Microbial Nitrate Reduction in Freshwater Sediments

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Nitrate reductase activity was three to four orders of magnitude greater in freshwater sediments than in the overlying water. Viable (most probable number) counts of denitrifiers provided sufficient resolution to distinguish between anoxic and surface waters, and between these and sediments, but did not correlate with differences between or within sediment cores. Activity within the sediment depended on the electrode potential (E_h) profile, which in turn was related to the degree of turbulence and oxygen concentration in the overlying water. Sediments from the littoral zone or those in contact with oxygenated water were oxidizing to a depth of 5 to 10 mm and the E_h then decreased rapidly. In these sediments nitrate reductase activity was often at its maximum at a depth of 10 to 15 mm, on the E_h gradient, and coincided with a mean E_h value of 210 mV. Under reducing conditions the E_h gradient moved upwards and nitrate reductase activity was greatest at the sediment–water interface. These observations were supported by analyses of the nitrogen gas content of the sediments. Inhibition of the enzymes with chlorate indicated that approximately 60% of the activity was dissimilatory in sediments where the E_h was greater than +100 mV, and that this proportion increased to more than 90% when the E_h fell below +50 mV. Although the evidence was not conclusive, there was also some indication that nitrate reductase activity in aerobic surface sediments was greater in the larger ($> 250 \mu\text{m}$) particle size fraction, which suggested that these particles might act as microsites for nitrate respiration.

INTRODUCTION

The numbers and activity of bacteria in freshwater sediments are three to four orders of magnitude greater than in the overlying water. The sediment is therefore an important site of microbial activity and has received particular attention in studies on denitrification in freshwaters. Field experiments with $^{15}\text{NO}_3^-$ have demonstrated that 63% of the label was lost through denitrification within 4 d (Chen *et al.*, 1972) and field measurements have shown 90% (Tiren *et al.*, 1976) and 95 to 97% (Kessel, 1977*a*) conversion of nitrate to N_2 . The effects of nitrate concentration (Andersen, 1977*a, b*; Kessel, 1977*b*, 1978) and temperature (Andersen, 1977*b*; Kaplan *et al.*, 1977; Kessel, 1977*b*) on denitrification have also been investigated. The role of oxygen in denitrification has been the centre of a long standing controversy (Brezonik, 1977; Delwiche & Bryan, 1976) and its potential controlling effect has therefore been considered in some detail (Kessel, 1977*b*; Focht & Verstraete, 1977). Tiren *et al.* (1976) pointed out that denitrification could occur in sediments even when the overlying water contained 6 to 8 mg $\text{O}_2 \text{ l}^{-1}$. Brezonik (1977) and Focht & Verstraete (1977) considered anoxic microzones, such as those within particles, as possible sites of denitrification in what appeared to be aerobic sediment.

It would not be possible to discuss the effect of oxygen tension on denitrification without considering the related role of electrode potential (E_h). Microbial processes are controlled by E_h and the nitrate concentration in a sediment can, in turn, affect this potential. It is

not surprising, therefore, that the inter-relationship of E_h and denitrifying activity has attracted so much attention. Kessel (1978) concluded that denitrification occurred below the oxidized sediment where the E_h was not greater than +100 mV. This agreed well with the findings of Johnston *et al.* (1974) who observed an E_h shift from +340 to +100 mV as oxygen was used up and a poisoning of the E_h at this lower level as the nitrate was consumed. Graetz *et al.* (1973) reported that nitrate stabilized the sediment E_h at about +200 to +100 mV. These values for the overall effects of nitrate and denitrification on the sediment are somewhat lower than the values observed in cultures. Focht & Verstraete (1977), in their review, cite an E_h range of +350 to +50 mV for nitrite reduction, poisoning at +200 mV being apparently due to N_2O .

