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Nitrification rates in
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J. McEvoy.
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Interim Report R&D Project 305

Nitrification Rates in Rivers and Estuaries

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October 1992
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NITRIFICATION RATES IN RIVERS AND ESTUARIES

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EXECUTIVE SUMMARY

This interim report details the work carried out between June and September 1992 relating to the construction and performance of two identical chemostats during the initial 75 days of operation.

The process of complete nitrification is evidenced in both chemostats after two days of operation, as shown by the stoichiometric conversion of ammonia to nitrate. Dilution of the original seeding material resulted in the onset of a progressive increase in ammonia levels in both chemostats after approximately 20 days.

For chemostat No.1, nitrate concentrations remained relatively constant up to Day 60, with a subsequent increase in response to a decrease in ammonia concentration. These results confirm that successful selection of nitrifying bacteria is progressing in chemostat No.1 under the conditions of operation. Although a stable nitrifying coculture is present in chemostat No.1, a steady-state has not yet been established.

During the first 55 days, operational data for chemostat No.2 paralleled that of chemostat No.1. Subsequent analysis has shown a marked increase and decrease of the nitrite and nitrate levels, respectively. These data suggest that for chemostat No.2, a stable coculture of ammonia and nitrite oxidisers is not fully established at present.

The recommendations arising from the study to date are discussed in relation to the initial and overall objectives of the project.

An outline of future work is presented for discussion.

KEYWORDS

nitrification, ammonium, chemostat, *Nitrosomonas* sp., *Nitrobacter* sp.

1. INTRODUCTION

1.1 Background

The biological oxidations of ammonia to nitrite and nitrite to nitrate, collectively referred to as nitrification, are carried out by two physiological Gram-negative genera, *Nitrosomonas* and *Nitrobacter*. The use of generic names allows the simplified grouping of all nitrifiers into two classifications of micro-organisms. *Nitrosomonas* usually refers to the group capable of oxidising ammonia to nitrite. *Nitrobacter* are able to further oxidise nitrite to nitrate. The organisms in both groups fix carbon dioxide via the Calvin cycle (Campbell *et al* 1966) for their major source of cell carbon and derive their energy and reducing power either from the oxidation of ammonia or nitrite. With the exception of a few strains of *Nitrobacter*, the nitrifying bacteria are obligate chemolithotrophs.

Nitrifying bacteria play an important role in the turnover of nitrogen in fresh water and marine habitats, and in most other aerobic environments where organic compounds are mineralised. In nature their growth is controlled by substrate concentration, temperature, pH and oxygen tension (Watson *et al* 1974). The process of ammonia oxidation has also been shown to be irreversibly inactivated by photo-oxidation (Hooper and Terry 1973). In pure culture, most strains of nitrifying bacteria grow optimally at substrate concentrations of 1-25 mM, a pH between 7.5 and 8.0 and a temperature of 25-30 °C. However, many environments with suboptimal conditions still support the growth of nitrifying bacteria. For example, nitrifying bacteria are strict aerobes, yet they can be isolated from sewage-disposal aeration tanks that are extremely low in oxygen (Chartrain *et al* 1983). The highest concentration of nitrifying bacteria in rivers and streams is found at the sediment-water interface (Curtis *et al* 1975, Matulewich and Finstein 1978, Van Rijn *et al* 1987) and in sewage disposal systems, attached to the sides of the aeration tanks (Watson *et al* 1974). In the marine environment they are localised in the upper 200 m of the water column or at the sediment-water interface (Watson *et al* 1971; Belser, 1979; Helder and DeVries, 1983).

The capacity of a freshwater or marine environment to maintain and propagate a balanced aquatic habitat is reflected by the concentration of dissolved oxygen which is regarded as an important measure of water quality and a significant factor in the evaluation of pollution impact. The oxygen demand of the ammonia oxidation process can theoretically constitute a considerable part of the total oxygen demand, especially for environments receiving sewage and/or low grade treated effluents containing high concentrations of ammonia. The circumstances under which nitrification may consume significant amounts of dissolved oxygen have been described as "...where the effluent discharges to an estuary or to the upper reaches of a river of long retention time, or where effluent makes up a large portion of total flow of a river" (Anon. 1971). Previous studies have demonstrated the influence of nitrification on stream oxygen balance and clearly indicated the necessity of considering inorganic nitrogen oxidation in the study of water quality models for river systems (O'Connor 1967, Stratton and McCarty 1967, Wezernak and Gannon 1968, Huang and Hopson 1974, Schwert and White 1974, Curtis *et al* 1975, Ruane and Krenkel 1978, Schultz 1988, Warwick and Shetty 1988, Barcia 1991).

Researchers have developed various models to describe nitrification in rivers under a variety of conditions using a number of different parameters. Huang and Hopson (1974) reported that the rate of oxidation of ammonia nitrogen was linear with time i.e. obeys zero-order kinetics. Another study showed that nitrification of surface waters under natural environmental conditions can be expressed by first order kinetics (Wazernak and Gannon 1968). Pure culture laboratory studies have revealed differing sensitivities of *Nitrosomonas* and *Nitrobacter* species to ammonia (Anthonison *et al* 1976, Suthersan and Ganczarczyk 1986). In the latter study the authors concluded that nitrite oxidation would be selectively inhibited at elevated pH values in response to an increase in the concentration of unionised ammonia.

The yield of nitrifier biomass relative to the quantity of ammonia oxidised is relatively small. The maximum specific growth rate of the nitrifying bacteria is the most crucial parameter describing the growth of autotrophic biomass. Its importance, in relation to wastewater treatment, is attributed to the fact it determines the mean cell residence time, below which washout of nitrifiers would occur. Furthermore, this parameter is required whenever a model is to be used to simulate a nitrification process (Henze *et al* 1986).

Since the specific growth rate is a strong function of the prevailing temperature and pH, a functional relationship accounting for this dependence can be used to evaluate the *in situ* nitrification process for a range of these environmental parameters. Previous works (Engel and Alexander 1958, Hopwood and Downing 1965, Knowles *et al* 1965, Jenkins *et al* 1967, Loveless and Painter 1968, Hall 1974, Quinlan 1980, Painter and Loveless 1983; Antoniou *et al* 1990) have shown that the optimal nitrification rate is achieved at a pH in the range of 7.0-8.2 and is an increasing function of temperature in the range of practical interest (less than 30 °C). Experimentally determined effects of temperature and pH on the rate of nitrification have been assessed separately or in combination by the previous researchers and in many cases equations are provided.

The effect of dissolved oxygen concentration on the rate of nitrification has been investigated by a number of researchers using both pure and mixed cultures found in wastewater treatment systems (Bragstad and Bradney 1937, Downing and Scragg 1958, Wuhrman 1963, Knowles *et al* 1965, Loveless and Painter 1968, *et al* 1969, Stenstrom and Poduska 1980). The maximum bacterial growth rate from both stages of nitrification are reported to be affected by dissolved oxygen concentration over the range of 0.3 mg l^{-1} to as much as 4.0 mg l^{-1}. In some instances, it has been reported that a dissolved oxygen concentration in excess of 4.0 mg l^{-1} is required to achieve maximum nitrification rates, whilst other investigators have found that only 0.5 to 1.0 mg l^{-1} is required. It has been proposed that several factors are responsible for the wide range of reported nitrification rates with varying dissolved oxygen concentrations. Among these factors are the effects of oxygen diffusion in flocs, variation in the measured results due to steady-state and dynamic measuring techniques, and double-substrate limited kinetics.

