Dissimilatory Nitrate Reduction and Nitrification in Estuarine Sediments

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Enumeration of populations of nitrate respiring bacteria in estuarine sediments of the River Tay, Scotland, showed that bacteria capable of dissimilating NO_3^- to NH_4^+ predominated over those denitrifying NO_3^- to N_2 . On the other hand, seasonal data and depth profile studies, using $^{15}\text{NO}_3^-$, showed that denitrification was the principal route of dissimilatory NO_3^- reduction (78–90% of NO_3^- respired), with maximum rates of both processes occurring in the summer. Population densities of both populations of NO_3^- respiring bacteria were highest in the 0–2 cm horizon in Tay estuary mud-flats where maximum rates of NO_3^- respiration were also recorded [$28.56 \mu\text{g N d}^{-1}$ (g dry wt sediment) $^{-1}$]. Autotrophic nitrification rates in Tay estuary sediments showed a distinct seasonality, highest rates [$0.93 \mu\text{g N d}^{-1}$ (g dry wt sediment) $^{-1}$] occurring during the summer. Nitrification rates declined rapidly with sediment depth and were not detectable below the oxidized zone (3 cm). Population densities of autotrophic NH_4^+ and NO_2^- oxidizing bacteria followed a similar pattern of distribution. Heterotrophic nitrification appears to play an insignificant role in Tay estuary sediments.

INTRODUCTION

The respiratory reduction of NO_3^- to NH_4^+ by bacteria in estuarine and inshore marine sediments has been considered by a number of workers to be potentially important (Koike & Hattori, 1978*a*; Sørensen, 1978; Herbert *et al.*, 1980; Macfarlane & Herbert, 1982). The ecological significance of this process is that the NH_4^+ produced as the end-product of nitrate respiration conserves nitrogen in a readily utilizable form, whereas the alternative process, denitrification, leads to the production of gaseous products (N_2O , N_2) from nitrate, and these are rapidly lost from the sediment.

The series of nitrogen intermediates involved in the anaerobic dissimilation of nitrate to ammonia are the reverse of those involved in nitrification, the aerobic oxidation of ammonia to nitrate by nitrifying bacteria. Sharp discontinuities in O_2 tension occur in marine and estuarine sediments over extremely small vertical distances or within micro-niches and so it is probable that both nitrification and nitrate respiration may proceed simultaneously. Experimental evidence to support this hypothesis has been provided by several workers. Koike & Hattori (1978*b*) using ^{15}N -dilution techniques demonstrated the simultaneous processes of nitrification and nitrate respiration (denitrification and NO_3^- dissimilation to NH_4^+) in Japanese marine sediments, whilst Grundmaris & Murray (1977) reported that nitrate produced by nitrification in oxidized sediments in Puget Sound was subsequently denitrified in the deeper anoxic zone.

The objectives of the present study were to investigate the relationships between populations of nitrate respiring (denitrifying bacteria and those respiring NO_3^- to NH_4^+) and nitrifying bacteria in estuarine sediments in respect of the physico-chemical factors which influence their activities.

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Abbreviation: MPN, most probable number.

METHODS

Physical characteristics of Kingoodie Bay sediments. Measurements of pH, E_h , temperature, salinity and dissolved O_2 tension were made *in situ* in 1 cm increments from the sediment surface to a depth of 5 cm, at monthly intervals at Kingoodie Bay, Tay estuary, NE Scotland. Depth profiles of pH and E_h were determined using a Pye model 293 pH meter and appropriate pH and E_h electrodes (Russell pH, Auchtermuchty, Fife, Scotland) which had been previously calibrated against reference standards. Dissolved O_2 concentrations were measured using an EIL O_2 electrode coupled to an EIL model 15A dissolved oxygen meter.