The methods used to study denitrification in the natural environment have included measurements of changes in nitrate concentration, ^{15}N -labelling (Brezonik & Lee, 1968), ^{13}N -labelling (Gersberg *et al.*, 1976), gas chromatographic measurement of N_2O reduction (Garcia, 1974) and acetylene inhibition (Sorensen, 1978*a*). Measurement of nitrate reductase has been used in marine systems to study denitrification (Packard *et al.*, 1977) and the nitrogen nutrition of phytoplankton (Eppley *et al.*, 1969; Collos & Slawyk, 1977).

This paper describes the use of a nitrate reductase assay to determine the depth distribution of nitrate reduction in freshwater sediments, and to assess the relative importance of denitrification in relation to the electrode potential of the sediment.

METHODS

Sampling. Water samples were taken with a Friedinger water bottle and transferred to glass bottles which had been sterilized by dry heat (180 °C for 1 h). Sediment samples were taken with a Jenkin surface mud corer and sub-sampled on extrusion using the device described by Jones (1976). The sediment samples came from the deepest points of the South Basin of Windermere, Grasmere and Blelham Tarn, under 40 m, 21.5 m and 14.5 m of water, respectively. Details of other sites used are provided in the text. The lakes are listed in order of increasing eutrophication and consequently of hypolimnetic deoxygenation and sediment reduction. The organic sediments in all three lakes are oxidized at the mud-water interface in spring. The E_h discontinuity layer, or gradient, gradually moves upwards during the summer and actually enters the overlying water in Grasmere and Blelham Tarn.

E_h measurements. The sediment E_h (cell potential plus the potential of the reference electrode) was measured at 1 mm depth intervals with a bright platinum electrode and a fast-flow calomel reference electrode, using the equipment and procedure described by Jones (1979).

Nitrate reductase assay. The assay was essentially that of Lowe & Evans (1964) except that methyl viologen (Sigma) was used as electron donor. The incubation mixture contained equal volumes of enzyme extract, substrate solutions and dithionite/bicarbonate solution. The first of these was prepared by sonicating a 10^{-1} dilution of sediment in extraction solution at an amplitude of 12 μm for 2 min in an ice bath. The extraction solution contained 1.7% (v/v) Triton X-100, 0.15% (w/v) polyvinylpyrrolidone and 75 μM - $MgSO_4$ in 0.1 M-phosphate buffer, pH 7.5. After sonication the samples were centrifuged at 4000 g for 15 min at 0 °C and the supernatant (enzyme extract) was then removed. All solutions were kept in ice/water baths and all manipulations before incubation were performed at a similar temperature. Incubation was for 1 h at 35 °C and after termination the colour was allowed to develop for 10 min before reading at 540 nm. For chlorate inhibition studies, the substrate solution contained 4.92 g $KClO_3$ l^{-1} . The reproducibility of the nitrate reductase assay, as applied to freshwater sediments, was tested before detailed studies were started. Variability within a sediment core was low (95% confidence limits within 4% of the mean) but that between cores was considerably larger (25%). Use of chlorate inhibitor increased the variability by about 3% and expression of results on a dry weight rather than a volume basis increased within- and between-core variabilities to 6 and 37%, respectively. Water samples were analysed by concentrating the plankton on to glass-fibre filters (GF/F, Whatman) and then sonicating the filters in extraction buffer as above. Variability under these conditions was usually about 30%. The detection limits for the assay, as defined in Jones & Simon (1979), were 1.72 μg NO_3-N l^{-1} h^{-1} for water and 1.70 μg NO_3-N ml^{-1} h^{-1} for sediment. The highest enzyme activity was obtained with phosphate buffer at pH 7.5; increasing the pH or using Tris/EDTA buffer lowered the yield. Addition of NADH to the substrate solution had no effect but inclusion of dithiothreitol in the extraction solution resulted in greater activity in most samples. Unfortunately, the SH group protective agent could not be used for chlorate inhibition studies due to an unidentified interference. Linearity of reaction rate with enzyme concentration and time (beyond the normal duration of