Previous studies have detailed the response of nitrifying species to a change in salinity (Helder and DeVries 1983). In pure culture studies, both *Nitrosomonas* and *Nitrobacter* species remained unaffected with respect to their specific growth rates over a salinity range of 0 to 35. However, salinity has been shown to affect the rate of nitrification by controlling the release of nitrogen from sediments (Gardiner *et al* 1991). These authors

demonstrated that vertical distribution of ammonium out of the sediment is hindered by cation exchange (or sorption) interactions with sediment particles to a greater extent in fresh water than in estuarine systems. The resulting relatively long residence time, and potentially high levels of particle-bound ammonia in the freshwater sediments were thought to favour nitrification as the major ammonia removal process. By contrast, ion pair formation of ammonium with seawater anions and blockage of sediment cation exchange sites with seawater cations was deemed responsible for diffusion of ammonium out of estuarine sediments before extensive nitrification could take place.

In summary, the ultimate fate of ammonia, introduced by direct discharge or derived as the result of *in situ* anaerobic metabolism, and hence the rate of nitrification, is dependent on a variety of environmental factors including substrate concentration, temperature, pH, salinity, light, dissolved oxygen, biomass concentration and spatial location. Furthermore, *in situ* nitrification is a dynamic process whose quantification is possible only in the presence of other nitrogen cycling processes.

1.2 Study objectives

To assess the impact of a polluting discharge on a river or estuary it is imperative that the NRA is able to predict the combined effect of the different oxygen demands created. The oxygen demand of the nitrification process can constitute a considerable part of the total oxygen demand of the receiving water. Previous studies within the UK relating to the process of nitrification have been centred on the efficiency of nitrogen removal within wastewater treatment systems. To date, the factors influencing the fate of nitrogen-containing compounds in surface waters have received little attention in relation to the overall problem they may represent.

Field observations have shown that the difficulties associated with predicting the impact of nitrification on the overall oxygen demand are related to the varying characteristics of the receiving water. WRc was asked to carry out a laboratory based study to assess the influence of nitrification on various environmental factors. By carrying out laboratory studies it is hoped to provide reference data to assist in interpretation of field studies. The environmental factors which are known to be affect the nitrification process have been identified as substrate concentration, temperature, dissolved oxygen, salinity, suspended solids and light.

The strategy which has been deployed to study the effect of the environmental factors on the process of nitrification is outlined below:

1. Set-up two small scale continuous culture vessels to provide a reliable source of *Nitrosomonas* and *Nitrobacter*. An environmental source of these organisms will be used.

2. Using the chemostats for supplies of nitrifying bacteria design an experimental procedure for determining the influence of various factors on nitrification.

3. Because nitrifying bacteria grow relatively slowly, a system based on continuous culture would be prohibitively expensive. As a consequence it is envisaged that the effect of environmental factors will be studied in small scale batch culture experiments. Essentially, a series of vessels each containing media designed to grow both the ammonia and nitrite oxidising species will be inoculated from the chemostats. The vessel will then be incubated under the influence of the environmental factor being studied.

4. Two alternative methods for determining nitrification rate will be assessed. The first will be based on determining the concentration of ammonia, nitrite and nitrate both before and after an optimised incubation period. In the second, the rate of ^{14}C-bicarbonate uptake will be assessed. This latter method may prove more sensitive and as a consequence shorter incubations may be required.

5. Once the system has been optimised with regard to growth requirements, incubation period and method of measuring nitrification, it will be used to study the influence of ammonia concentration, temperature, dissolved oxygen, salinity, suspended solids and light. Study of light may require the system to be modified.

6. Further modifications and developments may be required before the effect of short term changes in environmental factors can be assessed.

1.3 Resumé of contents

This report contains a brief description of the methodology associated with the set-up of two chemostats as outlined in a) above. Reference is made to a previous interim report (R&D 305/3/NW) for detailed explanation of the set-up procedure.

Data are presented and discussed with respect to the performance of two chemostats during the first 75 days of operation.

The recommendations arising from this work to date are discussed in relation to the achievement of the initial and overall objectives of the study.

An outline of future work is presented for discussion.

2. METHODOLOGY

This section outlines the procedures adopted for the provision of a nitrifying biomass. It is proposed to use continuously cultured nitrifying bacteria as a standardised source of inoculum during studies to determine the effect of environmental factors on the process of nitrification.

2.1 Previous continuous culture experiments

Previous attempts to provide a stable source of nitrifying bacteria have met with limited success. A detailed account of previous continuous culture experiments is contained in the interim report R&D 305/3/NW. The selection of media, the operation procedures and the outcome of previous continuous culture experiments are summarised in Table 2.1.

Previous efforts have concentrated on the separate enrichment and continuous culture of ammonia and nitrite oxidising bacteria. These studies have resulted in, a) the identification of a selective media capable of supporting simultaneous ammonia and nitrite oxidation, and b) the operating conditions required for maintenance of a stable nitrifying biomass.

2.2 Present continuous culture experiment

This study is presently dedicated to the enrichment of a mixed population of ammonia and nitrite oxidising bacteria. The previous trials have shown that complete nitrification can be achieved using a media containing ammonium sulphate and sodium carbonate as the sole nitrogen and carbon sources, respectively (Soriano and Walker, 1968). It has also been shown that the origin of the inoculum together with the dilution rate have a profound effect on the stabilisation of a continuous flow nitrification process, as reflected by the time required to achieve conditions of steady-state (Table 2.1).

During this phase, two identical 10 litre chemostats were constructed as detailed in sections 3.2.3 and 3.2.4 of the previous interim report (R&D 305/3/NW). It was decided to operate both chemostats in an identical fashion to allow provision for additional available culture for use in subsequent batch studies. Both chemostats were inoculated with 5 litres of activated sludge (obtained from Wargrave Sewage Treatment Works) prior to the addition of 5 litres of ammonia oxidation enrichment media according to Soriano and Walker (1968). Both chemostats were operated at a maximum dilution rate of approximately 0.004 hr^{-1}, equivalent to a 10 day hydraulic retention time.

The chemostats were monitored for ammoniacal and inorganic nitrogen, pH, dissolved oxygen, temperature, dilution rate and air flow as previously described (section 3.2.3, Interim Report R&D 305/3/NW).