Chemical characteristics of Kingoodie Bay sediments. Sediment samples were taken at monthly intervals from Kingoodie Bay using a sterile perspex corer as previously described (Herbert, 1975) and returned to the laboratory within 30 min of collection. The sediment cores were aseptically sectioned into 1 cm segments, from the surface to a depth of 5 cm. NO_3^- , NO_2^- and NH_4^+ in the sediment sections were extracted using 1 M-KCl, according to the method of Sørensen (1978). Total NH_4^+ (soluble, interstitial NH_4^+ and adsorbed exchangeable NH_4^+) was determined by the phenol/hypochlorite method (Solorzano, 1969), NO_2^- according to the method of Barnes & Folkard (1951) and NO_3^- by the method of Elliot & Porter (1971).

Enumeration of nitrate respiring and nitrifying bacteria in Kingoodie Bay sediments. Sediment samples were taken at monthly intervals and sectioned as previously described. From each sediment horizon, sub-samples were removed and serial ten-fold dilutions prepared in sterile one-quarter strength Ringer's solution.

Population densities of bacteria respiring nitrate to gaseous products (denitrification) and those dissimilating nitrate to NH_4^+ were determined using the most probable number (MPN) technique (Alexander, 1965). Nitrate nutrient broth (nutrient broth + 10 mM-KNO₃) was used to enumerate denitrifying bacteria and gas production was demonstrated by the inclusion of Durham tubes. Bacteria dissimilating NO_3^- to NH_4^+ were enumerated using the medium of Jones *et al.* (1982); the tubes were incubated under anaerobic conditions for 7 d at 15 °C and NH_4^+ production was confirmed in the positive tubes with Nessler's reagent.

The micro-MPN method described by Rowe *et al.* (1977) was used to determine population densities of autotrophic NH_4^+ and NO_2^- oxidizing bacteria and heterotrophic nitrifiers. Sediment samples were prepared as previously described and used to inoculate the wells of 8 × 12, sterile micro-titre plates (Corning, New York, USA). The NH_4^+ /bicarbonate medium of Alexander & Clark (1965) was used to enumerate the NH_4^+ oxidizing populations, whilst the medium of Aleem & Alexander (1958) was used for the NO_2^- oxidizers. The micro-titre plates were incubated at 30 °C for 28 d and to reduce desiccation were placed in plastic bags. Heterotrophic nitrifying bacteria were enumerated using the medium of Gode & Overbeck (1972) with acetate as C-source. N-serve (2-chloro-6-trichloromethyl pyridine) was added at a concentration of 10 mg l⁻¹ to inhibit autotrophic nitrifying bacteria and the plates were incubated for 14 d at 30 °C. Following incubation for the appropriate time period the MPN micro-titre plates were spot tested for NO_2^- production/disappearance using Griess-Ilosvay's reagents (BDH). Production of NO_2^- in autotrophic and heterotrophic micro-titre plate wells was taken as positive for NH_4^+ oxidation; conversely, the disappearance of NO_2^- from the NO_2^- oxidizer wells was taken as positive for autotrophic NO_2^- oxidation. Uninoculated wells were included as controls.

Identification of the dominant nitrate respiring bacteria. The dominant nitrate respiring bacteria from Kingoodie Bay sediments were isolated and identified by methods described by Macfarlane & Herbert (1982). The end-products of nitrate respiration were determined in cultures grown anaerobically in the medium of Jones *et al.* (1982) under a gas atmosphere of 90% H₂/10% CO₂. Gas production was determined in Durham tubes whilst NO_2^- and NH_4^+ in the spent media were determined using Griess-Ilosvay's and Nessler's reagents, respectively.

Determination of rates of nitrate respiration using $^{15}NO_3^-$. Sediment samples were obtained and sectioned as previously described. Sub-samples (about 5 g) from each horizon were transferred into a triplicate series of 15 ml serum bottles, closed with suba-seals and flushed with argon for 5 min. Finally, 100 µl Na¹⁵NO₃ (99 atom % ¹⁵N) was injected into the bottles to give a NO_3^- concentration of approximately 2 µmol (g sediment)⁻¹ and the contents were thoroughly mixed on a rotamixer prior to incubation in the dark at 15 °C. Samples treated with 1 ml 0.1 M-HgCl₂ immediately after the addition of ¹⁵NO₃ served as controls.