the assay) was confirmed, and examination of temperature effects yielded an energy of activation of approximately 60 kJ mol^{-1} with a Q_{10} over the range examined (8°C to 35°C) of 2.4. Glass-fibre filters were chosen to concentrate plankton samples because the membrane filters used successfully for other enzyme studies (Jones & Simon, 1979) contained too much nitrite and nitrate, even after thorough rinsing. The enzyme yield from polycarbonate membranes was usually lower than that obtained with glass-fibre filters. Tests with sediments of known activity showed that the yield of enzyme was reduced by about 50% on passage through the glass-fibre. This may have been partly due to passage of bacteria through the pad (nominal pore size $0.7 \mu\text{m}$, median pore size $0.5 \mu\text{m}$; Sheldon, 1972) as well as to damage during filtration. Values for the water column were corrected for this loss.

Electron transport system (ETS) activity. This was used as a general measure of microbial energy-yielding metabolism, and was determined according to the method of Jones & Simon (1979).

ATP. This was measured using the firefly luciferin-luciferase bioluminescence method. Increased sensitivity was obtained by adopting the procedure of Jones & Simon (1977). Extractions were performed by injecting 0.5 ml of a 5×10^{-3} dilution of sediment into actively boiling Tris buffer at pH 7.8. Internal standards, in the form of bacterial mixed cultures, were used with every sample.

Direct counts of bacteria. Counts were performed by epifluorescence microscopy on black membrane filters using the procedure of Jones & Simon (1975). Sediment samples were diluted 10^{-3} with membrane-filtered water and sonicated at an amplitude of $11 \mu\text{m}$ for 30 s to break up clumps before staining with acridine orange at a final concentration of 10 mg l^{-1} .

Enumeration of denitrifying bacteria. The most probable number (MPN) of denitrifiers was estimated by preparing a fourfold dilution series of the samples for 12 dilutions with 8 replicates at each dilution. A preliminary dilution of 10^{-3} was used for all sediments. The medium of Stanier *et al.* (1966) was used and the samples were incubated at 15°C for 14 d under aerobic and anaerobic conditions. A drop each of sulphanilamide and naphthyl solutions were added to test for nitrite. Negative samples were reduced with activated cadmium [zinc rods treated with 20% (w/v) CdSO_4 solution and activated by stirring in 0.23 M-HCl]. If the samples remained negative then it was assumed that nitrate had been reduced to ammonia or N_2 . The dilutions were performed in 8×12 microtiter dishes (Sterilin) as described by Rowe *et al.* (1977) except that a fourfold dilution series was used. This was achieved by transferring $50 \mu\text{l}$ samples into wells containing $150 \mu\text{l}$ of medium with 8-channel Titertek piston pipettes (Jencons). The MPN was obtained from the tables of Harris & Sommers (1968) using the results from six dilutions with, when possible, the third or fourth dilution scoring approximately 80% positive.

Analysis of sediment gas content. Cores taken with the Jenkin corer at 1 cm intervals were stoppered with Suba-seals (William Freeman Co., Barnsley) and then brought to the laboratory and placed in a gas-tight glove box along with all the equipment necessary for handling the samples. The box was flushed with helium for 1 h and sediment samples were then withdrawn into syringes. A volume of degassed distilled water equal to that of the sediment was then drawn into each syringe, followed by 2 vol. helium. The syringe needle was stoppered, the contents shaken violently for 10 s and then allowed to equilibrate for 1 h. The syringes were then removed from the glove box and the head space was analysed by gas chromatography. A sub-sample was taken via a 5 ml gas-sampling valve and then separated on a 2 m column of 60 to 85 mesh Molecular Sieve 5A at an oven temperature of 100°C . The carrier gas was helium flowing at 40 ml min^{-1} at a pressure of 276 kPa and the thermal conductivity detector was operated at 200°C .

