

The limited work reported in the scientific literature on nitrate inhibition suggest that other nitrogen oxides may be more inhibitory than nitrate. In sediments nitrous oxide was the strongest inhibitor followed by nitric oxide, nitrite and nitrate (Balderston & Payne,1976). The intimate association of the bacteria involved in anaerobic decomposition with the nitrogen oxides produced during denitrification may be important in inhibiting methanogenesis. Concentrations of nitrite were not measured in batch culture studies on refuse decomposition in the presence of nitrate at 500 and 1000 mg NO_3^--N l^{-1} (Chapter 5), but if subjected to a sudden pulse of nitrate it is possible for some intermediates to build up. Very high concentrations of nitrate (3000 and 5000 mg NO_3^--N l^{-1}) produced detectable amounts of nitrite (1-2 mg NO_3^--N l^{-1}) in batch cultures (Chapter 5). Thus the oxides of nitrogen may have contributed to some inhibition of the methanogenic associations as well as the nitrate added to the vessel cultures.

It seems likely that decreases in the cellobiose available to the acetogenic bacteria in vessel 1 led to reductions in the activity of acetogens and the amount of volatile fatty acids produced. This could have been brought about by increased denitrification of cellobiose. Since no reductions in volatile fatty acid concentrations were observed more nitrate may have been required to have been denitrified in vessel 1 before reductions in the volatile fatty acids were observed. The energy released from the denitrification of cellobiose is significantly greater than that from acetate because of the size and potential energy yield of cellobiose compared to the acetate, propionate and butyrate present in the vessel 1 culture. The denitrification of cellobiose to carbon dioxide and N_2 will require considerably more nitrate than the volatile fatty acids present in the vessel 1 and 5 mM cellobiose was available to the denitrifying bacteria. Significant amounts of cellobiose will have to be oxidised and therefore nitrate denitrified before it impinges on acetogenesis. The quality and availability of substrate for denitrification may therefore be important in determining the effect of denitrification on the other anaerobic processes.

Although denitrification occurred in vessel 1 of the nitrate adjusted arrays the cellobiose concentration (5 mM), in terms of potential oxidisable equivalents available to the denitrifying bacteria, was considerable. Denitrification was not able to significantly affect acetogenesis and the quantities of volatile fatty acids produced. Longer periods of nitrate addition and denitrification would have been required to affect the concentrations of volatile fatty acids present. The substrate availability in the lower vessels was more restricted and denitrification of volatile fatty acids was observed. With this in mind the addition of nitrate to a landfill will not necessarily affect the concentrations of fatty acids in the leachate because there is the opportunity for denitrification to oxidise larger and more energy rich compounds in the refuse. This was observed in batch culture studies where nitrate addition brought about only small decreases in the concentrations of volatile fatty acids.

8.6 Conclusions

- Pulsed additions of nitrate at 4 mM (57 mg NO_3^--N l^{-1}) severely inhibited the process of methanogenesis. Although initially inhibited, methanogenesis in the cultures quickly recovered following denitrification of nitrate, indicating that the methanogens remained viable within the culture. The inhibition of methanogenesis by nitrate appeared to be a result of the presence of the nitrate ion since the redox potential remained unchanged after the addition of nitrate.

- Methanogenesis and denitrification coexisted at continuous added nitrate concentrations of 0.4 mM (5.7 mg NO_3^--N l^{-1}) with relatively little inhibition of methanogenesis.

- Low concentrations of nitrate were only slightly inhibitory to methanogenesis. Continued increases in the supply of nitrate to methanogenic cultures led eventually to strong inhibition of methanogenesis, an increase in pH and an increase in the redox potential. The continued supply of nitrate to the lower two culture vessels lead to a reduction in volatile fatty acid concentrations as denitrification began to use these compounds as a carbon supply.

- There appeared to be preferential use of longer chain volatile fatty acids by denitrifying bacteria within the cultures. Propionate and butyrate were used as a carbon source in preference to acetate.

9. FINAL CONCLUSIONS

9.1 Nitrogen Balances in Landfills

Interviews were carried out with local landfill operators and representatives of the landfilling industry to determine their views pertaining to landfill nitrogen balances. So little was know about the nitrogenous components of refuse, the source of ammonia in the leachate and the expected amounts produced during decomposition that any research into this area was extremely welcome. The literature search revealed little information on the nitrogen content of landfill refuse despite the importance of ammonia as a landfill pollutant (Burton & Watson-Caik, 1996b). The first experiments in the study were chosen to satisfy this requirement.

The nitrogen content of landfilled refuse was analysed during decomposition. The proportion of free ammonia-N compared to the total organic nitrogenous pool (3.86% N dry weight), the source of ammonia, was relatively small, between 5% to 10%, indicating that by the end of the active phase of ammonia production the bulk of ammonia was retained in the refuse and only a small proportion (5%) was released into the leachate during anaerobic decomposition. Nitrate and nitrite were not found in the refuse studied and presumably have no role in the mineralisation of landfilled refuse.

One of the objectives of the project was to suggest suitable methods of landfill management likely to lead to the rapid and permanent reduction of the ammonia content of landfill leachate. Some operators were cautious about any changes to their treatment or landfilling regimes. Without any perceived problems or impending legislation there was no impetus to apply strategies to accelerate the decomposition of landfill refuse. However, some operators were receptive to possible recirculation and ammonia treatment strategies and welcomed suggestions which involved simple adjustments to their procedures which could accelerate refuse decomposition. In order to keep the present study of relevance to landfill operators simple low cost methods of accelerating refuse stabilisation were considered during the interviews. One low cost option considered was nitrifying the ammonia in leachate by aeration in lagoons and pumping the nitrate containing leachate back to the landfill where it would be anaerobically denitrified to nitrogen gas and lost to the atmosphere, thus removing the need and cost to treat nitrate in the leachate anaerobically. Aerating the leachate would also allow some treatment of residual organic compounds which do not decompose anaerobically in the landfill. The bacterial process of denitrification may also promote the decomposition of organic components when nitrate is supplied to the landfill. Recirculation of leachate will accelerate the decomposition, keep the refuse moist, assist in the solubilisation of the degradable components of refuse and promote flushing of toxic components from the refuse.