The rate of denitrification was determined by measuring the production of ¹⁵N gas. Using a gas-tight syringe, 100 µl samples from each bottle were injected into the inlet of a VG Micromass MM 601 mass spectrometer. To determine the rate of ¹⁵NH₄⁺ production the sediment slurries were extracted with 5 ml 1.0 M-KCl as described by Sørensen (1978). NH_4^+ was separated and collected by steam distillation according to the method of Bremner & Keeney (1965). The NH_4^+ in the distillate was oxidized to N₂ by the Dumas method (Vose, 1980). The total quantity of NH_4^+ in the distillate was determined as described above.

Determination of nitrification rates in Kingoodie Bay sediments. Sediment samples were obtained and sectioned as described above. Nitrification rates were determined in the presence and absence of 5 mg N-serve l⁻¹ (final concentration), to discriminate between autotrophic and heterotrophic nitrification, using the [¹⁴C]bicarbonate dark uptake method described by Billen (1975).

Chemicals. N-serve was a gift from the Dow Chemical Co., and Na¹⁵NO₃ was obtained from BOC Prochem, London, UK. All other chemicals were obtained from BDH.

Table 1. *Typical physico-chemical profiles recorded in Kingoodie Bay sediments during February and July 1982*

Results are mean values of three samples.

Depth (cm)	Month	pH value	E_h value (mV)	O_2 concn (mg l ⁻¹)	Concn (nmol ml ⁻¹):		
					NH_4^+	NO_2^-	NO_3^-
0-1	Feb	6.9	+390	8.8	110	20	49
	Jul	6.4	+320	8.7	50	19	95
1-2	Feb	7.0	+310	8.1	128	ND	40
	Jul	6.9	+200	7.0	460	25	85
2-3	Feb	7.2	+200	5.7	256	ND	10
	Jul	7.1	+50	4.7	510	20	74
3-4	Feb	7.4	+39	3.3	265	ND	ND
	Jul	7.3	-100	1.1	516	12	21
4-5	Feb	7.5	-60	ND	280	ND	ND
	Jul	7.4	-190	ND	530	ND	ND

ND, Not detected.

RESULTS

Physico-chemical characteristics of Kingoodie Bay sediments

The surface sediments in Kingoodie Bay were markedly more oxidized during the winter months due to a combination of lowered microbial activity, as a consequence of low temperatures (mean temperature 4.5 °C), and increased resuspension and oxygenation of the sediments (Table 1). Below 4 cm the sediments were reduced and anoxic. The total NH_4^+ concentration was high compared with those for NO_3^- and NO_2^- and increased rapidly with depth. In contrast, NO_3^- concentrations were highest at the surface and decreased with depth. These data indicate that *in situ* O_2 and NO_3^- respiration were localized in the upper 0-4 cm horizon of these sediments, and this conclusion is substantiated by data on the spatial distribution of nitrifying and NO_3^- respiring bacteria (Figs 1 and 2).

Spatial and seasonal distribution of NO_3^- respiring and nitrifying bacteria

Cell population densities of autotrophic and heterotrophic nitrifying bacteria in Kingoodie Bay sediments showed a marked seasonality, with two distinct peaks in cell numbers of autotrophic organisms being recorded in early spring and July (Fig. 1). Both increases in cell populations were different in character. The increase in populations of NH_4^+ oxidizing bacteria in the spring was followed by a smaller increase in the NO_2^- oxidizing population, suggesting that the oxidation of NH_4^+ to NO_2^- stimulated the growth of these bacteria. However, in the summer, populations of both groups of bacteria increased concurrently with NO_2^- oxidizers being present, if anything in greater numbers. These results indicate that a source of NO_2^- other than that derived from autotrophic NH_4^+ oxidation may be involved. Depth profiles of autotrophic nitrifying bacteria showed that highest cell populations were present in the 0-2 cm horizon of the sediments and below this depth there was a rapid decrease in numbers. Population densities of heterotrophic nitrifying bacteria were always lower than those recorded for the autotrophs; this supports the view that these bacteria play an insignificant role in nitrification processes in estuarine sediments. This conclusion was further supported by the finding that the addition of 5 mg N-serve l⁻¹ almost totally inhibited nitrification irrespective of whether it was measured by the [¹⁴C]bicarbonate dark uptake method or NO_2^- production.