RESULTS AND DISCUSSION

Nitrate reductase activity was far greater in the sediment than in the overlying water column. Results from a stratified lake (Grasmere) in midsummer gave values of 0 to $0.36 \mu\text{g NO}_2\text{-N l}^{-1} \text{ h}^{-1}$ for the epilimnion, 3.8 to $5.6 \mu\text{g NO}_2\text{-N l}^{-1} \text{ h}^{-1}$ for the hypolimnion and $20000 \mu\text{g NO}_2\text{-N l}^{-1} \text{ h}^{-1}$ for the top 1.5 cm of sediment. On an area basis these values were equivalent to a summer average of 1.9, 18.7 and $287 \text{ mg NO}_2\text{-N m}^{-2} \text{ h}^{-1}$, respectively, for the 10 m basin of Grasmere where the samples originated. These and other measures of denitrification in Grasmere showed that the rates were at least an order of magnitude higher than those for nitrification; nitrogen fixation was not observed unless samples were amended with a source of carbon or phosphorus (G. H. Hall & K. L. Jones, personal communication). MPN estimates of denitrifiers for these samples were 7.0×10^3 (1.9×10^3), 7.3×10^3 (4.1×10^4) and 5.6×10^8 (1.7×10^9) bacteria ml^{-1} , respectively (the values in parentheses were anaerobic counts). Clearly, over this range of values, there was agreement between the measurements of activity and bacterial counts, and the increased activity in the anoxic hypolimnion could be attributed to anaerobic denitrifiers. These results were in good agreement with earlier

Fig. 1. Analyses of sediments from the littoral (*a, c*) and the profundal (*b, d*) zones of the South Basin of Windermere in June 1978. Overlying water depths were 10 m and 40 m, respectively, and the water was fully oxygenated. (*a, b*) E_h profile (—) and ATP content (●). (*c, d*) Nitrate reductase activity (●) and direct counts of bacteria (○).

work (Jones, 1976; Jones & Simon, 1979) which showed that microbial activity was three to four orders of magnitude greater in the sediment than in the overlying water column. The activity in the water column was greatest in the anoxic hypolimnion, presumably due to bacterial activity. Similar results were obtained by Packard *et al.* (1977) who attributed a peak of nitrate reductase activity in deeper marine waters to bacterial denitrification.

When the water overlying the sediment was oxygenated, and conditions were oxidizing at the surface, the sediment nitrate reductase activity was often highest at a depth of several millimetres. This was clearly seen in sediments from the South Basin of Windermere in early summer (Fig. 1). The water in this basin does not become anoxic and the E_h at the sediment surface indicated oxidizing conditions. The peak in nitrate reductase activity was usually between 12 and 15 mm below the surface. Estimates of microbial biomass (ATP) and direct counts of bacteria did not peak at these depths (Fig. 1), and so the increased nitrate reduction could not be attributed simply to changes in the size of the microbial population. It may, therefore, represent metabolic adaptation to the conditions within the sediment. Clearly, the stratification in nitrate reductase activity required further investigation at a site where a greater variety of sediment conditions could be found. Grasmere is a lake with a relatively shallow mean depth (7.7 m) but with one basin as deep as 21.5 m. Prevailing winds and development of anoxic conditions at the deepest point ensured that a greater variety of sediment E_h profiles would be obtained (Fig. 2). The two sites (5 m and 16 m) at which the E_h gradient (or discontinuity layer) started at some point below the sediment–water interface also had a nitrate reductase peak at depths of 10 to 15 mm. These were comparable to the profiles illustrated in Fig. 1. When conditions were more reducing

Fig. 2. Analyses of sediments from four sites in Grasmere in May 1978. The depth and dissolved oxygen concentration in the water overlying the sediment are shown. E_h (—), nitrate reductase activity (●) and ETS activity (○).

at the sediment surface (as at the 10 m and 21 m sites) and the E_h decreased immediately with depth, the highest nitrate reductase activities were obtained at the sediment surface, being greater at the site (21 m) with the more pronounced gradient. The nitrate reductase activities did not correspond to any general measure of microbial activity or population density, for example ETS activity, again suggesting that the enzyme activity represented a degree of metabolic adaptation to conditions in the sediment.