Table 2.1 The selection of media, the operation procedures and the outcome of previous nitrifying bacteria continuous culture studies

Media type	Operational procedures	Outcome of study
Run No.1		
Soriano and Walker[1]	* 1 litre chemostat * N-source $(NH_4)_2SO_4$ * Inoculum-estuarine sample	Stable complete nitrification achieved after 60 days of operation
Schmidt and Belser[2]	* 1 litre chemostat * N-source KNO_2 * Inoculum-estuarine sample	Stable nitrite oxidation after 20 days of operation days of operation
Run No.2		
Soriano and Walker	* 10 litre chemostat * N-source $(NH_4)_2SO_4$ * Inoculum-secondary effluent	Nitrification not achieved after 36 days
Schmidt and Belser	* 10 litre chemostat * N-source KNO_2 * Inoculum-secondary effluent	Limited nitrite oxidation after 33 days
Run No.3		
Soriano and Walker	* 10 litre chemostat * N-source $(NH_4)_2SO_4$ * Inoculum-activated sludge	Partial nitrification achieved after day 60 (experiment stopped on day 90)
Schmidt and Belser	* 10 litre chemostat * N-source KNO_2 * Inoculum-activated sludge	Partial nitrite oxidation after day 80 (experiment stopped on day 90)

1 Soriano and Walker (1968). Media constituents as follows (all g l^{-1}): $(NH_4)_2SO_4$ 0.5, KH_2PO_4 0.2, 0.04, $MgSO_4.7H_2O$ 0.04, Na_2CO_3 0.15, chelated iron 0.0005.

2 Schmidt and Belser (1981). Media constituents as follows (all mg l^{-1}): KNO_2 8.5, $CaCl_2.2H_2O$ 13.4, $MgSO_4.7H_2O$ 200, K_2HPO_4 34.8, KH_2PO_4 27.2, chelated iron 2.5, $Na_2MoO_4.2H_2O$ 0.1, $MnCl_2.4H_2O$ 0.3, $CoCl_2.6H_2O$ 2.0, $ZnSO_4.7H_2O$) 0.1, $CuSO_4.5H_2O$ 2.0.

3. RESULTS

Data are presented with respect to the operation of two chemostats during the first 75 days of operation.

The variation in the rate of substrate addition to each chemostat during the first 75 days of operation is shown in Figure 3.1. The average dilution rate (D) applied to chemostats No.1 and No.2 during this phase of the study was 0.004 and 0.0038 hr^{-1}, respectively. Variation in the dilution rate was primarily caused by air entrainment within the inlet tubing resulting in uneven flow characteristics.

The concentration of dissolved oxygen was maintained above 6 mg l^{-1} at all times in both chemostats during this phase of the study (Figure 3.2). Similar dissolved oxygen levels were measured in each chemostat throughout this phase with an average concentration maintained in excess of 8 mg l^{-1}.

During the first 15 days of operation, the pH of the medium in chemostat No.1 was controlled between 7.5 and 8.0 by the automatic addition of sodium carbonate. Operational failure of the pH controlling unit after day 15 resulted in manual addition of carbonate solution between day 15 and 75. The pH of the medium in chemostat No.2 was controlled manually from the start of the experiment. Figure 3.3 shows the variation in pH for both chemostats during the first 75 days of operation. The results presented from Day 15 onwards were measurements taken after the addition of carbonate solution. In general, the pH of the medium in both chemostats decreased from approximately 8.0 to below 7.0 after two days of operation as a result hydrogen ion release during the oxidation of ammonia to nitrite.

The changes in the ammonia, nitrite and nitrate concentrations for chemostats No.1 and No.2, during continuous operation with ammonia as the sole nitrogen source are shown in Figures 3.4 and 3.5, respectively. The results shown in these latter figures are expressed as equivalent nitrogen concentrations to provide a clear estimation of the nitrogen budget as presented in Figure 3.6.

The process of complete nitrification is shown in both chemostats after 2 days of operation, as evidenced by the stoichiometric conversion of ammonia to nitrate. Dilution of the original seeding material resulted in the onset of elevated ammonia levels in both chemostats after approximately 20 days of operation. A progressive increase in the ammonia concentration was observed in both chemostats between Days 20 and 62, after which time ammonia concentrations gradually decreased.

For chemostat No.1, nitrate concentrations remained relatively constant up to Day 60, with a subsequent increase in response to a decrease in ammonia concentration. These results confirm that successful selection of nitrifying bacteria is being carried out in chemostat No.1 under the conditions of operation. The concentration of nitrite in chemostat No.1 gradually increased between after Day 30 from below 1 to above 10 mg-N l^{-1}. The increase in nitrite and the high residual ammonia and nitrate

concentrations in chemostat No.1 indicate that, although a stable nitrifying coculture is present, a steady-state has not yet been established.

During the first 55 days, operational data for chemostat No.2 paralleled that of chemostat No.1. Subsequent analysis has shown a marked increase and decrease of the nitrite and nitrate levels, respectively. These data suggest that for chemostat No.2, a stable coculture of ammonia and nitrite oxidisers is not fully established at present.

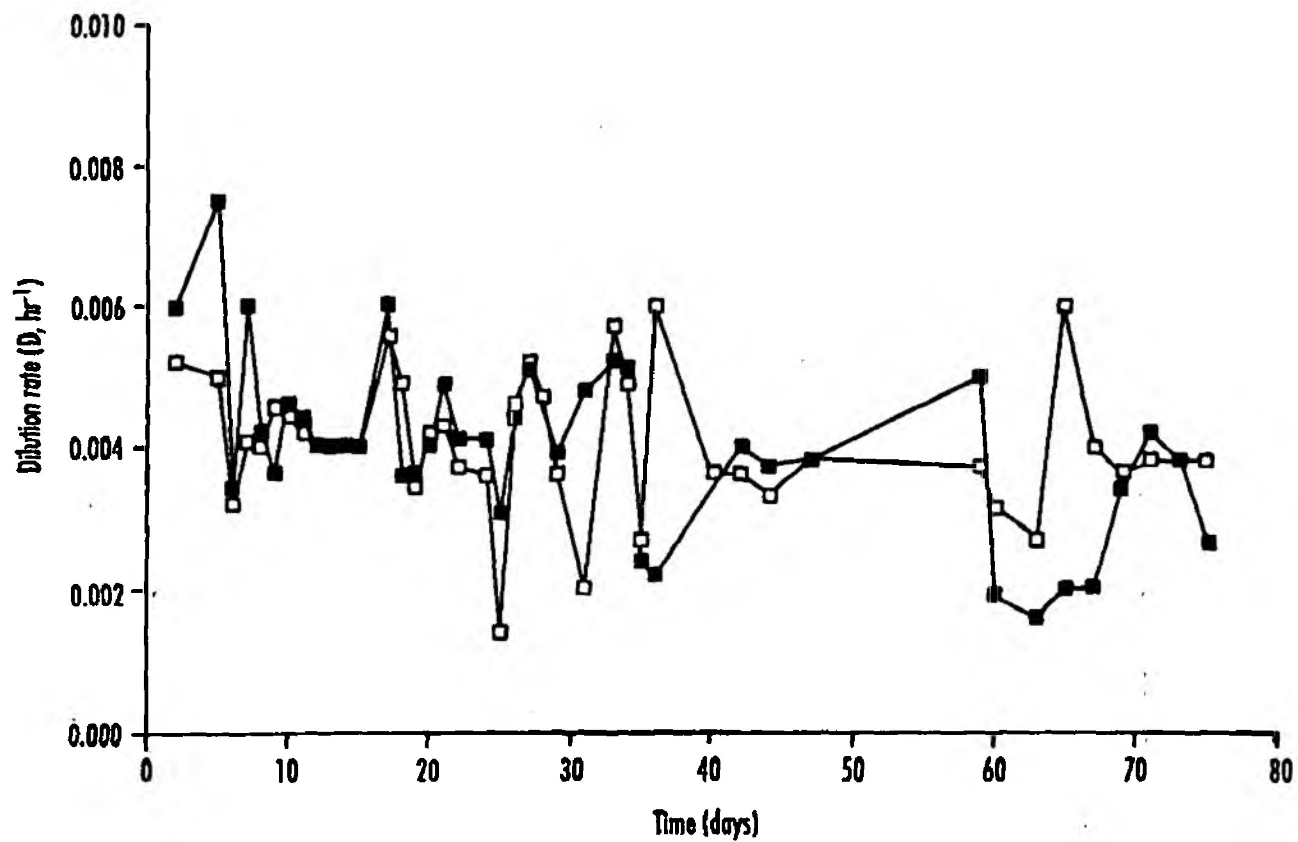

Figure 3.1 Variation in dilution rate (D) measured during operation of chemostats # 1 and # 2 (days 0-75). ■ chemostat # 1, □ chemostat # 2.