The wet/flushing bioreactor strategies discussed by Harris *et al.* (1994) require a considerable change in the way refuse is landfilled, and recirculating nitrified leachate may also have a role to play in accelerating refuse decomposition in these systems. Research into the fate of nitrate during refuse decomposition, the process of denitrification and the possible effects of this strategy may have on the decomposition are of relevance both to current and future landfill processes. The effect and fate of nitrate in refuse during decomposition was therefore chosen as an important part of the research in this study. High ammonia concentrations may also affect the decomposition processes and these were also studied.

9.2 The Effect of Nitrate on Methanogenesis

If a strategy of recirculating treated landfill leachate is adopted then the leachate returned to the landfill will contain nitrate, since leachate ammonia taken from the landfill will be converted to nitrate as a result of the bacterial process of nitrification in the lagoon or reactor. The effects of nitrate on the refuse degradation processes prior to this research were poorly understood.

Experiments involving the addition of nitrate to anaerobically degrading refuse were performed. Nitrate (500-1000 mg NO_3^--N l^{-1}) was rapidly lost from refuse cultures such that after a period of 6 days no nitrate was present. The onset of methanogenesis was delayed compared to distilled water controls indicating inhibition of methanogenesis. Refuse at 80% moisture content was capable of quickly removing the nitrate, presumably by denitrification and the release of nitrogen gas. Results suggested that methanogenesis will only recover once the nitrate has been denitrified. No ammonia accumulated as a result of the consumption of nitrate by refuse cultures suggesting that the reduction of nitrate to ammonia was insignificant and will not lead to increasing leachate ammonia concentrations if recirculation of nitrified leachate is attempted. Concentrations of volatile fatty acids in nitrate treated refuse cultures were similar, albeit with smaller net concentrations of propionate during nitrate consumption.

The addition of nitrate to methanogenic cultures inhibited further methane production for 20 days before methanogenesis began to recover. The addition of nitrate to a landfill will inhibit methane production in the short term if applied at similar concentrations (500-1000 mg NO_3^--N l^{-1}) in nitrified or treated landfill leachate. Volatile fatty acid concentrations were higher in the nitrate-treated cultures suggesting that the inhibition of methanogenesis encourages the persistence of volatile fatty acids in solution. Nitrate additions at high concentrations (500-1000 mg NO_3^--N l^{-1}) appeared toxic to the methanogenic associations since methanogenesis took 20 days to recover.

In refuse cultures at 95% moisture content large amounts of nitrate in the form of potassium nitrate, containing 3000 mg NO_3^--N l^{-1} and above, soured the refuse cultures. Methanogenesis was not observed, neither was the nitrate completely removed, probably because of the increases in pH value produced both by the process of denitrification and the presence of potassium. These nitrate concentrations represented the capacity of 7.6 g (dry weight) of refuse to consume nitrate without pH adjustment. They could also represent the limit at which the process of denitrification becomes limited by the concentrations of readily degradable organic components of the refuse. Cultures containing increased phosphate concentrations suggested that methanogenesis was not phosphate limited.

A closer and more sensitive inspection of the reduction of nitrate by refuse, using heavy isotopes, revealed a pathway capable of reducing 4 -7% of nitrate (50 mg NO_3^--N l^{-1}) to ammonia. This reductive pathway from nitrate to ammonia was initially felt to be caused by the anaerobic conditions of landfill refuse and reduction on the surfaces of metals and materials in the refuse. Autoclaving experiments suggested a microbial enzymatic or biochemical process. Greater portions were reduced to ammonia when the indigenous bacteria were destroyed by gamma irradiation, suggesting that this pathway will compete with the denitrification for nitrate.

Experiments on the component microbial groups involved in refuse decomposition, using refuse models, revealed consumption of volatile fatty acids by denitrification. Acetogenic culture vessels showed little volatile fatty acid consumption whereas cultures at neutral pH values showed rapid denitrification and removal of volatile fatty acids. Low levels of nitrate (50 mg NO_3^--N l^{-1}) in keeping with the experiments with heavy isotopes allowed relatively rapid recoveries in methanogenesis. Lower concentrations (5.7 mg NO_3^--N l^{-1})enabled methanogenesis and denitrification to coexist.

The results of experiments with refuse cultures and fermenter systems suggested that concentrations of 500 mg NO_3^--N l^{-1} and above were toxic to methanogenic associations whereas concentrations of 50 mg NO_3^--N l^{-1} were inhibitory, with methanogenesis recovering once the nitrate had been consumed. Methanogenesis and denitrification coexisted when nitrate was supplied at concentrations of 5.7 mg NO_3^--N l^{-1}. Limiting the concentrations of nitrate added to a landfill to 50 mg NO_3^--N l^{-1} would enable methanogenesis to rapidly recover once the nitrate had been consumed. Supplying concentrations of 500 mg NO_3^--N l^{-1} may lead to stratification of the waste and the development of zones of methanogenesis and denitrification.

9.3 Ammonia and Ammonia Toxicity in Landfills

Ammonia is released during the anaerobic decomposition of proteins. No microbial transformations were identified which could significantly reduce the concentrations of ammonia in refuse. Ammonia consequently appears trapped in the refuse. Only by pumping and treating the leachate from the landfill will ammonia concentrations in the landfill be reduced. Significant volatilisation of ammonia appears unlikely as the refuse is generally acetogenic and of a low pH value during emplacement, and capped when methanogenic, and able to volatilise ammonia only at high pH values. The utilisation of the ammonia produced during the decomposition of landfilled refuse appears insignificant. Biomass does not accumulate in anaerobic systems to the extent of aerobic systems. There was no significant increase in the total nitrogen concentrations of refuse perfused by leachate modelling acetogenic leachate (Chapter 7) nor were there significant losses identified in refuse cultures (Chapter 5).

