Microbiological Sources of Ammonia in Freshwater Lake Sediments

By J. GWYNFRYN JONES,* BERNARD M. SIMON AND
RICHARD W. HORSLEY

*The Freshwater Biological Association, Windermere Laboratory, The Ferry House, Ambleside,
Cumbria, LA22 0LP, U.K.*

(Received 9 March 1982; revised 21 May 1982)

During summer stratification ammonia is released from the profundal sediments of Blelham Tarn (English Lake District). The quantity of ammonia released exceeds the consumption of nitrate in the hypolimnion. Nitrate dissimilation may be a component in the generation of ammonia, but only during early summer when nitrate is still available. The remainder of the ammonia arises largely from the deamination of proteins, amino acids and urea. Population estimates of bacteria which produced ammonia from nitrate, amino acids and urea were of the same order of magnitude. Numbers of bacteria which produced ammonia from nitrate increased with sediment depth, and urea decomposers were more numerous in the profundal (deep water) sediments. While nitrate was available in the water column, surface sediments converted nitrate almost exclusively to nitrogen gas. After depletion of the nitrate, the release of ammonia from washed sediment particles was largely microbiological, whereas there was a significant chemical component to the release from intact sediment cores. Chemical binding of ammonia by the sediments was demonstrated and this hindered calculations of inorganic nitrogen metabolism based on changes in water chemistry. Trace additions of ^{14}C -labelled protein, amino acids and urea to sediments showed that urea was turned over the most rapidly, but more reliable estimates of available protein in the sediments are required before decomposition rates can be treated with confidence.

INTRODUCTION

Differences in the inorganic nitrogen metabolism of littoral (shallow water, oxygenated) and profundal (deep water, anoxic) lake sediments were recently demonstrated by Chan & Campbell (1980) and Jones & Simon (1981). The most marked difference was the greater release of ammonia from the profundal zone and of nitrogen gas from the littoral site. The release of ammonia from deep water sediments was first described in detail by Mortimer (1941, 1942) but only recently has attention focussed on its possible origin. Sørensen (1978) demonstrated that a potential existed for conversion of nitrate to ammonia in coastal marine sediments, and that its significance relative to denitrification increased with the depth of the sediment. Buresh & Patrick (1981) showed that conversion of nitrate to ammonia increased with decreasing redox potential; it also accounts for 60–70% of the nitrate dissimilated in digested sludge (Kaspar *et al.*, 1981). The pathway has also been the subject of several recent studies with pure cultures, particularly with members of the family *Enterobacteriaceae* (Cole & Brown, 1980; Dunn *et al.*, 1979) which have included a demonstration of energy conservation via nitrite reduction by formate (Pope & Cole, 1982). In eutrophic lakes, nitrate conversion to ammonia has been shown to be an order of magnitude lower in activity than denitrification (Stewart *et al.*, 1982). However, such sediments may rapidly become depleted of nitrate, and under such circumstances ammonia may be generated primarily by the deamination of proteins, amino acids and urea (Molongoski & Klug, 1980). Although it is possible to demonstrate the presence of proteolytic bacteria and protease enzyme activity in sediments and water (Jones, 1971), little is known about the turnover

of proteins in freshwater ecosystems. Similarly ammonifying bacteria are known to be diverse and to exhibit little substrate specificity (Sepers, 1981) yet studies on their activity have been largely confined to the turnover of amino acids in sea water (Hoppe, 1976; Gocke, 1977; Billen *et al.*, 1980). Urea, an end product of phytoplankton decomposition (Satoh, 1980) is known to be metabolized rapidly in zones such as shallow sea fronts (Floodgate *et al.*, 1981) but there is no information available on urea as a source of ammonia in sediments. This paper examines the relative importance of these sources of ammonia in freshwater sediments, and discusses how changes in physico-chemical conditions affect the release of the ammonia into the overlying water column.

METHODS

Sampling. Sediment and water samples were taken from the profundal and littoral zones and the hypolimnion of Blelham Tarn in the English Lake District during summer stratification, 1981. The sites were described in more detail by Jones & Simon (1981). Sediment samples were taken with a Jenkin surface-mud sampler and the sealed cores were returned to the laboratory and sampled within 2 h. Water samples were taken with a peristaltic pump sampler at fixed distances from the sediment-water interface, determined by an opto-electronic detector (Cunningham & Davison, 1980).

Sample preparation. Wherever possible, samples were handled according to Hungate procedures to maintain strictly anaerobic conditions. The use of serum bottles and purification of gases followed the general methods described by Latham & Wolin (1978). Subsamples of sediment were taken using the extrusion device described by Jones (1976) into serum bottles which had been flushed with oxygen-free gas (N_2/CO_2 , 95:5, v/v, or He). Details of dilutions and other sample preparation varied with the experiment being undertaken, but in general the procedure was as follows. Sediment was diluted 10^{-1} with hypolimnetic, core or interstitial water, it was allowed to settle and the water removed by careful syphoning. This process was repeated twice if reduction of background ammonia levels was required. The diluted sediment was transferred to a serum bottle flushed with gas (350 ml min^{-1}) for 5 min and the bottle then sealed with a slotted butyl rubber stopper. Sediment was also subsampled into small cores as described by Jones & Simon (1981) except that modified 20 ml polypropylene syringes were used as the cores. The flange of the syringe barrel was removed to leave a chamfered edge and the small cores removed after sealing with a rubber stoppered 21 gauge needle and replacing the piston.