Populations of those bacteria dissimilating NO_3^- to NH_4^+ were always substantially greater (up to a factor of 10²) than those denitrifying NO_3^- to gaseous products (Fig. 2). In general, population densities of the nitrate respiring bacteria were more stable and less variable, on a seasonal basis, than those recorded for the autotrophic nitrifying bacteria. Depth profiles of nitrate dissimilating bacteria in Kingoodie Bay sediments showed that during the winter

Fig. 1. Populations of autotrophic NH_4^+ oxidizing (a), NO_3^- oxidizing (b) and heterotrophic nitrifying bacteria (c) in Kingoodie Bay sediments, December 1981 to October 1982. ●, 0–1; ■, 2–3; ▲, 4–5 cm depth.

Fig. 2. Populations of NO_3^- dissimilating (a) and denitrifying (b) bacteria in Kingoodie Bay sediments, December 1981 to October 1982. ●, 0–1; □, 1–2; ■, 2–3; ○, 3–4; ▲, 4–5 cm depth.

months maximum cell populations were present in the 1–2 cm horizon but during the spring and summer months there was an apparent migration into the 0–1 cm horizon. These data are in agreement with the recorded changes of the redox (E_h) profiles of the sediments (Table 1) in which the surface layers became progressively more reduced during the summer.

Fig. 3. Nitrifying activity in Kingoodie Bay sediments, December 1981 to October 1982. ●, 0–1; □, 1–2; ■, 2–3 cm depth.

Table 2. Generic distribution and products of NO_3^- reduction by dominant NO_3^- respiring bacteria isolated from Kingoodie Bay sediments

Bacterial groups were characterized according to the scheme of Dunn *et al.* (1980). One hundred colonies were tested.

Bacterial group	No. of colonies	No. of colonies with products of NO_3^- respiration:		
		NH_4^+	NO_2^-	N_2 and/or N_2O
<i>Aeromonas/Vibrio</i>	50	50	0	0
<i>Enterobacteria</i>	8	8	0	0
<i>Pseudomonas</i>	15	0	3	12
<i>Bacillus</i>	4	0	3	1
<i>Micrococcus</i>	2	0	1	1
<i>Alcaligenes</i>	21	0	3	18

End-products produced by NO_3^- respiring bacteria

Sediment samples were diluted 10-fold in one-quarter Ringer's solution. Volumes (0.1 ml) were then plated out on the corresponding agar medium and from these, 100 isolates were selected at random. The dominant NO_3^- dissimilating bacteria (50% of the isolates) were identified, on the basis of limited morphological and biochemical tests, as belonging to the genera *Aeromonas/Vibrio* (Table 2). Ammonia was the principal product of dissimilatory NO_3^- reduction by these bacteria as with the eight isolates identified as members of the family *Enterobacteriaceae*. Fluorescent pseudomonads (15 isolates) and *Alcaligenes* spp. (21 isolates) were the dominant denitrifying bacteria isolated and they produced N_2 and/or N_2O as the end products of nitrate respiration. These results are in good agreement with those obtained by Dunn *et al.* (1980) and demonstrate the predominance of fermentative bacteria capable of respiring NO_3^- in these sediments.

Nitrification rates in Kingoodie Bay sediments

Depth profiles of nitrifying activity in Kingoodie Bay sediments, as measured by the indirect [^{14}C]bicarbonate uptake method, showed that maximum rates occurred in the 0–1 cm horizon and decreased markedly with depth (Fig. 3). No significant nitrifying activity could be detected at depths greater than 3 cm and these results are in agreement with the bacterial counts (Fig. 1). Maximum rates of nitrification [$0.93 \mu\text{g N d}^{-1} (\text{g dry wt sediment})^{-1}$] occurred during the summer months when the sediments were warmest (19°C), whereas lowest activities were recorded during the winter (mean temperature 5°C). Maximum rates of nitrification occurred

Table 3. *Rates of denitrification and NO_3^- dissimilation to NH_4^+ in Kingoodie Bay sediments as determined using $^{15}\text{NO}_3^-$*

Rates are expressed as $\mu\text{g N d}^{-1}$ (g dry wt sediment) $^{-1}$ and are mean values of three samples.