This research report comes soon after the release of the Department of the Environment Review on Landfill Leachate Composition by Mr. Howard Robinson of Aspinwall & Company (DoE, 1995) and provides an opportunity to compare laboratory results with landfill data. Of particular interest to this study was the bank of leachate ammonia data. Concentrations of ammonia present in leachate from 72 United Kingdom landfills were examined. The majority of sites had leachate ammonia concentrations of below 2000 mg $NH_{3/4+}$-N l^{-1}. A summary table is shown (Figure 9.1).

Domestic refuse	Domestic and industrial
462 (SD 413) mg N l^{-1}(total nitrogen) 429 (SD 391) mg $NH_{3/4+}$-N l^{-1} (ammonia) 2.5 (SD 4.1) mg PO_4^{2-} l^{-1} (phosphate) 3.0 (SD 7.4) mg NO_3^--N l^{-1} (nitrate) 0.2 mg (SD 0.3) NO_2^--N l^{-1} (nitrite)	684 (SD 360) mg N l^{-1} (total nitrogen) 635 (SD 316) mg $NH_{3/4+}$-N l^{-1} (ammonia) 4.1 (SD 4.8) mg PO_4^{2-} l^{-1} (phosphate) 1.1 (SD 1.1) mg NO_3^--N l^{-1} (nitrate) 0.2 (SD 0.2) mg NO_2^--N l^{-1} (nitrite)

Figure 9.1 Comparison of the leachate analysed from 30 United Kingdom landfills receiving either domestic refuse or domestic refuse with some industrial waste.
(Calculated from data presented in the Review of the Composition Leachates from Domestic Wastes in Landfill Sites by Mr. Howard Robinson, Aspinwalls & Company (DoE, 1995)).
(SD represents the standard deviation of the mean)

The presence of industrial waste in the refuse was reflected in the ammonia concentrations in the leachate, which were slightly higher. However, taking into consideration the results of experiments where artificially high concentrations of ammonia were maintained (Chapter 5), the ammonia concentrations reported from United Kingdom landfill sites receiving domestic and some industrial waste were not such that the ammonia in the leachate would dramatically inhibit methanogenesis and the decomposition of the waste. Experiments where refuse columns were perfused with leachate containing 500 mg $NH_{3/4+}$-N l^{-1} released only slightly reduced quantities of methane in the leachate (Chapter 7). Many of the landfills receiving either domestic refuse or domestic refuse and some commercial waste had similar concentrations of ammonia in the leachate to the refuse studied in this report (DoE, 1995).

It was observed from the anaerobic decompositon of refuse batch cultures that leachate ammonia concentrations in the leachate could be increased during both preparation of the cultures and the anaerobic decomposition of refuse (Chapter 5). Mixing the refuse and increasing the moisture content of refuse allowed much higher ammonia concentrations to develop compared to liquid squeezed and analysed from sorted refuse at 62% moisture content. Leachate ammonia concentrations also doubled after anaerobic decomposition (Chapter 5). Ammonia has the ability to bind to refuse particles (Chapter 6) and consequently landfill leachate concentrations do not necessarily reflect the ammonia content of the waste. Bound ammonia can also persist within the refuse particles. Only mixing and blending the waste at a high moisture content gives a true representation of the ammonia content. Refuse cations like sodium and potassium can interchange with and thus solubilise the ammonia, bound as NH_4^+ to the negatively charged refuse. However, the perfusion of leachate through refuse did not remove all the ammonia since direct distillation of refuse revealed some ammonia after perfusion (Chapter 7). This binding of ammonia suggests why landfills release ammonia for prolonged periods.

During the acetogenic phase of refuse decomposition there is most probably enhanced leaching due to both the effect of H^+ ions at low pH values (~5) displacing some bound ammonia in the waste and the rapid ammonification of the easily degraded proteinaceous portions of the food waste. A demonstration of the binding of NH_4^+ was seen during experiments with heavy isotopes at near neutral pH values, when reductions in pH probably led to the formation of $^{15}NH_4^+$ from the $^{15}NH_3$ in solution which facilitated the binding of $^{15}NH_4^+$ to negatively charged refuse particles and a reduction in the aqueous ammonia analysed (Chapter 6). There is the possibility that bound NH_4^+ could be released by interchange with H^+ in acidic conditions (~pH 5) or cations. Experiments are currently in

progress in the laboratory to identify any release or binding of ammonia in recirculated leachate during the transition from acetogenesis to methanogenesis under Environment Agency funding.

Ammonia concentrations in United Kingdom landfill leachate will most probably affect the rate of decomposition in the short term only when combined with a reasonable proportion of bound ammonia present in the waste. Waste will also have the potential to release ammonia during the decomposition of proteins. On a microscale, the bacteria, which are responsible for the decomposition of the waste, will have to contend with higher local concentrations of ammonia than observed in the sump of United Kingdom landfill leachate. How the binding of ammonia to refuse particles on a microscale reduces the availability and ability of ammonia to inhibit bacterial decomposition is not known. Protection from inhibition by surfaces and the production of slimes are two mechanisms which bacteria may use to avoid inhibition from ammonia. Acid production by acetogenic bacteria will encourage the release of bound ammonia at low pH from the refuse particles by interchange with produced H^+ ions, but will also encourage the formation of less toxic NH_4^+ in neutral and alkaline conditions which will reduce ammonia toxicity. Ammonification of proteins by bacteria during decomposition will contribute to an increase in ammonia concentrations on a microscale. pH value have an important role in determining the binding of ammonia in refuse (Chapter 6). Ammonia, which inhibits methanogenesis (Chapter 5), probably also inhibits acetogenic bacteria, reducing the production of volatile fatty acids, which in turn reduces methanogenesis. The corrosive properties of ammonia in the form of NH_3 can contribute to the inhibition of all but the most hardy of bacteria. The higher the pH the greater the proportion of NH_3 to NH_4^+. There is a tenfold increase in the concentrations of NH_3 with each increase in pH unit above the pKa value (pKa = 9.2) as NH_3 is formed from NH_4^+ (Burton & Watson-Craik, 1996b). Since methanogenic leachate has generally a higher pH value than acetogenic leachate it will have a greater ammonia toxicity not just to bacteria but to other organisms. Experiments on methane production by refuse cultures containing increased concentrations of ammonia (0-5000 mg $NH_{3/4+}$-N l^{-1}) revealed ammonia inhibition of methanogenesis (Chapter 5). The bacteria present in the refuse were not immune to increases in ammonia concentration although the theoretical concentration of ammonia in the refuse at 62% moisture content was just under 2.5 times that of cultures prepared for experimentation at 80% moisture content (517-565 mg $NH_{3/4+}$-N l^{-1}).