The depth of the E_h gradient in a freshwater sediment is controlled to a large degree by the oxygen content and the degree of turbulence in the overlying water, and the reducing power of the sediment (Gorham, 1958). Thus, sediments from the littoral zone have a deeper oxidized layer and the E_h gradient is not as steep as that in profundal muds. Sub-surface peaks of denitrification have been observed in marine sediment by Sorensen (1978*b*) using the acetylene inhibition technique; however, E_h measurements were not made. These results conform with the conventional view of a sediment in which aerobic respiration occurs at

Fig. 3. Gas chromatographic analysis of the depth distribution of nitrogen gas in profundal sediments from Blelham Tarn, sampled in April 1978 (○) at the onset of thermal stratification, and in August 1978 (●) when the hypolimnion was anoxic.

Table 1. *Depth distribution of denitrifying bacteria at four sites in Grasmere (corresponding to those illustrated in Fig. 2) in May 1978*

Incubation conditions	Depth in sediment (cm)	$10^{-9} \times \text{Most Probable Number}^* \text{ g}^{-1}$			
		Depth of water above sediment (m)			
		5	10	16	21
Aerobic	0-1	7.4	6.3	7.6	6.9
	1-2	8.3	28.1	5.8	19.3
	2-3	9.7	9.7	4.8	10.6
	3-4	5.7	16.6	3.0	12.5
	4-5	12.1	18.9	1.2	7.9
Anaerobic	0-1	17.9	18.6	38.6	42.2
	1-2	18.3	7.4	31.4	40.3
	2-3	16.4	7.5	4.6	8.9
	3-4	7.8	2.3	0.9	21.6
	4-5	10.7	14.5	13.8	11.3

* The standard error on log MPN = 0.15 and 95 % confidence limits = ± 0.304 . Results are significantly different when $(\log \text{MPN}_1/0.2128) - (\log \text{MPN}_2/0.2128) > 2.0$.

the surface. With increasing depth below the surface there are zones of denitrification, sulphate reduction and methanogenesis (Fenchel & Jørgensen, 1977). As conditions become more reducing, the E_h gradient and the point of maximum nitrate reductase activity move up to the sediment surface. Similar seasonal changes in sulphate reduction have been observed by Jørgensen (1977) in marine sediments. Thus, the zones of certain anaerobic respirations become compressed as anoxic conditions develop. Analyses for nitrogen gas in the sediments (Fig. 3) confirmed this trend of upward movement of nitrate reduction, and thus nitrogen accumulation, as anoxic conditions developed. Greater quantities of gas were also observed in the absence of oxygen.

While the sediment was oxidized at the surface the peak of nitrate reductase activity was observed (Fig. 2) at a mean E_h of +210 mV (s.d. 70 mV). This is, of course, lower than the redox potential for the $\text{NO}_3^-/\text{NO}_2^-$ couple (+420 mV) and slightly higher than the E_h at which denitrification and nitrate poisoning of sediments was reported by Kessel (1978), Johnston *et al.* (1974) and Graetz *et al.* (1973), although nitrite reduction has been reported in cultures by Focht & Verstraete (1977) over the range +350 to +50 mV. Mortimer (1941, 1942) also considered that an E_h of +200 mV represented the boundary between oxidizing and reducing conditions.