Depth (cm)	Nov 1981			Mar 1982			July 1982			Oct 1982		
	N_2	NH_4^+	Total	N_2	NH_4^+	Total	N_2	NH_4^+	Total	N_2	NH_4^+	Total
0-1	6.2	1.1	7.3	12.5	2.7	15.2	18.9	4.6	23.5	15.4	4.7	20.1
1-2	9.7	2.1	11.8	16.1	4.6	20.7	24.4	4.1	28.5	18.0	5.9	23.9
2-3	5.2	6.1	11.3	12.4	2.8	15.2	11.6	3.5	15.1	8.0	1.7	9.7
3-4	2.2	1.3	3.5	8.4	4.6	13.0	4.7	2.4	7.1	6.5	2.8	9.3
4-5	2.3	1.0	3.3	3.1	2.7	5.8	4.4	1.7	6.1	2.7	3.0	5.7

during the summer months and showed little correlation with seasonal maxima for autotrophic NH_4^+ and NO_2^- oxidizing bacteria (Fig. 1), indicating that temperature probably exerts a greater influence on nitrifying activity than on cell numbers. The addition of 5 mg N-serve l^{-1} totally inhibited nitrification, indicating that heterotrophic nitrification was not significant in the NH_4^+ oxidation processes in these sediments. These results are in keeping with the conclusions of Painter (1977), that heterotrophic nitrifiers are much less important than autotrophs in most ecosystems.

Nitrate respiration rates in Kingoodie Bay sediments

Initially, experiments were undertaken to determine whether N_2O was a major end-product of denitrification by incubating sediment samples in the presence and absence of 0.7% acetylene, which blocks the reduction of N_2O to N_2 (Yoshinari & Knowles, 1976). The rate of N_2O gas production in acetylene amended sediments was similar and paralleled the production of $^{15}\text{N}_2$ in control experiments. Total gas production at the end of these experiments was also similar, indicating that N_2O was not a major end-product of denitrification in these sediments. Denitrification rates were therefore determined solely on the production of $^{15}\text{N}_2$ in the gas phase of the serum bottles.

Maximum rates of the combined activities of nitrate respiration [$28.5 \mu\text{g N d}^{-1}$ (g dry wt sediment) $^{-1}$] occurred at the 1-2 cm sediment horizon during July 1982 (Table 3). Whilst rates were lower in the 0-1 cm horizon, there was still substantial activity (82% of that recorded at 1-2 cm depth in July). It is also evident that denitrification is the principal process of nitrate reduction in Kingoodie Bay sediments (83.2% of NO_3^- respired reduced to N_2 at 0-2 cm in July 1982). Initially this would appear paradoxical since populations of denitrifying bacteria were substantially smaller than those capable of respiring NO_3^- to NH_4^+ (Fig. 2). However, these results are similar to the findings of Sørensen (1978) for Danish coastal marine sediments and those of Koike & Hattori (1978a) for marine sediments in Japan. It was only with increasing depth (2-5 cm) that an increasing proportion of the NO_3^- was reduced to NH_4^+ , but the total quantity of NO_3^- respired was substantially less than that dissimilated in the surface sediments (0-2 cm). Thus, whilst denitrification is the predominant process in the surface sediments (0-2 cm), NO_3^- reduction to NH_4^+ is not an inconsequential route of NO_3^- dissimilation in Kingoodie Bay sediments.