Long-term experiments were not carried out on ammonia toxicity in this study, and the acclimatisation of bacteria to high ammonia concentrations over considerable periods of time could not therefore be assessed. It is proposed to study this in more detail in future experiments. Only bacteria present in the sampled waste were studied, although bacteria from landfills with higher ammonia contents may be predisposed to higher ammonia concentrations and the rate of methanogenesis may be considerably higher.

Although lags in the development of methanogenesis were not observed there is a reasonable chance that bacteria would acclimatise to the higher concentrations. Increases from 500 to 1000 mg $NH_{3/4+}$-N l^{-1} did not affect the soluble or headspace methane concentrations in a refuse column supplied with 25 mM acetate. It is therefore possible that the methanogeneic association acclimatised to higher ammonia concentrations over several days so that differences could not be distinguished between the different treatments. Resistant strains of bacteria may eventually become dominant over longer periods of time, so increasing refuse decomposition and methanogenesis at higher ammonia concentrations. The addition of 3000 mg $NH_{3/4+}$-N l^{-1} may have been too great an increase in the ammonia concentration and the

methanogenic association became inhibited such that the perfusion of leachate could not proceed further.

Ammonia toxicity was not considered to be an important factor when the literature was consulted prior to the start of this study (Burton & Watson-Craik, 1996b). Observations from previous studies suggested methanogenesis could operate at extremely high ammonia concentrations. Closer inspection of published material on ammonia toxicity and methanogenesis did not always reveal the long term data required to determine the volumes of methane produced by the different ammonia treatments. The four stage system (Chapter 8) or similar vessels would be an ideal system for measuring the long term effects of ammonia concentrations on anaerobic decomposition by increasing the concentrations of ammonia in the influent.

9.4 Conclusions

1. The proportion of ammonia-N compared to the total organic nitrogenous pool (3.86% N dry weight), the source of ammonia was relatively small, between 5% to 10%, indicating that by the end of the active phase of ammonia production the bulk of ammonia was retained in the refuse and only a small proportion (5%) was released into the leachate during anaerobic decomposition.

2. Nitrate and nitrite were not found in the refuse studied and presumably have no role in the mineralisation of landfilled refuse.

3. In closed refuse cultures, the reduction of nitrate to ammonia at high initial concentrations of nitrate (500-1000 mg NO_3^--N l^{-1}) appeared insignificant and would not lead to increasing leachate ammonia concentrations if recirculation of nitrified leachate is attempted.

4. The addition of nitrate to a landfill will inhibit methane production in the short term if applied in concentrations similar to those in nitrified or treated acetogenic landfill leachate. Methanogenesis will only recover once the nitrate has been denitrified.

5. High ammonia concentrations had a considerable effect on the rates of methane production by refuse cultures. Better rates of methane production and anaerobic degradation may result from controlling the ammonia concentration in refuse.

6. The anaerobic decomposition by refuse cultures was not phosphate or nitrogen limited.

7. A reductive nitrogen transformation pathway which converted nitrate to ammonia was found during incubation of refuse cultures. This was capable of reducing 4 - 7% of the nitrate added. Autoclaving experiments suggested that an enzymatic/biochemical process was involved. The reductive pathway competed with the denitrification of nitrate. Greater proportions were reduced when the indigenous populations of bacteria were destroyed by gamma irradiation.

8. Binding of ammonia to refuse in refuse cultures appeared to be controlled by pH value, suggesting greater leaching at higher pH values.
9. Incorporation of the ammonia of the biomass produced during decomposition appeared insignificant, in experiments using heavy isotopes in model landfill systems. Leachate

ammonia accumulated during anaerobic decomposition in batch cultures. Nitrogen in refuse did not accumulate significantly during the growth of anaerobic bacteria in refuse columns when supplied with combinations of ammonia and volatile fatty acids. Utilisation of ammonia during bacterial growth is probably not an important route for the nitrogenous components in decomposing landfill refuse.

10. Ammonia leached from refuse columns during perfusion of distilled water with significant concentrations appearing in the leachate during the first 1 - 10 bed volumes. Significant proportions of bound ammonia remained after perfusion in the refuse which could be analysed by direct distillation.

11. The supply of ammonia in distilled water to refuse columns appeared to very slightly delay the onset of methanogenesis and reduced the concentrations of soluble methane in the effluent.

12. Increases in the concentration of ammonia from 500 to 1000 mg $NH_{3/4+}$-N l^{-1} did not appear to affect the soluble or headspace methane concentrations a refuse column receiving 25 mM acetate, suggesting limited effects of ammonia on methanogenesis at these concentrations.

13. Pulsed additions of nitrate at 4 mM (57 mg NO_3^--N l^{-1}) severely inhibited methanogenesis. However, methanogenesis in the cultures quickly recovered following denitrification of nitrate, indicating that the methanogens remained viable within the culture. The inhibition of methanogenesis by nitrate appeared to be a result of the presence of the nitrate ion since the redox potential remained unchanged after the addition of nitrate.