The numbers of denitrifying bacteria, as estimated by a viable count (MPN) technique, did not correlate in any way with the observed distribution of nitrate reductase activity. The

Fig. 4. Analyses of a sediment core from the 21 m site of Grasmere when the oxygen concentration in the overlying water was less than 1 mg l^{-1} . (a) E_h profile. (b) Nitrate reductase activity in the presence (○) and absence (●) of chlorate inhibitor ($4.92 \text{ g KClO}_3 \text{ l}^{-1}$) in the substrate solution.

peaks of enzyme activity at 10 to 15 mm at the 5 m and 16 m sites (Fig. 2) were not reflected in the counts obtained (Table 1), nor were the differences between the sites. Anaerobic counts were, as might be expected, higher than aerobic estimates. The standard error of log MPN is given by $0.55\sqrt{(\log a/n)}$, where a is the dilution ratio (4) and n is the number of replicates at each dilution (8). The count at 1 to 2 cm depth at the 10 m and 21 m sites was significantly different from those at 0 to 1 cm, but only at the 10 m site was it also significantly different from the 2 to 3 cm count. The results of these tests are included merely to provide some indication of the variability of the MPN data even with a relatively low dilution ratio and high degree of replication. The medium used for the MPN procedure gave higher counts of bacteria than that of Focht & Joseph (1973), and the estimates obtained were considerably higher than those of Johnston *et al.* (1974) and May (1976) from similar freshwater sites. The absence of correlation with nitrate reductase activities within and between sediments may reflect the inadequacy of a viable count procedure for such a study, or the differences in activity may be due, in part, to organisms other than bacteria.

The nitrate reductase assay used in this investigation measured both the assimilatory and dissimilatory enzymes, yet only the latter would be of interest with regard to denitrification processes. The assimilatory enzyme is inhibited by chlorate ions, and the results of some inhibitor studies on a sediment with a steep E_h gradient are shown in Fig. 4. A greater proportion of the enzyme activity was inhibited at the sediment surface where assimilatory processes might be expected to be more important. Approximately 60% of the total activity was dissimilatory in samples where the E_h was $> +100 \text{ mV}$. Between $+100$ and $+50 \text{ mV}$ the dissimilatory portion increased rapidly to about 90%. One might conclude, therefore, that although the enzyme assay demonstrated the region of maximum nitrate reduction in a sediment, a significant proportion of this activity may be linked to assimilatory processes, although such an interpretation of chlorate inhibition should, perhaps, be forwarded with caution (Stouthamer, 1976). There was, however, some additional information which supported the view that the chlorate inhibitor studies provided a fairly accurate estimate of assimilation and dissimilation. At the site where these studies were performed, gas traps had been installed to measure loss of gaseous nitrogen from the profundal zone. At the same time, changes in nitrate concentrations in the overlying water were measured. The total loss of gaseous nitrogen, on an area basis, was equivalent to 54% of the nitrate-nitrogen removed from the water column. This was in good agreement with the estimate of 60% dissimilatory nitrate reductase in the surface sediment. The role of the dissimilatory enzymes in the deeper sediments, particularly the formation of ammonia rather than N_2 as end-product (Sorensen, 1978a) deserves further attention.

An examination of the micro-zonation of enzymic activity in aerobic sediments produced results which were less conclusive (Table 2). Particles from an aerobic sediment were

Table 2. *Effect of sediment particle size on microbial biomass and metabolic activity in aerobic surface sediments from the South Basin of Windermere*

	Particle size range (μm)		Ratio (B/A)
	A 45–125	B 250–500	
Nitrate reductase ($\text{mg NO}_3\text{-N g}^{-1} \text{h}^{-1}$)	0.66	38.43	58
ETS activity ($\text{mg O}_2 \text{g}^{-1} \text{h}^{-1}$)	0.14	11.69	81
ATP content ($\mu\text{g g}^{-1}$)	75.9	359.3	4.7

separated into two size classes by a graded wet sieve procedure. Biomass, nitrate reductase and ETS activity were higher in the 250 to 500 μm particles than in the 45 to 125 μm size range but the ratio of metabolic activity between large and small particle sizes was far greater than it was for biomass. Clear-cut results such as these were not always obtained but they were sufficiently frequent to suggest that large sediment particles with internal E_h gradients might provide conditions conducive to energy metabolism (Jones, 1979), particularly anaerobic respiration. Jørgensen (1977) has already demonstrated that sediment particles can act as micro-niches for sulphate reduction in aerobic sediments. The results for nitrate reductase were not as conclusive, but suggested that microsites of anaerobic respiration could contribute to the nitrate reductase activity of aerobic surface sediments.