DISCUSSION

The concentrations of interstitial NO_3^- , NO_2^- and NH_4^+ in vertical profiles of Kingoodie Bay sediments showed the generation of distinct gradients (Fig. 1). The principal species of inorganic-N present was NH_4^+ and concentrations increased with depth. Qualitatively, NH_4^+ profiles found in Tay estuary sediments were similar to those reported for other estuarine and inshore marine sediments which are produced as a consequence of ammonification (Focht & Verstraete, 1977; Sørensen, 1978; Stanley *et al.*, 1981). Ammonium generated, in smaller quantities, as an end-product of NO_3^- dissimilation (Table 3) supplemented that derived by ammonification. Conversely, NO_3^- and NO_2^- maxima as well as highest dissolved O_2

concentrations were recorded in the surface sediments (0–1 cm) and rapidly decreased with depth. Similar nitrogen profiles have been reported for sediments in the Limfjorden (Sørensen, 1978), the North Sea (Vanderborght & Billen, 1975) and Scottish west coast sea lochs (Stanley *et al.*, 1981). Vanderborght & Billen (1975) considered that the NO_3^- profiles in the sluice dock sediments at Ostend resulted from nitrification in the upper layers of the sediment and denitrification in the lower horizons. Populations of autotrophic nitrifying bacteria (Fig. 1) and nitrification rates (Fig. 3) provide experimental evidence to substantiate the mathematical models of Vanderborght & Billen (1975). Further evidence to support this hypothesis has been obtained (G. T. Macfarlane & R. A. Herbert, unpublished data) using a modification of the gel stabilized system developed by Wimpenny *et al.* (1981). Gel systems established in the absence of the nitrification inhibitor N-serve generated concentration gradients of NO_3^- , NO_2^- and NH_4^+ which closely matched those in Kingoodie Bay sediments, whereas in the presence of N-serve (10 mg l^{-1}) these gradients failed to develop.

The dominant autotrophic NH_4^+ and NO_2^- oxidizing bacteria were identified as strains of *Nitrosomonas* and *Nitrobacter* (Macfarlane & Herbert, 1984); these genera are the most commonly isolated representatives of autotrophic NH_4^+ and NO_2^- oxidizing nitrifiers (Belser, 1979; Watson *et al.*, 1981). Maximum nitrification rates [$0.93 \mu\text{g N d}^{-1} (\text{g dry wt sediment})^{-1}$] were recorded in the 0–1 cm horizon in summer, when sediment temperatures were highest (19°C). Nitrification rates decreased steadily with depth, however, and no activity was measured below 3 cm. The rates of nitrification recorded in Kingoodie Bay sediments are comparable to those reported by Christofi *et al.* (1979) for the Eden estuary [$0.32 \mu\text{g N d}^{-1} (\text{g dry wt sediment})^{-1}$], but are lower than those reported by Billen (1975) for North Sea sediments [$6.72 \mu\text{g N d}^{-1} (\text{ml sediment})^{-1}$]. Whilst heterotrophic nitrifying bacteria are present in Kingoodie Bay sediments, the experimental data obtained using $5 \text{ mg N-serve l}^{-1}$ to inhibit autotrophic nitrification indicate that they play an insignificant role in NH_4^+ oxidation. These conclusions are consistent with those of Tate (1980) who demonstrated that large populations of heterotrophic nitrifying bacteria (10^2 – 10^3 times greater than those recorded in Kingoodie Bay sediments) did not contribute significantly to NH_4^+ oxidation in soils.