14. Low concentrations of nitrate were only slightly inhibitory to methanogenesis in fermenter models of landfill refuse decomposition. Methanogenesis and denitrification coexisted at nitrate concentrations of 0.4 mM (5.7 mg NO_3^--N l^{-1}) with relatively little inhibition of methanogenesis. Continued increases in the supply of nitrate to methanogenic cultures led eventually to strong inhibition of methanogenesis, an increase in pH and a increase in the redox potential. The continued supply of nitrate to fermenter systems, modelling the methanogenic decomposition of refuse which contained volatile fatty acids at approximately neutral pH, led to a reduction in volatile fatty acid concentrations as these compounds were used as a carbon supply for denitrification.

15. There appeared to be preferential use of longer chain volatile fatty acids by denitrifying bacteria within anaerobic cultures modeling landfill refuse decomposition. Propionate and butyrate were used as a carbon source in preference to acetate.

16. Concentrations of nitrate (500-1000 mg NO_3^--N l^{-1}) were toxic to methanogenic associations whereas concentrations of 50 mg NO_3^--N l^{-1} were inhibitory, with methanogenesis recovering once the nitrate had been consumed. Methanogenesis and denitrification could coexist in model landfill systems when nitrate was supplied at concentrations of 5.7 mg NO_3^--N l^{-1}. Limiting the concentrations of nitrate added to a landfill to 50 mg NO_3^--N l^{-1} would enable methanogenesis to recover once the nitrate had been consumed. Supplying concentrations of 500 mg NO_3^--N l^{-1} may lead to stratification of the waste and the development of zones of methanogenesis and denitrification.
17. Based on the results of short term experiments, ammonia concentrations in United Kingdom landfill leachate will most probably affect the rate of decomposition when

combined with bound ammonia and potential ammonia in the proteins present in the waste. Concentrations of ammonia were inhibitory but not such that actively reducing the ammonia content of leachate would bring significant increases to refuse decomposition. Ammonia concentrations higher than the leachate concentrations seen in the refuse in this study and in the leachate studied in current reviews published by the former DoE exerted a much more pronounced effect on methanogenesis. The addition of industrial waste with a high protein content will add considerably to the ammonia potential of refuse. Higher concentrations of ammonia will most probably develop as a result, with detrimental and significant effects on methanogenesis and the creation of concentrated leachates.

9.5 Recommendations

- Anaerobically decomposing landfilled refuse rapidly removes nitrate and represents an effective method of removing nitrate from treated nitrified leachate by recirculation, without the expense of secondary anaerobic leachate treatment. This strategy should be tested on an operational landfill or test cell to ensure there are not significant effects on gas production. (Experiments are currently in progress by the authors under Environment Agency funding).

- To prevent reductions in methanogenesis, additions of 500 mg NO_3^--N l^{-1} and above should be avoided during recirculation of shallow landfills. Deep landfills may allow the stratification of the waste into zones of denitrification and methanogenesis and experiments should be carried out to determine whether this will affect the gas production of these landfills.

- Industrial waste of a high protein content, when landfilled with domestic refuse, may represent a possible threat to the rapid decomposition of landfill refuse.

- Experiments on ammonia inhibition were carried out over relatively short time scales and we suggested further research on ammonia inhibition over longer periods. (Long-term experiments currently being carried out in our laboratory under Environment Agency funding).

GLOSSARY

Acetogenesis: The production or formation of acetate in refuse.

Acetogenic phase: The period during the fermentation of refuse when acetate and volatile fatty acids are formed and the pH of the leachate released is usually below 7.

Amonification: The biochemical removal of an amino group (NH_2^-) from protein or other nitrogenous organic compounds.

Anaerobic bacteria: Bacteria that do not use oxygen for growth and are inhibited by an oxygen rich atmosphere.

BOD (Biochemical Oxygen Demand): A measure of the oxygen used by microorganisms to breakdown organic compounds in a water sample. The higher the BOD the greater the amount of biodegradable compound present in the sample. The BOD is normally measured by comparing the dissolved oxygen concentration in a water sample with that of an identical sample which has been incubated in the dark (to prevent plant growth), at 20^oC for 5 days.

C/N Ratio: The ratio of the carbon content to the nitrogen content.

COD (Chemical oxygen demand): A measure of the amount of oxygen required to oxidise organic compounds present in a water sample using the chemical potassium dichromate. The higher the COD the greater the amount of organic compound in the sample, though a portion of these may be non biodegradable. The method requires the separation of organic compounds from inorganic compounds in the sample which may interfere with the reaction. Measurement of a sample's COD and BOD are rarely equal. However, a sample with a high BOD will typically have a high COD.

Deamination: The biochemical removal of an amino group (NH_2^-) from protein or other nitrogenous organic compounds.

Denitrification: The biochemical reduction of nitrates to nitrogen gas. Can be expanded to include the stepwise reduction of nitrate, nitrite, nitrous oxide and nitric oxide to nitrogen gas during the oxidation of organic compounds by denitrifying bacteria for energy.

Facultatively anaerobic bacteria: Bacteria, which do not require oxygen for growth (but may use it if available), which grow well under aerobic and anaerobic conditions, and to which oxygen is not toxic.

Hydrolysis: The process by which a compound is split to form products through the intervention of a molecule of water.

Nitrification: The biological oxidation of ammonia to nitrite and nitrite to nitrate by ammonia oxidising bacteria and nitrite oxidising bacteria. The process may also be used to describe the release of nitrite and nitrate by certain fungi.

Oxidation: A chemical process involving the combination of oxygen with a compound or substance. The loss of electrons or hydrogen from a substance. Example Iron (Fe) rusts by combination with oxygen in water to form iron oxide ($Fe_2O_3.H_2O$) also known as ferric III

oxide. Iron from its chemical formula has also lost electrons during the oxidation process to become positively charged (Fe to Fe^{3+}).