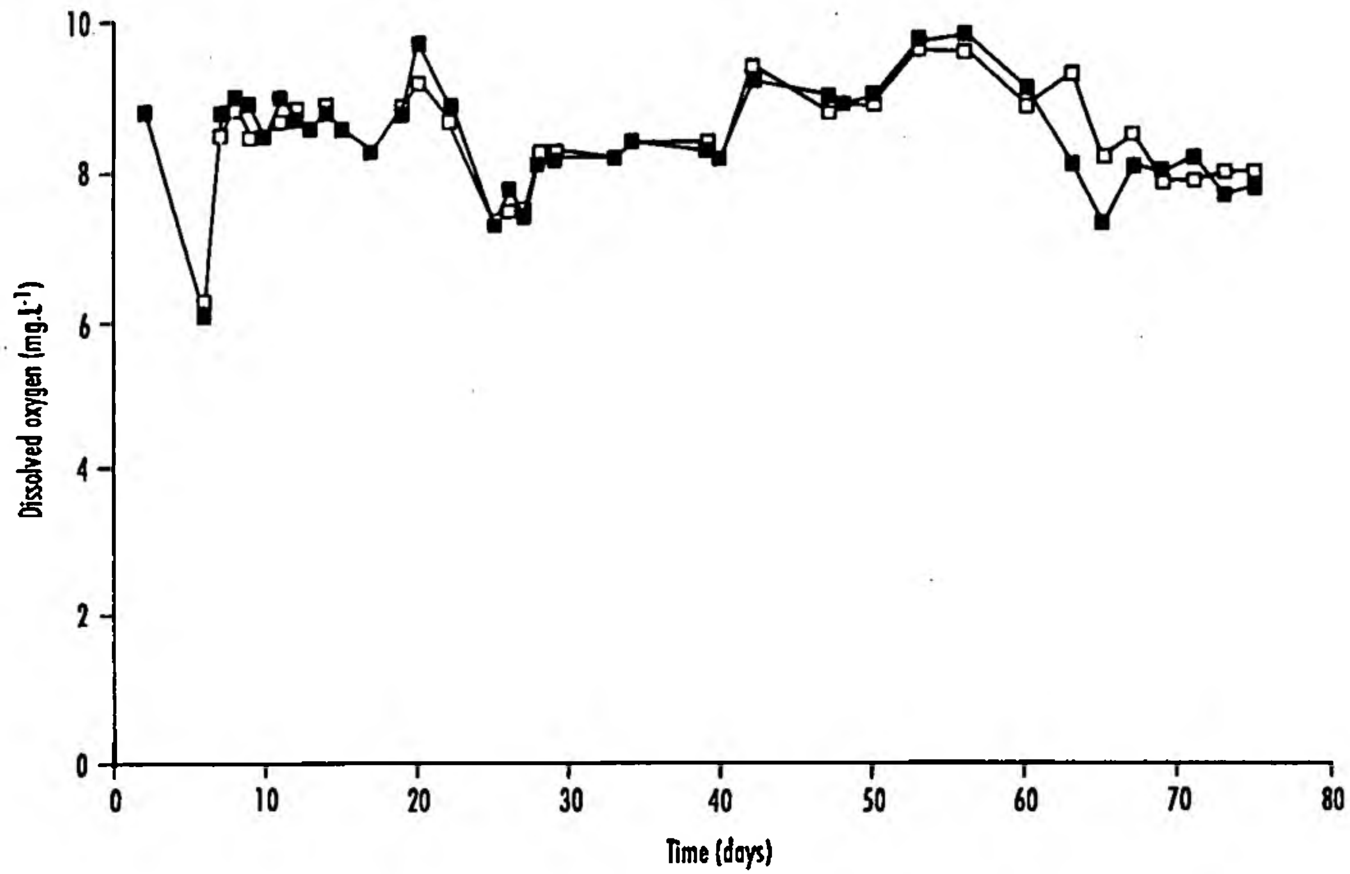

Figure 3.2 Dissolved oxygen concentrations measured during operation of chemostats # 1 and # 2 (days 0-75). ■ chemostat # 1, □ chemostat # 2.