Substantial populations of nitrate respiring bacteria (denitrifiers and bacteria dissimilating NO_3^- to NH_4^+) were also present in the 0–2 cm horizon of Kingoodie Bay sediments and decreased with increased depth. The results in Table 3 show that denitrification was the principal process of NO_3^- reduction occurring in these sediments. These results are in agreement with those of Sørensen (1978) for the Limfjorden sediments and Koike & Hattori (1978a) for coastal marine sediments. Sørensen (1978) recorded a maximum rate of NO_3^- reduction of $22.6 \mu\text{g N d}^{-1} (\text{ml sediment})^{-1}$ in the 0–3 cm horizon of Limfjorden sediments, and of this $10.6 \mu\text{g N d}^{-1} (\text{ml sediment})^{-1}$ (46.8%) was in the form of NH_4^+ . NO_3^- dissimilation to NH_4^+ in Kingoodie Bay sediments accounted for between 15.5 and 24.9% of the NO_3^- respired in the 0–2 cm horizon (Table 3), but, in a manner similar to that reported by Sørensen (1978), the proportion of NO_3^- dissimilated to NH_4^+ increased with depth (27.6 to 47% at 4–5 cm depth) although the total quantity of NO_3^- reduced was less. Koike & Hattori (1978a) showed that the organic content of the sediments influenced the products of NO_3^- respiration. Organically rich sediments produced NH_4^+ as the principal end-product of NO_3^- dissimilation, whereas the nutritionally poorer sediments produced only 16% and 7% NH_4^+ , respectively. Kingoodie Bay sediments have a low organic content (Herbert, 1975); the NO_3^- respiration rates and end-products produced reflect this and are similar to those recorded for Simoda Bay and Tokyo Bay sediments (Koike & Hattori, 1978a).

The experimental results presented in this paper show that not only are maximum populations of nitrifying and nitrate respiring bacteria present in the 0–2 cm horizon of these sediments but that this zone is also the site of maximum activity. While the rates of nitrate respiration are substantially greater than those recorded for nitrification, these results nonetheless indicate that both processes are occurring simultaneously in the oxidized surface sediments and corroborate and extend the field experiments of Sørensen (1978) and Koike & Hattori (1978b). Studies are now required to establish unequivocally the relationships between these physiologically distinct groups of bacteria which appear to operate an internal nitrogen cycle to their mutual advantage.