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REFERENCES

- ANDERSEN, J. M. (1977*a*). Importance of the denitrification process for the rate of degradation of organic matter in lake sediments. In *Interactions Between Sediments and Fresh Water*, pp. 357–362. Edited by H. L. Golterman. The Hague: Dr W. Junk.
- ANDERSEN, J. M. (1977*b*). Rates of denitrification of undisturbed sediment from six lakes as a function of nitrate concentration, oxygen and temperature. *Archiv für Hydrobiologie* **80**, 147–159.
- BREZONIK, P. L. (1977). Denitrification in natural waters. *Progress in Water Technology* **8**, 373–392.
- BREZONIK, P. L. & LEE, G. F. (1968). Denitrification as a sink in Lake Mendota. *Environmental Science and Technology* **2**, 120–125.
- CHEN, R. L., KEENEY, D. R., GRAETZ, D. A. & HOLDING, A. J. (1972). Denitrification and nitrate reduction in Wisconsin lake sediments. *Journal of Environmental Quality* **1**, 158–163.
- COLLOS, Y. & SLAWYK, G. (1977). Nitrate reductase activity as a function of *in situ* nitrate uptake and environmental factors of euphotic zone profiles. *Journal of Experimental Marine Biology and Ecology* **29**, 119–130.
- DELWICHE, C. C. & BRYAN, A. B. (1976). Denitrification. *Annual Review of Microbiology* **30**, 241–262.
- EPPLEY, R. W., COATSWORTH, J. L. & SOLORZANO, L. (1969). Studies of nitrate reductase in marine phytoplankton. *Limnology and Oceanography* **14**, 194–205.
- FENCHEL, T. M. & JØRGENSEN, B. B. (1977). Detritus food chains of aquatic ecosystems: the role of bacteria. *Advances in Microbial Ecology* **1**, 1–58.
- FOCHT, D. D. & JOSEPH, H. (1973). An improved method for the enumeration of denitrifying bacteria. *Proceedings of the Soil Science Society of America* **37**, 698–699.
- FOCHT, D. D. & VERSTRAETE, W. (1977). Biochemical ecology of nitrification and denitrification. *Advances in Microbial Ecology* **1**, 135–214.
- GARCIA, J. L. (1974). Réduction de l'oxyde nitreux dans les sols de rizières du Sénégal: mesure de l'activité dénitrifiante. *Soil Biology and Biochemistry* **6**, 79–84.
- GERSBERG, R., KROHN, K., PEEK, N. & GOLDBERG, C. R. (1976). Denitrification studies with ^{15}N -labelled nitrate. *Science* **192**, 1229–1231.
- GORHAM, E. (1958). Observations on the formation and breakdown of the oxidized microzone at the mud surface in lakes. *Limnology and Oceanography* **3**, 291–298.
- GRAETZ, D. A., KEENEY, D. R. & ASPIRAS, R. B. (1973). Eh status of lake sediment–water systems in relation to nitrogen transformations. *Limnology and Oceanography* **18**, 908–917.
- HARRIS, R. F. & SOMMERS, L. E. (1968). Plate-dilution frequency technique for assay of microbial ecology. *Applied Microbiology* **16**, 330–334.
- JOHNSTON, D. W., HOLDING, A. J. & MCCLUSKIE, J. E. (1974). Preliminary comparative studies on denitrification and methane production in Loch