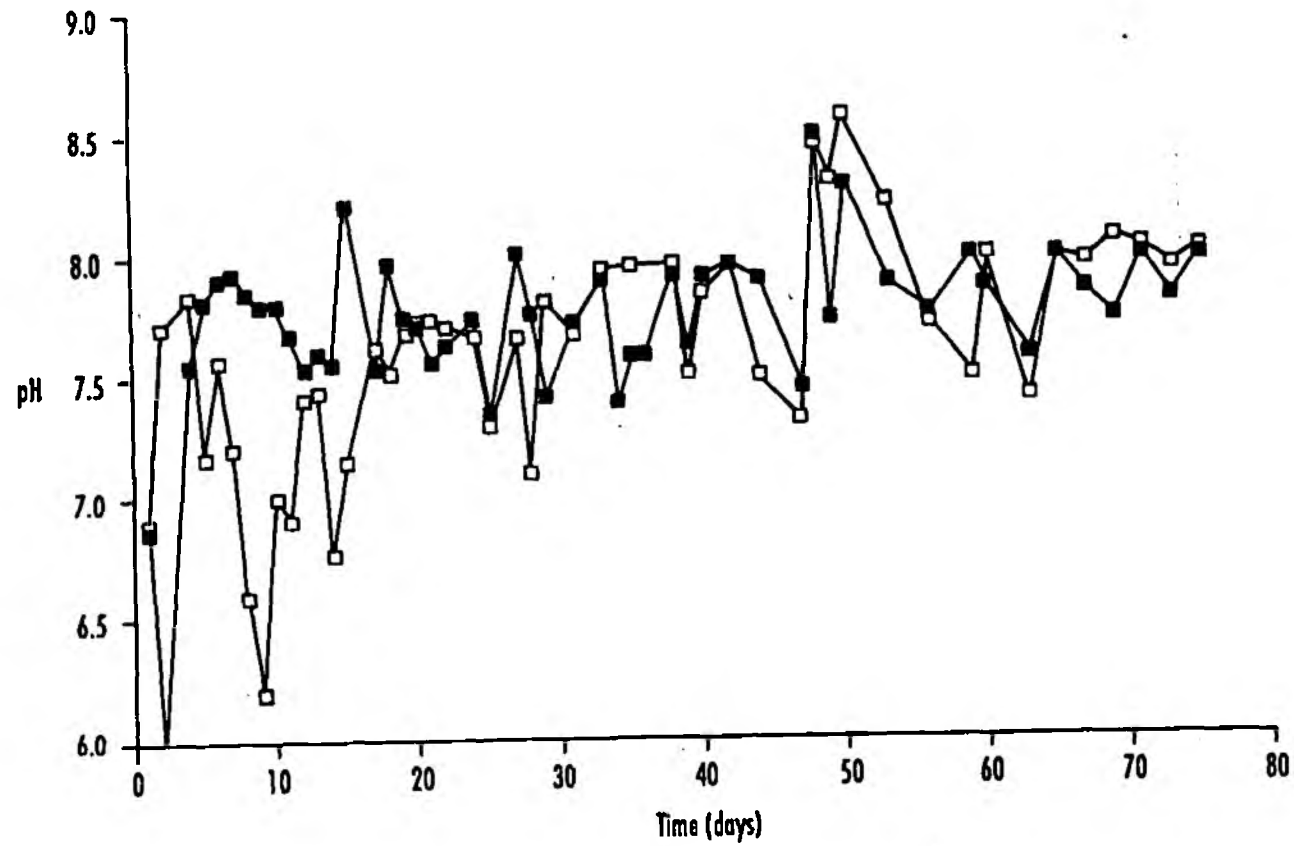

Figure 3.3 pH measurement during operation of chemostats # 1 and # 2 (days 0-75). ■ chemostat # 1, □ chemostat # 2.

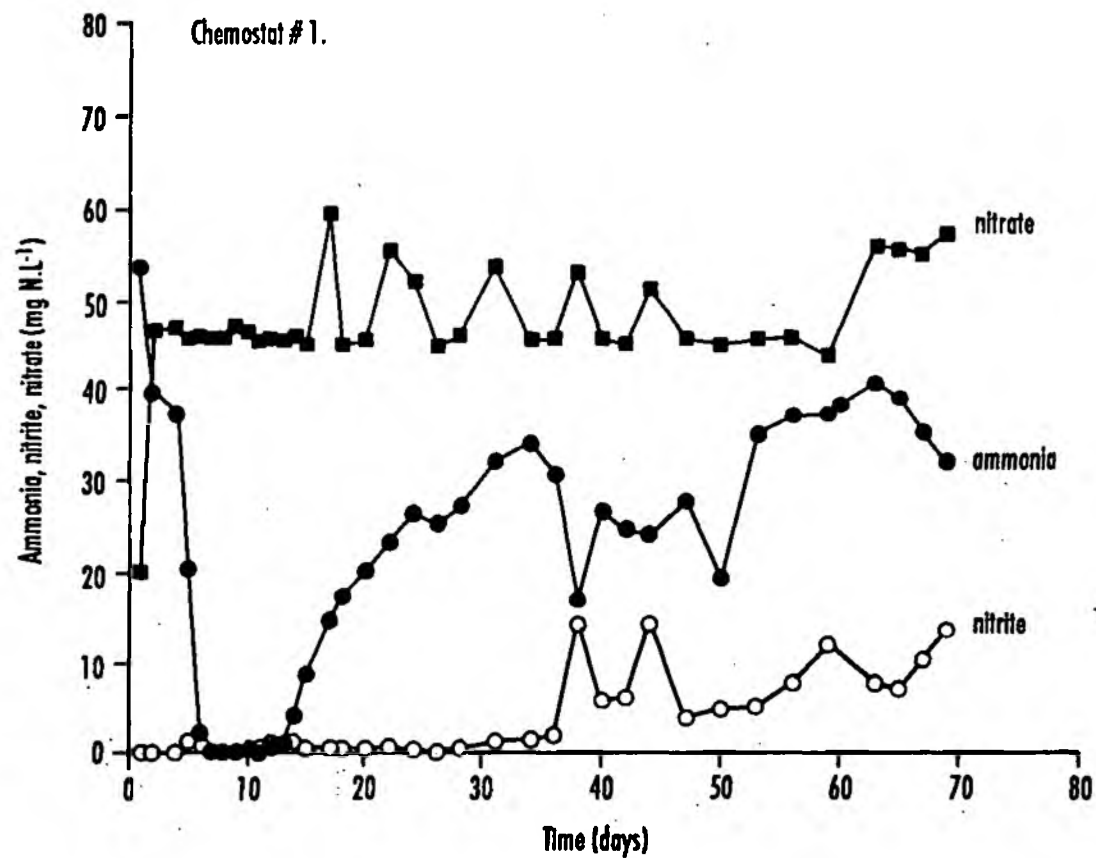

Figure 3.4 Changes in ammonia, nitrite and nitrate concentrations in chemostat # 1 when operated in continuous culture with $(NH_4)_2SO_4$ as N-source at 20°C. $D = 0.004\ hr^{-1}$. ● ammonia, ○ nitrite, ■ nitrate.