Reduction: A chemical process involving the removal of oxygen, the addition of hydrogen or the gain of electrons.

REFERENCES

Archer, D. B. and Powell, G. E. (1985). Dependence on the specific growth rate of methanogenic mutualistic cocultures on the methanogen. Archives of Microbiology 141, 133-137

Archer, D., Reynolds, P. and Blakey, N. C. (1995). A review of landfill microbiology research. Contractor Report: WRc plc, ETSU B/LF/00416/REP. Harwell, Oxfordshire.

Balderston W. L. and Payne, W. J. (1976). Inhibition of methanogenesis in salt marsh sediments and whole-cell suspensions of methanogenic bacteria by nitrogen oxides. Applied and Environmental Microbiology 32, 264-269.

Barber, C. and Maris, P. J. (1992), Leachate recirculation: Full scale experience. In Landfilling of Waste: Leachate, pp 381-401. Edited by T. H.

Christensen, R. Cossu and R. Stegman. Elsevier Applied Science. London.

Barlaz, M. A., Ham, R. K. and Schaefer, D. M. (1990). Methane production from municipal refuse: A review of enhancement techniques and microbial dynamics. Critical Reviews in Environmental Control 19, 557-584.

Barlaz, M. A., Milke, M. W. and Ham, R. K. (1987). Gas production parameters in sanitary landfill simulators. Waste Management and Research 5, 27-39.

Blackmer, A. M. and Bremner, J. M. (1978). Inhibitary effect of nitrate on reduction of N_2O to N_2 by soil microorganisms. Soil Biology and Biochemistry 10, 187-191.

Blaut, M. (1994). Metabolism of the methanogens. Antonie van Leeuwenhoek 66, 187-208.

Bogner, J. E. (1990). Controlled study of landfill biodegradation rates using modified BMP assays. Waste Management and Research 8, 329-352.

Brooks, P. D., Stark, J. M., McInteer, B. B. and Preston, T. (1989). Diffusion method to prepare soil extracts for automated nitrogen -15 analysis. Soil Science Society of America Journal 53, 1707-1711.

Bruntland Report - Our Common Future: Report of the World Commission on Environment and Development (OUP, 1987).

Burton, S. A. Q. (1993). Ureolytic nitrification at low pH. PhD Thesis. University of Aberdeen.

Burton, S. A. Q. and Watson-Craik, I. A. (1996a). Nitrogen balances in landfill refuse. In: *Proceedings of the1995 Society for Chemical Industry Conference, Current Trends in Contaminated Land*, SCI, London.

Burton, S. A. Q. and Watson-Craik, I. A. (1996b) Nitrogen balances in landfills. ETSU. Department of the Environment Contractor Report. Report N^{o} CWM A125/96. Waste Management Information Bureau, Abingdon, Oxfordshire.

Caskey, W. H. and Tiedje, J. M. (1980). The reduction of nitrate to ammonia by a *Clostridium* sp. isolated from soil. Journal of General Microbiology 119, 217-223.

Chen K-C, and Lin, Y-F (1993). The relationship between denitrifying bacteria and methanogenic bacteria in a mixed culture system of acclimated sludges. Water Research 27, 1749-1759.

Cole, J. A. and Brown, C. M. (1980). Nitrite reduction to ammonia by fermentative bacteria; a short circuit in the biological nitrogen cycle. FEMS Microbiology Letters 7, 65-72.

Coutts, D. A. P., Senoir, E. and Balba, M. T. M. (1987). Multi-stage chemostat investigations of intersepecies interactions in a hexanoate-catabolising microbial association isolated from anoxic landfill. Journal of Applied Bacteriology 62, 251-260.

Department of the Environment (1994a). Landfill Completion. Waste Management Paper 26A. HMSO, London.

Department of the Environment (1994b). Licensing of Waste Management Facilities. Waste Management Paper 4. HMSO, London.

Department of the Environment (1994c). The Technical Aspects of Controlled Waste Management. National Household Waste Analysis Project-Phase 2, Volume 1. Report on Composition and Weight Data. CWM 082/94.

Department of the Environment (1994d). The Technical Aspects of Controlled Waste Management. National Household Waste Analysis Project-Phase 2, Volume 3. Chemical Analysis Data. CWM 087/94.

Department of the Environment (1995). A Review of the Composition of Leachates from Domestic Wastes in Landfill Sites. Environment Agency CWM/072/95

Diekert, G. and Wohlfarth, G. (1994). Metabolism of homoacetogens. Anntonie van Leeuwenhoek 66, 209-221.

Drtil, M., Nemeth, P., Klucman, K., Bodik, I. and Kasperek, V. (1995). Acidobasic balances in the course of hetrotrophic denitrification. Water Research 29, 1353-1360.

Ehrig, H. -J. (1989). Leachate quality. In Sanitary Landfilling: Process Technology and Environmental Impact, pp. 213-229. Edited by T. H. Christensen, R. Cossu and R. Stegmann. Academic Press, London.

Enzminger, J. D., Robertson, D., Ahlert, R. C. and Kosson, D. S. (1987). Treatment of landfill leachates. Journal of Hazardous Materials 14, 83-101.

Farquhar, G. J. and Rovers, F. A. (1973). Gas production during refuse decomposition. Water Soil and Air Pollution 2, 483-495.

Harmsen, J. (1983). Identification of organic compounds in leachate from a waste tip. Water Research 17, 699-705.

Harris, R. C., Knox, K. and Walker, N. (1994). A strategy for the development of sustainable landfill design. IWM Proceedings January p26-29.

James A. G. and Watson-Craik I. A. (1993). Elucidation of refuse interspecies interaction by use of laboratory models. Department of Trade and Industry Contractor Report. Report N^{o} ETSU B/B2/00148/REP Waste Management Information Bureau, Abingdon, Oxfordshire.