- Leven, Kinross and other freshwater lakes. *Proceedings of the Royal Society of Edinburgh* B74, 123-133.
- JONES, J. G. (1976). The microbiology and decomposition of seston in open water and experimental enclosures in a productive lake. *Journal of Ecology* 64, 241-278.
- JONES, J. G. (1979). Microbial activity in lake sediments with particular reference to electrode potential gradients. *Journal of General Microbiology* 115, 19-26.
- JONES, J. G. & SIMON, B. M. (1975). An investigation of errors in direct counts of aquatic bacteria by epifluorescence microscopy, with reference to a new method for dyeing membrane filters. *Journal of Applied Bacteriology* 39, 317-329.
- JONES, J. G. & SIMON, B. M. (1977). Increased sensitivity in the measurement of ATP in freshwater samples with a comment on the adverse effect of membrane filtration. *Freshwater Biology* 7, 253-260.
- JONES, J. G. & SIMON, B. M. (1979). The measurement of electron transport system activity in freshwater benthic and planktonic samples. *Journal of Applied Bacteriology* 46, 305-315.
- JØRGENSEN, B. B. (1977). Bacterial sulfate reduction within reduced micro-niches of oxidized marine sediments. *Marine Biology* 41, 7-17.
- KAPLAN, W. A., TEAL, J. M. & VALIELA, I. (1977). Denitrification in salt marsh sediments: evidence for seasonal temperature selection among populations of denitrifiers. *Microbial Ecology* 3, 193-204.
- KESSEL, J. F. (1977a). The immobilization of nitrogen in a water-sediment system by denitrifying bacteria as a result of nitrate respiration. *Progress in Water Technology* 8, 155-160.
- KESSEL, J. F. (1977b). Factors affecting the denitrification rate in two water-sediment systems. *Water Research* 11, 259-267.
- KESSEL, J. F. (1978). The relation between redox potential and denitrification in a water-sediment system. *Water Research* 12, 285-290.
- LOWE, R. H. & EVANS, H. J. (1964). Preparation and some properties of a soluble nitrate reductase from *Rhizobium japonicum*. *Biochimica et biophysica acta* 85, 377-389.
- MAY, E. (1976). *Studies of denitrification in some Scottish freshwater lochs*. Ph.D. thesis, University of Dundee.
- MORTIMER, C. H. (1941). The exchange of dissolved substances between mud and water in lakes. I and II. *Journal of Ecology* 29, 280-329.
- MORTIMER, C. H. (1942). The exchange of dissolved substances between mud and water in lakes. III and IV. *Journal of Ecology* 30, 147-201.
- PACKARD, T. T., DUGDALE, R. C., GOERING, J. J. & BARBER, R. T. (1977). Nitrate reductase activity in the subsurface waters of the Peru Current. *Journal of Marine Research* 36, 59-76.
- ROWE, R., TODD, R. & WAIDE, J. (1977). Micro-technique for most-probable-number analysis. *Applied and Environmental Microbiology* 33, 675-680.
- SHELDON, R. W. (1972). Size separation of marine seston by membrane and glass-filters. *Limnology and Oceanography* 17, 494-499.
- SØRENSEN, J. (1978a). Capacity for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. *Applied and Environmental Microbiology* 35, 301-305.
- SØRENSEN, J. (1978b). Denitrification rates in marine sediment as measured by the acetylene inhibition technique. *Applied and Environmental Microbiology* 36, 139-143.
- STANIER, R. Y., PALLERONI, J. J. & DOUDOROFF, M. (1966). The aerobic pseudomonads: a taxonomic study. *Journal of General Microbiology* 43, 159-271.
- STOUTHAMER, A. H. (1976). Biochemistry and genetics of nitrate reductase in bacteria. *Advances in Microbial Physiology* 14, 315-375.
- TIREN, T., THORIN, J. & NOMMIK, H. (1976). Denitrification measurements in lakes. *Acta agriculturae scandinavica* 26, 175-184.