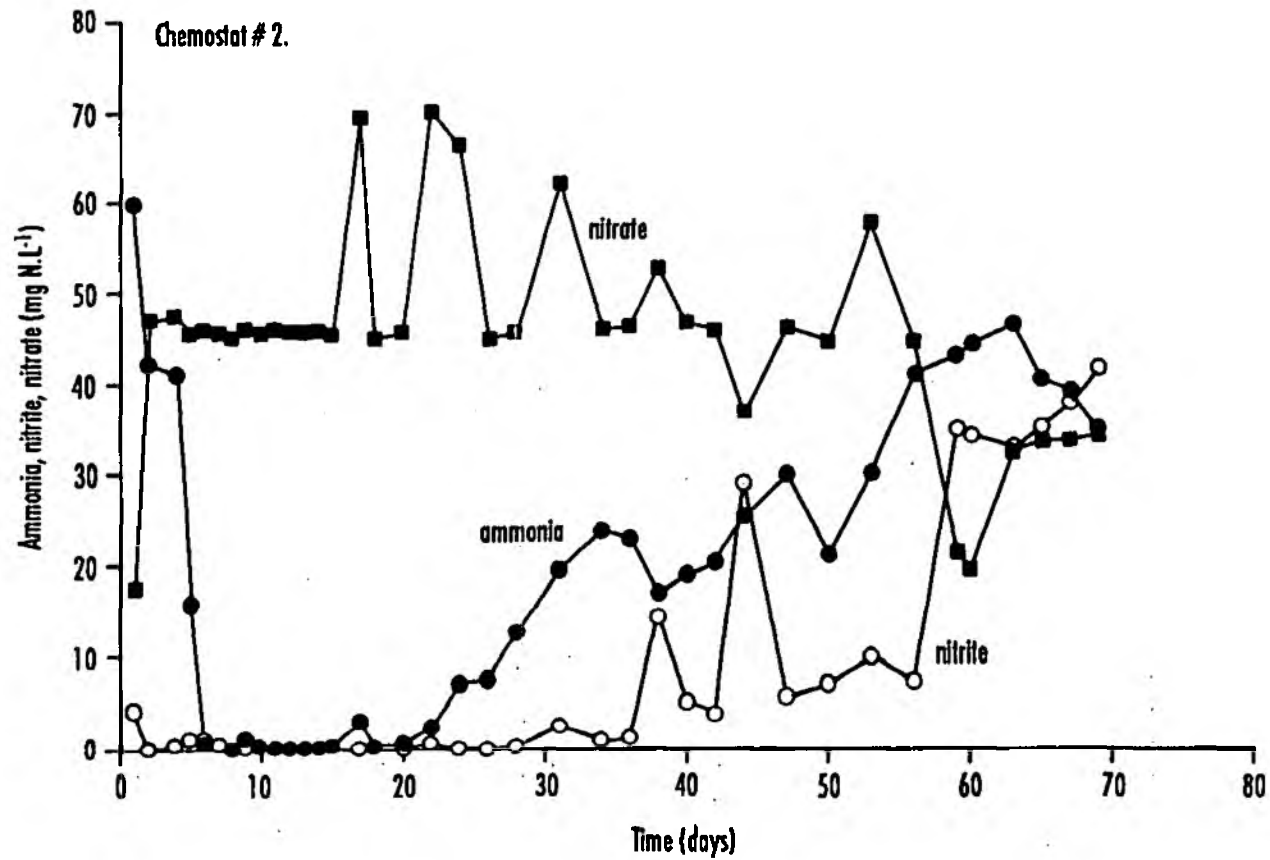

Figure 3.5 Changes in ammonia, nitrite and nitrate concentrations in chemostat # 2 when operated in continuous culture with $(NH_4)_2SO_4$ as N-source at 20°C. $D = 0.0038\ hr^{-1}$. ● ammonia, ○ nitrite, ■ nitrate.

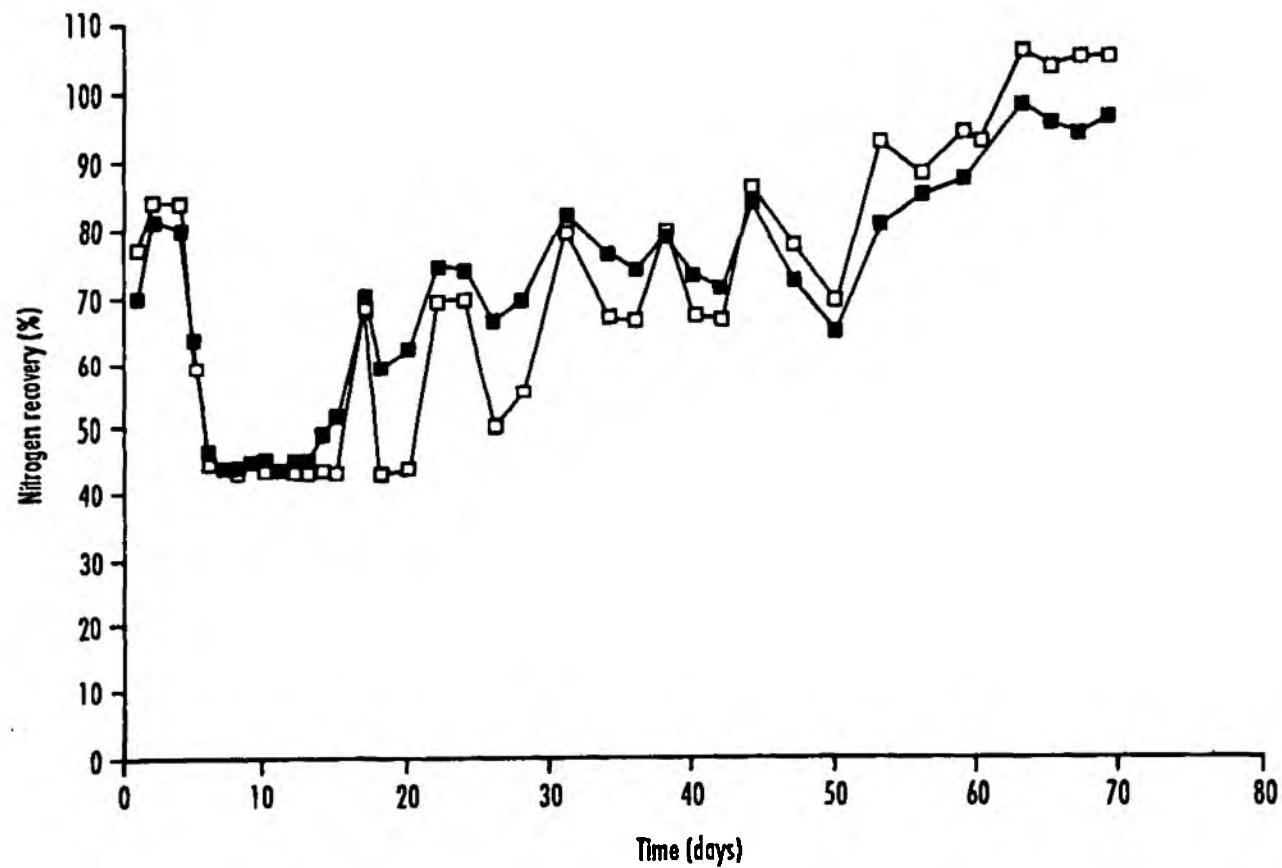

Figure 3.6 Total nitrogen recovery measured in chemostats # 1 and # 2 during continuous culture with $(NH_4)_2SO_4$ as N-source at 20°C. D = 0.004 hr^{-1} (# 1), 0.0038 hr^{-1} (# 2). ■ chemostat # 1, □ chemostat # 2.

4. DISCUSSION

4.1 Continuous culture studies

The initial aim of this present study is to establish a stable bacterial coculture capable of performing simultaneous oxidation of ammonia to nitrite and nitrite to nitrate. Future use of this coculture is to provide a consistent supply of nitrifying bacteria for assessing the effect of environmental factors on the rate of nitrification. Data presented in this report show that the establishment of a stable coculture is a slow process. This is not surprising since it is widely accepted that an inevitable consequence of growth on inorganic compounds is a relatively low growth yield. Generation times of nitrifiers are generally measured in hours even under ideal conditions, and are almost certainly in the order of days in most soils and sediments (Watson *et al* 1974).

It is clear from the data presented that the inhibition of nitrite oxidation results in the accumulation of nitrite as a consequence of continued ammonia oxidation. The oxidation of nitrite has been shown to be inhibited by the concentration of nitrite only in the presence of a high concentration of ammonia (Gee *et al* 1990). Nitrite formation from ammonia is accompanied by a release of hydrogen ions that decrease the pH to an extent related to the buffering capacity of the system (Anthonisen *et al* 1976). The media used in this study has limited buffering capacity as shown by the continual requirement for extra carbonate addition. Furthermore, there is no provision in the media for the inclusion of trace metals which are essential for the growth of nitrite oxidising bacteria. These facts, coupled to the high concentration of residual ammonia, may account for the low activity of the nitrite oxidising bacteria present in chemostat No.2.