Jones, R. L. and Watson-Craik I. A. (1994). Assessment of the Role of Surfaces in Refuse Degradation. Department of Trade and Industry Contractor Report. Report N^{o} CWM 113/94L Waste Management Information Bureau, Abingdon, Oxfordshire.

Knox, K. (1985). Leachate treatment with nitrification of ammonia. Water Research 19, 895-904.

Kristensen, P. (1992). Ecotoxicological characteristics of landfill leachate. In Landfilling of Waste: Leachate, pp. 89-105. Edited by T. H. Christensen, R. Cossu and R. Stegmann. Elsevier Applied Science. London.

Latham, B. (1996). Personal communication. Aspinwall & Company, Baschurch, Shrewsbury.

Ljungdahl, L. G. (1986). The autotrophic pathway of acetate synthesis in acetogenic bacteria. Annual Review of Microbiology 40, 415-50.

Mah, R. A., Ward, D. M., Baresi, L. and Glass, T. L. (1977). Biogenesis of methane. Annual Review of Microbiology 31, 309-41.

Mullin, J. B. and Riley, J. P. (1955). The spectrophotometric determination of nitrate in natural waters, with particular reference to seawater. Analytica Chimica Acta 12, 464-480.

Pirt, S. J. (1978). Aerobic and anaerobic microbial digestion in waste reclamation. Journal of Applied Chemistry and Biotechnology 28, 232-236.

Postgate, J. R. (1984). The Sulphate Reducing Bacteria. 2nd Edition. Cambridge University Press, Cambridge.

Preston, T. and Barrie, A. (1991). Recent progress in continuous flow isotope ratio mass spectrometry. International Laboratory September, 31-34.

Rand, M. C, Greenberg, A. E. and Taras, M. J. (eds). *1975 Standard Methods for the Analysis of Water and Wastewater (14th Edition).* American Public Heath Association, Washington.

Rees, J. F. (1980). The fate of carbon compounds in the landfill disposal of organic matter. Journal of Chemical Technology and Biotechnology 30, 161-175.

Robinson, H. D., Barber, C. and Maris, P. J. (1982). Generation and treatment of leachate from domestic wastes in landfills. Journal of the Water Pollution Control Federation 52, 465-478.

Robinson, H. D. and Maris, P. J. (1985). The treatment of leachates from domestic waste in landfill sites. Journal of the Water Pollution Control Federation 57, 30-38.

Robinson, H. D., Barr, M. J. and Last, S. D. (1992). Leachate collection, treatment and disposal. Journal of the Institute of Water and Environmental Management 6, 321-332.

Robinson, H. D. (1995). Role of leachate in landfill. In Proceeding of the ETSU Landfill Gas Microbiology Workshop, March 1995, Solihull.

Robinson, H. D. (1996). Personal communication. Aspinwall & Company, Baschurch, Shrewsbury.

Senior, E. and Balba, M. T. N. (1987). Landfill biotechnology. In Bioenvironmental Systems: Vol. II. Edited by D. L. Wise: CRC Press, Boca Raton, Florida.

Sinclair, K. J. (1994). The co-disposal of sewage sludge with domestic refuse and potential importance of landfill nitrogen transformations. PhD Thesis. University of Strathclyde, Glasgow.

Skinner, F. A. and Walker, N. (1961). Growth of *Nitrosomonas europaea* in batch and continuous culture. Archiv für Mikrobiologie 38, 339-473.

Sulisti (1994). Studies on the co-disposal of *o*-cresol with municipal refuse by use of laboratory models. PhD Thesis. University of Strathclyde, Glasgow.

Sulisti and Watson-Craik, I. A. (1996). Studies on the co-disposal of *o*-cresol with municipal refuse. Journal of Chemical Technology and Biotechnology 65, 72-80.

Tittlebaum, M. E. (1982). Organic carbon content stabilization through landfill leachate recirculation. Journal of the Water Pollution Control Federation 54, 428-433.

Watson-Craik, I. A. (1987). Landfill as an anaerobic filter for the co-disposal of phenolic wastewaters. PhD Thesis. University of Strathclyde, Glasgow.

Watson-Craik, I. A. and Senior, E. (1989a). Landfill co-disposal: hydraulic loading rate considerations. Journal of Chemical Technology and Biotechnology 45, 203-212.

Watson-Craik, I. A. and Senior, E. (1989b). Treatment of phenolic wastewaters by co-disposal with refuse. Water Research 23, 1293-1303.

Watson-Craik, I. A. and Senior, E. (1989c). Quantitative analysis of solubilised methane in refuse leachate. Letters in Applied Microbiology 8, 49-53.

Watson-Craik, I. A. and Senior, E. (1989d). Effects of phenol wastewater co-disposal on the attenuation of the refuse leachate molecule hexanoic acid. Letters in Applied Microbiology 9, 227-232.

Watson-Craik, I. A. and Senior, E. (1990). Landfill co-disposal of phenol bearing wastewaters: organic load considerations. Journal of Chemical Technology and Biotechnology 47, 219-233.

Winter, J. U. and Wolfe, R. S. (1980). Methane formation from fructose by syntrophic associations of *Acetobacterium woodii* and different strains of methanogens. Archives of Microbiology 124, 73-79.

Wolin, M. J. (1974). Metabolic interactions among intestinal microorganisms. The American Journal of Clinical Nutrition 27, 1320-1328.

Yoshinari, T. and Knowles, R. (1976). Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochemical and Biophysical Research Communications 69, 705-710.

APPENDICES

Appendix 1 Description of ammonia

To avoid confusion this report refers simply to ammonia as a combination of NH_3 and NH_4^+ dissolved in landfill leachate. Both are present in varying amounts according to the pH value of the solution. The report when it refers to ammonia does not differentiate between NH_3, known as 'free ammonia,' 'un-ionised ammonia' or simply 'ammonia' or NH_4^+, known as 'ammonium' or 'ionised ammonia,' when it refers to ammonia in the text. All analyses of leachate made in this study analyse both NH_3 and NH_4^+. When not dissolved in leachate and present in the atmosphere it is referred to as ammonia gas which has the formula NH_3.