REFERENCES

- ALEEM, M. I. H. & ALEXANDER, M. (1958). Cell free nitrification by *Nitrobacter*. *Journal of Bacteriology* **76**, 510–514.
- ALEXANDER, M. (1965). Most-probable-number method for microbial populations. In *Methods of Soil Analysis*, pp. 1467–1472. Edited by C. A. Black. Madison: American Society of Agronomy.
- ALEXANDER, M. & CLARK, F. E. (1965). Nitrifying bacteria. In *Methods of Soil Analysis*, pp. 1477–1483. Edited by C. A. Black. Madison: American Society of Agronomy.
- BARNES, H. & FOLKARD, A. R. (1951). Determination of nitrites. *Analyst* **76**, 599–603.
- BELSER, L. W. (1977). Nitrate reduction to nitrite, a possible source of nitrite for growth of nitrite-oxidising bacteria. *Applied and Environmental Microbiology* **34**, 403–410.
- BELSER, L. W. (1979). Population ecology of nitrifying bacteria. *Annual Review of Microbiology* **33**, 309–333.
- BILLEN, G. (1975). Evaluation of nitrifying activity in sediments by dark ^{14}C -bicarbonate incorporation. *Water Research* **10**, 51–57.
- BREMNER, J. M. & KEENEY, D. R. (1965). Steam distillation methods for determination of ammonium, nitrate and nitrite. *Analytica chimica acta* **32**, 485–496.
- CHRISTOFI, M., OWENS, N. J. P. & STEWART, W. D. P. (1979). Studies on nitrifying microorganisms of the Eden Estuary, Scotland. In *Proceedings of the 13th European Marine Biology Symposium*, pp. 259–266. Edited by E. Naylor & R. G. Hartnoll. Oxford: Pergamon Press.
- DUNN, G. M., WARDELL, I. N., HERBERT, R. A. & BROWN, C. M. (1980). Enrichment, enumeration and characterization of nitrate reducing bacteria present in the sediments of the River Tay. *Proceedings of the Royal Society of Edinburgh* **78B**, 47–56.
- ELLIOT, R. J. & PORTER, A. G. (1971). A rapid cadmium reduction method for the determination of nitrate in bacon and curing brines. *Analyst* **96**, 522–527.
- FOCHT, D. D. & VERSTRAETE, W. (1977). Nitrification and denitrification. In *Advances in Microbial Ecology*, vol. 1, pp. 137–214. New York: Plenum Press.
- GODE, P. & OVERBECK, J. (1972). Untersuchungen zur heterotrophen Nitrifikation in See. *Zeitschrift für allgemeine Mikrobiologie* **12**, 567–574.
- GRUNDMARIS, V. & MURRAY, J. W. (1977). Nitrification and denitrification in marine sediments from Puget Sound. *Limnology and Oceanography* **22**, 804–813.
- HERBERT, R. A. (1975). Heterotrophic nitrogen fixation in shallow estuarine sediments. *Journal of Experimental Marine Biology and Ecology* **18**, 215–225.
- HERBERT, R. A., DUNN, G. M. & BROWN, C. M. (1980). The physiology of nitrate dissimilatory bacteria from the Tay Estuary. *Proceedings of the Royal Society of Edinburgh* **78B**, 137–144.
- JONES, J. G., SIMON, B. M. & HORSLEY, R. W. (1982). Microbiological sources of ammonia in freshwater lake sediments. *Journal of General Microbiology* **128**, 2823–2831.
- KOIKE, I. & HATTORI, A. (1978a). Denitrification and ammonia formation in anaerobic coastal sediments. *Applied and Environmental Microbiology* **35**, 278–282.
- KOIKE, I. & HATTORI, A. (1978b). Simultaneous determinations of nitrification and nitrate reduction in coastal sediments by a ^{15}N -dilution technique. *Applied and Environmental Microbiology* **35**, 853–857.
- MACFARLANE, G. T. & HERBERT, R. A. (1982). Nitrate dissimilation by *Vibrio* spp. isolated from estuarine sediments. *Journal of General Microbiology* **128**, 2463–2468.
- MACFARLANE, G. T. & HERBERT, R. A. (1984). Comparative study of enrichment methods for the isolation of autotrophic nitrifying bacteria from soil, estuarine and marine sediments. *FEMS Microbiology Letters* (in the Press).
- PAINTER, H. A. (1977). Microbial transformations of inorganic nitrogen. *Proceedings in Water Technology* **8**, 3–29.
- ROWE, R., TODD, R. & WAIDE, J. (1977). Micro-technique for most-probable-number analysis. *Applied and Environmental Microbiology* **33**, 675–680.
- SOLORZANO, L. (1969). Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography* **14**, 799–801.
- SØRENSEN, J. (1978). Capacity for denitrification and reduction of NO_3^- to NH_4^+ in a coastal marine sediment. *Applied and Environmental Microbiology* **35**, 301–305.
- STANLEY, S. O., LEFTLEY, J. W., LIGHTFOOT, A., ROBERTSON, M., STANLEY, I. M. & VANCE, I. (1981). The Loch Eil project: sediment chemistry sedimentation and the chemistry of the overlying water in Loch Eil. *Journal of Experimental Marine Biology and Ecology* **55**, 299–314.
- TATE, R. L. (1980). Variation in heterotrophic and autotrophic nitrifier populations in relation to nitrification in organic soils. *Applied and Environmental Microbiology* **40**, 75–79.
- VANDERBORGHT, J. P. & BILLEN, G. (1975). Vertical distribution of NO_3^- concentration in interstitial water of marine sediments with nitrification and denitrification. *Limnology and Oceanography* **20**, 953–961.
- VOSE, P. B. (1980). *Introduction to Nuclear Techniques in Agronomy and Plant Biology*, pp. 151–176. Oxford: Pergamon Press.
- WATSON, S. W., VALOIS, F. W. & WATERBURY, J. B. (1981). The Family *Nitrobacteraceae*. In *The Prokaryotes*, pp. 1005–1022, Edited by M. P. Starr, H. Stolp, H. G. Truper, A. Balows & H. H. Schlegel. New York, Heidelberg, Berlin: Springer-Verlag.
- WIMPENNY, J. W. T., COOMBS, J. P., LOVITT, R. N. & WHITTAKER, S. G. (1981). A gel-stabilized model ecosystem for investigating microbial growth in spatially ordered solute gradients. *Journal of General Microbiology* **127**, 277–287.
- YOSHINARI, T. & KNOWLES, R. (1976). Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. *Biochemical and Biophysical Research Communications* **69**, 705–710.