The results to date suggest that the complete oxidation of ammonia is limited by the concentration of ammonia and nitrite oxidisers present within each chemostat. Since the rate of removal of ammonia is a function of active bacterial concentration, it is not surprising that complete ammonia oxidation was rapidly achieved at the start of the experiment in the presence of a large inoculum. Consequently, washout of the seeding material would result in a decrease in the extent of ammonia oxidation, as observed in both chemostats. The maximum ammonia and nitrite oxidation rates, derived from previous continuous culture experiments, are approximately 2 and 5 mg-N/mg volatile suspended solids/day, respectively (Gee *et al* 1990). The application of these substrate utilisation rates to this present study would predict that a twofold increase in the bacterial concentration is required to achieve complete ammonia removal within each chemostat.

4.2 Future Studies

4.2.1 Evaluation of techniques to determine the rate of nitrification

Very few methods have been developed for direct evaluation of nitrification rates. To date, three isotope techniques have been employed to carry out these measurements: [^{15}N]nitrate dilution, N-serve sensitive [^{14}C]bicarbonate incorporation, and [^{15}N]ammonium oxidation to nitrite and nitrate. Each of the three methods are known to

have disadvantages. It has been suggested by Enoksson (1986) that either of the latter two methods be used, separately, or in conjunction, for accurate estimation of nitrification rates. An alternative non-radioactive method may be employed based on the measurement of ammonia, nitrite and nitrate before and after an optimised incubation period. It is common practice to develop any such methodology using bacterial cultures cultivated under laboratory-controlled conditions before use with environmental samples. In our study, the chemostats will be used to provide a source of nitrifying bacteria for assessing the suitability and practicality of both the [^{14}C]bicarbonate isotope technique, and the direct non-radioactive measurement of ammonia reduction.

4.2.2 Design of experimental procedure for determining the influence of various factors on nitrification

The provision of continuously cultured bacteria for the study of biogeochemical processes has been commonly practised over a number of decades. The extrapolation of data derived from artificial laboratory experiments is, however, always questionable. To understand the role of biological processes in any biogeochemical process in a given ecosystem, dynamical data, i.e. estimations of the fluxes of matter associated with these processes, are required. Only measurements of the *in situ* rates of these biological activities can provide these data. The effect of environmental factors was originally envisaged to be carried out using small batch culture experiments, with bacteria originating from the chemostats. The applicability of results obtained from such experiments will always be subject to debate, as stated above. It is proposed to meet the requirements of the NRA, as stated in the study objectives, by broadening the scope of the present study and detailing the effect of environmental factors on environmental samples. The use of environmental over laboratory derived samples will have the following advantages:

- Nitrification rate measurements will be performed using bacteria adapted to their environment. Bacteria cultured in the laboratory may not represent species found in the environment;
- The rate of nitrification will be measured in the presence of all bacteria involved in biological oxidation and reduction reactions;
- A change in the environmental conditions will affect all trophic groups present in an environmental sample. The interdependence of bacterial species involved in biogeochemical cycling will not be reflected using specifically derived laboratory cultures;
- Valid comparisons can be made between environmental samples whereas laboratory derived samples have limited reference;
- The complexity of models to predict the fate of nitrogenous compounds in the environment can only be fully understood using environmental samples.

In a previous report entitled "Determination of Nitrification Rates in the Tidal Mersey" (Bealing *et al* 1990) it was concluded that no single physical or chemical parameter emerged as a clearly dominant factor controlling nitrification rates. The validity of nitrification rate data presented in this previous report could not be verified since parallel

determinations based on either oxygen or [^{14}C]bicarbonate uptake gave significantly different results. It is clear from the previous report that laboratory studies are first required to determine the precision and sensitivity of nitrification rate measurements before use with environmental samples. As stated above, chemostats will be used to provide a source of nitrifying bacteria for assessing the suitability and practicality of two nitrification rate measurement techniques.

To determine the influence of various factors on nitrification, it is proposed to use environmental samples obtained from three fixed points along the estuary and tidal reaches of the Mersey corresponding to freshwater, intertidal and marine zones as previously reported (Bealing *et al* 1990). Freshwater samples will be taken from Walton Bridge, intertidal samples from Oglet/Ince, and marine samples from New Brighton. Samples will be taken from the water column at each location and analysed for ammonia concentration, dissolved oxygen, temperature, salinity and suspended solids content. Site specific data generated in the study conducted by Bealing *et al* (1990) will provide valuable reference during this study. On return to the laboratory, prior to nitrification rate measurements, samples will be altered with respect to the environmental factors under study as follows:

- NH_4^+ concentration ranging from measured value to 100 mg l^{-1}
- Dissolved oxygen ranging from measured *in situ* value to full saturation;
- Temperature ranging from 4 to 30 °C;
- Salinity ranging from measured value to 35°/oo;
- Suspended solids ranging from measured value to a 10-fold increase achieved by sample filtration and resuspension;
- Light ranging from 0 to 420 lux.

To provide a measure of the specific activity of each sample, estimation of ammonia and nitrite oxidisers will be carried out using most probable number techniques as previously described (Bealing *et al* 1990).

Data collected from this proposed study may provide a valuable comparison to two other related programmes currently underway in the UK:

1. JoNuS Programme (Joint Nutrient Study)

   Funded by the Department of the Environment, and collaborating with Anglia NRA, MAFF (Lowestoft), University of Essex, University of East Anglia and Plymouth Marine Laboratory.

   The work is being carried out in the River Ouse, flowing into the Wash. The University of Essex are responsible for examining sediment/water column exchange of nutrients, and the fate of the N compounds in the sediment i.e. nitrification/denitrification. In this study the water column in the estuary is not

being examined. Techniques for measuring the processes include the use of selective inhibitors and ^{15}N tracer techniques.

2. NERC Programme

The University of Essex are investigating N fluxes along the Colne estuary. This study is concentrated on the processes of nitrification and denitrification in the sediment, and sediment/water exchange of N compounds. Particular emphasis has been placed on the formation of N-gases (N_2, N_2O) during denitrification.

Collaboration with other researchers at the University of Essex would provide a valuable comparison with regard to a) techniques employed for the determination of nitrification rate measurements, b) the nitrification rates measured within the sediment, and c) the influence of denitrification on the rate of nitrification.

5. RECOMMENDATIONS

In order to obtain a stable bacterial population for the evaluation of nitrifying activity measurements, provision will be made for the inclusion of both automatic pH control to both chemostats, and the addition of trace metals to the medium in accordance with Schmidt and Belser (1981).

It is further recommended that in order to meet the operational requirements of the NRA, set out earlier in the study objectives, emphasis should shift to determining the effect of environmental factors on environmental samples in place of laboratory enriched cultures.

Collaboration with other researchers in the UK is suggested to provide a valuable comparison with regard to a) techniques employed for the determination of nitrification rate measurements, b) the nitrification rates measured within the sediment, and c) the influence of denitrification on the rate of nitrification.

REFERENCES

Anon. (1971) Nitrification in the BOD test. *Notes on Water Pollution* **52.** Water Pollution Research Laboratory, Stevenage, UK.

Anthonisen C., Loehr R.C., Prakasam T.B.S. and Srinath E.G. (1976) Inhibition of nitrification by ammonia and nitrous acid. *Journal of Water Pollution Control Federation* **48**, 835-851.

Antoniou P., Hamilton J., Koopman B., Jain R., Holloway B., Lyberatos G. and Svoronos S.A. (1990) Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. *Water Research* **24**, 97-101.