Other reports and scientific literature sometimes call the combination of NH_3 and NH_4^+ as ammoniacal nitrogen sometimes expressed as ammoniacal-N. This can be substituted for all references to ammonia.

When expressing the concentrations of ammonia the format mg $NH_{3/4+}$-N l^{-1} is used which refers to the milligrammes of the nitrogen present in the ammonia (both NH_3 and NH_4^+) in a litre. This is equivalent to µg $NH_{3/4+}$-N ml^{-1} or parts per million nitrogen (ppm N).

Appendix 2 The contents of the mineral medium (10 l) used for the growth of anaerobic microorganisms within the four stage continuous culture chemostat (Chapter 7)

Cellobiose	17.11 g
Na_2SO_4	1.42 g 1 mM SO_4^{2-}
K_2HPO_4	10 g
$NaH_2PO_4.2H_2O$	8.5 g
NH_4Cl	19.09 g (500 mg $NH_{3/4+}$-N l^{-1})
$MgCl_2.6H_2O$	2 g
$NaHCO_3$	2 g
Trace elements	10 ml
Trace minerals	10 ml
Nickel solution	10 ml of a 1 mM $NiCl_2.6H_2O$ solution in distilled water
Vitamins	10 ml
Resazurin stock solution	10 ml

Made up to 10 l with distilled water.
Typical pH value after mixing 6.6, adjusted to pH 7.0 with dilute NaOH.

Trace elements solution in 1 l of distilled water

NaCl	9000 mg
$FeCl_2.4H_2O$	1500 mg
$MnCl_2.4H_2O$	197 mg
$CaCl_2.2H_2O$	900 mg
$CuCl_2.H_2O$	17 mg
$ZnSO_4$	287 mg
$AlCl_3.6H_2O$	50 mg
H_3BO3	62 mg
$NiCl_2.6H_2O$	24 mg

Trace minerals solution in 1 l of distilled water

$NaMoO_4.2H_2O$	48.4 mg
$NaSeO_3.xH_2O$ (31% Se)	2.55 mg
$NaWO_4.2H_2O$	3.3 mg

Vitamins solution in 1 l of distilled water

biotin	10
p-amino benzoic acid	19
α-lipoic acid	20
folic acid	10
pyridoxine HCl	20
thiamine HCl	20
riboflavin	30
nicotinic acid	50
D(+) Ca pantothenate	30
cyanocobalamine	20

Appendix 3 Effect of moisture on methane production in batch culture

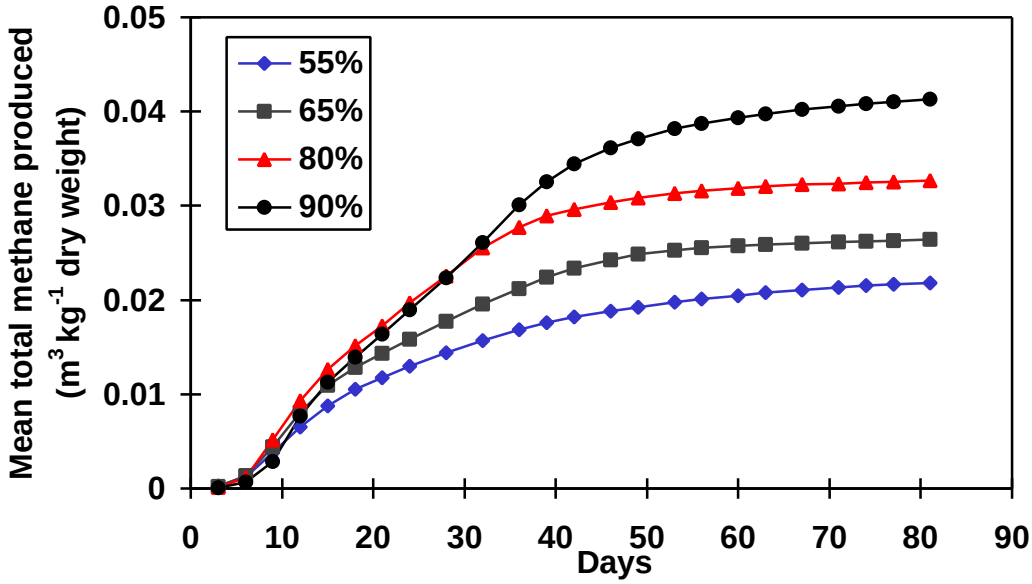
Experiments using heavy nitrogen isotopes required increasing the moisture content of the refuse beyond what would normally be experienced in a landfill so that appropriate nitrogen concentrations could be caught by microdiffusion. The effect of moisture content on methane production was explored in batch culture experiments with refuse. Refuse cultures containing 7.6 g dry weight were adjusted to moisture contents of 55%, 65%, 80% and 90% w/w and incubated at 30 ^{o}C in triplicate in 500 ml bottles sealed by a Suba-Seal (Merck) under a helium atmosphere. The refuse was analyzed for methane every 3 or 4 days using a Perkin Elmer 8500 Gas Chromatograph, after which time the bottles were degassed with helium for 20 minutes. The majority of the analyses in these experiments were carried out by Mr. Marc Jacobs during his research placement.

The mean headspace volumes in each of the bottles at each moisture content are tabulated below:

Moisture content	Mean headspace volume
55%	524 ml
65%	520 ml
80%	503 ml
90%	465 ml

The mean volume of a stoppered bottle was 538 (SD 3.5) ml, the mean refuse density was 1.28 (SD 0.048) g ml^{-1} after sorting.

Total methane production by refuse at 55(◆), 65(■), 80(▲) and 90%(●) moisture content. Total methane produced was expressed as m^{3} kg^{-1} dry weight.



Conclusions

Methane production by refuse batch cultures was dependent on the moisture content.