Barcia J. (1991) Deformation of the nitrogen cycle in a lake receiving excessive loadings of ammonia. *Environmental Pollution* **1**, 795-801.

Bealing D.J., Wilson V. and Sidorowicz S.V. (1990) Determination of nitrification rates in the tidal Mersey. Final report to North West Water February 1989 to March 1990. WRc Report No. UC 828.

Belser L.W. (1979) Population ecology of nitrifying bacteria. *Annual Revue of Microbiology* **33**, 309-333.

Bragstad R.E. and Bradney L. (1937) Packing house waste and sewage treatment at Sioux Falls, South Dakota. *Sewage Works Journal* **9**, 959.

Campbell A.E., Hellebust J.A. and Watson S.W. (1966) Reductive pentose phosphate cycle in *Nitrocystis oceanus*. *Journal of Bacteriology* **91**, 1178-1185.

Chartrain M., Rizet M. and Faup G. (1983) Isolation of *Nitrobacter* from sewage and drinking water. *Water Research* **17**, 831-832.

Curtis E.J.C., Durrant K. and Harman M.M. (1975) Nitrification in rivers in the Trent Basin. *Water Research* **9**, 255-268.

Downing A.L. and Scragg L.J. (1958) The effect of synthetic detergents on the rate of aeration in diffused air activated sludge plants. *Water and Waste Treatment Journal* **7**, 102.

Engel M.S. and Alexander M. (1958) Growth and autotrophic metabolism of *Nitrosomonas europaea*. *Journal of Bacteriology* **76**, 217.

Enoksson V. (1986) Nitrification rates in the Baltic Sea: comparison of three isotope techniques. *Applied and Environmental Microbiology* **51**, 244-250.

Gardiner W.S., Seitzinger S.P. and Malczyk J.M. (1991) The effect of sea salts on the forms of nitrogen released from estuarine and freshwater sediments: Does ion pairing affect ammonia flux? *Estuaries* **14**, 157-166.

Gee C.S., •Suidan M.T. and Pfeffer J.T. (1990) Modelling of nitrification under substrate-inhibiting conditions. *Journal of Environmental Engineering* **116**, 18-31.

Hall I.R. (1974) Some studies on nitrification in the activated-sludge process. *Water Pollution Control* **73**, 538-547.

Helder W. and DeVries R.T.P. (1983) Estuarine nitrite maxima and nitrifying bacteria (Ems-Dollard Estuary). *Netherlands Journal of Sea Research* **17**, 1-18.

Henze M., Grady C.P.L., Gujer W., Marais G.R. and Matsuo T. (1986) Final Report: Activated sludge model. *IAWPRC Scientific and Technical Reports*, **1**.

Hooper A.B. and Terry K.R. (1973) Specific inhibitors of ammonia oxidation in *Nitrosomonas*. *Journal of Bacteriology* **115**, 480-485.

Hopwood A.B. and Downing A.L. (1965) Factors affecting the rate of production and properties of activated sludge in plants treating domestic sewage. *Journal of the Proceedings of the Institute of Sewage Purification* **64**, 435-452.

Huang C.S. and Hopson N.E. (1974) Nitrification rate in biological processes. *Journal of Environmental Engineering. Division of the American Society of Civil Engineers* **100**, 409-422.

Jenkins S.H., Keicht D.G., Snaddon X.V.M. and James K. (1967) Large-scale treatment of sewage at Minworth works from 1961 to 1966. *Journal of the Proceedings of the Institute of Sewage Purification* **66**, 334-361.

Knowles G., Downing A.L. and Barrett M.J. (1965) Determination for kinetic constants for nitrifying bacteria in mixed culture with the aid of an electronic computer. *Journal of General Microbiology* **38**, 263-278.

Loveless J.E. and Painter H.A. (1968) The influence of metal ions and pH value on the growth of *Nitrosomonas* strain isolated from activated sludge.
Journal of General Microbiology **52**, 1-14.

Matulewich V.A. and Finstein M.S. (1978) Distribution of autotrophic nitrifying bacteria in a polluted river (the Passaic). *Applied and Environmental Microbiology* **35**, 67-71.

O'Conner D.J. (1967) The temporal and spatial distribution of dissolved oxygen in streams. *Water Resources* **3**, 65-78.

Painter H.A. and Loveless J.E. (1983) Effect of temperature and pH value on the growth-rate constants of nitrifying bacteria in the activated-sludge process. *Water Research* **17**, 237-248.

Peeters T.L., Van Goal A.D. and Laudelout H. (1976) Kinetics study of oxygen limited respiration in nitrifying bacteria. *Bacterial Proceedings* **141**.

Quinlan A.V. (1980) The thermal sensitivity of nitrification as a function of the concentration of the nitrogen substrate. *Water Research* **14**, 1501-1507.

Ruane R.J. and Krenkel P.A. (1978) Nitrification and other factors affecting nitrogen in the Holston River. *Journal of the Water Pollution Control Federation* **50**, 2016-2028.

Schmidt E.L. and Belser L.W. (1981) Nitrifying bacteria. In: *Methods of Soil Analysis, Part 2 - Chemical and Microbiological Properties. Second Edition* ASA_SSSA, Madison, Wisconsin, USA.

Schultz D.J. (1988) Nitrogen dynamics in the tidal freshwater Potomac River Maryland and Virginia, Water Years 1979-1981. US Geological Survey, Water Supply Paper 2234-J.

Schwert D.P. and White J.P. (1974) Method for *in-situ* measurement of nitrification in a stream. *Applied Microbiology* **28**, 1082-1083.

Soriano S. and Walker N. (1968) Isolation of ammonia-oxidising bacteria. *Journal of Applied Bacteriology* **31**, 493-497.

Stenstrom M.K. and Poduska R.A. (1980) The effect of dissolved oxygen concentration on nitrification. *Water Research* **14**, 643-649.

Stratton F.E. and McCarty P.L. (1967) Prediction of nitrification effects on the dissolved oxygen balance of streams. *Environmental Science and Technology* **1**, 405-410.

Suthersan S. and Ganczarczyk J.J. (1986) Inhibition of nitrite oxidation during nitrification - some observations. *Water Pollution Research Journal of Canada* **21**, 257-266.

Van Rijn J., Diab S. and Shilo M. (1987) Phytoplankton succession in relation to the nitrogen regime in shallow brackish-water fishponds. *Archive für Hydrobiologie* **111**, 183-196.

Warwick J.J. and Shetty P. (1988) In-stream nitrification rate prediction. *Water Research* **22**, 723-732.

Watson S.W., Valois F.W. and Waterbury J.B. (1974) The family *Nitrobacteraceae*. In: *The Procaryotes*. Edited by M.P. Starr, H. Stolp, H.G. Truper, A. Balows and H.G. Schlegal. 1005-1022. Springer-Verlag. New York.

Wezernak C.T. and Gannon J.J. (1968) Evaluation of nitrification in streams. *Journal of Sanitation Engineering. Division of the American Society of Civil Engineers* **94**, 827-837.

Wuhrman K. (1963) Effect of oxygen tension on biochemical reactions in sewage purification plants. *Proceedings of the 3rd Manhattan Conference on Advances in Biological Waste Treatment*. MacMillan. New York.