Chemical analyses. Ammonia was determined by the method of Chaney & Marbach (1962) and nitrate by the hydrazine reduction method of Downes (1978) modified for use on a Model AC1 Automatic Chemistry Unit (Pye Unicam). In experiments involving the addition of formaldehyde, ammonia was determined with an electrode (Orion, model 95-10). Carbon dioxide was analysed by the headspace method of Stainton (1973) with the following modifications. A sample of headspace gas was flushed into a gas sampling valve fitted with a 3 ml loop, and the carbon dioxide content determined by gas chromatography. The carbon dioxide injected was analysed on a Hewlett Packard gas chromatograph fitted with a $1/8" \times 2'$ ($3 \times 610\text{ mm}$) stainless steel column of 80/100 mesh Carbosieve S and a thermal conductivity detector. A pre-column of 14-22 mesh magnesium perchlorate (BDH) external to the oven removed water from the gas sample. The oven temperature setting was 120°C and the helium flow rate 60 ml min^{-1} . Peak area was electronically determined with an Infotronics 308 computing integrator. Machine performance was checked by injection of a standard calibration gas mixture of 5% CO_2 in N_2 (Phase Separations). Gaseous nitrogen was determined with the same apparatus except that a 2 m column of 60-85 mesh Molecular Sieve 5A was used.

The urea content of the interstitial and overlying water was determined by the method of Newell *et al.* (1967) and primary amines by the fluorescamine method of Udenfriend *et al.* (1972), using the procedure of North (1975) for natural waters. The protein content was determined by a micro-modification (Kochert, 1978) of the Coomassie Brilliant Blue dye binding technique (Bradford, 1976). A gas chromatographic method, based on the ninhydrin reaction (Simon & Jones, 1982) was used to measure amino acids in untreated and acid-hydrolysed sediment.

Enumeration of bacteria. Numbers of bacteria which produced ammonia from nitrate, urea and amino acids were determined by a most probable number (MPN) procedure using a modification of the medium of Stanier *et al.* (1966). In this medium glycerol ($10^{-2}\text{ mol l}^{-1}$) was the C source, yeast extract was omitted, the minor salts of Patel *et al.* (1978) and the vitamin solution of Wolin *et al.* (1963) were included, and nitrate, urea or amino acids were added at a final concentration of 2 mmol l^{-1} . A tenfold dilution series, with five replicates at each dilution, was prepared. The ability of organisms to reduce nitrate to nitrite, and to produce ammonia from all the nitrogen sources, was tested after 4, 7, 11 and 14 d incubation at 25°C .

Core tube experiments. Sediment cores were taken from the profundal zone of Blelham Tarn before the onset of summer stratification. The water above the sediment was analysed for major ions, particularly potential electron acceptors. The water was then removed and replaced, with minimum disturbance of the sediment, by a chemically defined salts solution based on the above analysis. This contained ($\mu\text{mol l}^{-1}$ in distilled H_2O): $NaHCO_3$, 320;

K₂HPO₄, 0.25; CaCl₂, 250; MgSO₄·7H₂O, 110; (NH₄)₂SO₄, 10; KNO₃, 70. A second solution, in which the KNO₃ was replaced by KCl (70 µmol l⁻¹), was also prepared to determine the effect of nitrate on ammonia production. Unstoppered sediment cores were incubated for 16 h at 8 °C and the overlying water was replaced once again by the chemically defined solutions. It was hoped that this treatment would establish reproducible gradients of electron acceptors within the sediment while allowing the experiment to start with overlying water of known composition. The tubes were then stoppered after inserting magnetic stirring vanes (Jones, 1976). The upper stopper contained two wide-gauge syringe needles, one for removal of water samples and the other attached to a reservoir of the minerals solution which had been degassed and maintained under a slight pressure of N₂. The cores were incubated at 20 °C and the water stirred gently immediately before removal of small (≤1% of the volume of overlying water) samples for chemical analysis.

Core tubes were also used, as described above, to determine the release of ammonia and CO₂ from added protein (bovine serum albumin, 43.3 mg l⁻¹), and to determine the chemical component of the ammonia release by poisoning with formaldehyde (final concentration 2%, v/v).

Serum bottle experiments. Conversion of nitrate to nitrogen and ammonium was determined by incubation of 10⁻¹ dilutions of sediment to which KNO₃ (2 mmol l⁻¹) had been added. The bottles were incubated at 20 °C in a shaking water bath. To determine the effect of temperature on the release of ammonia from unamended sediments (no added nitrate), samples were incubated over the temperature range 10 °C–70 °C.

Turnover times of ¹⁴C-labelled protein, amino acids and urea were also determined in serum bottles. The sediment samples were diluted 10⁻¹ in glass-fibre (Whatman, GF/F) filtered core water, 5 ml aliquots were dispensed into 20 ml serum bottles and 50 µl of uniformly labelled ¹⁴C substrate was injected. The substrates, obtained from Amersham, were denatured protein [CF. B6, 56 mCi (atom C)⁻¹], amino acid mixture [CFB. 104, 58 mCi (atom C)⁻¹] and urea [CFA.41, 57 mCi mmol⁻¹] (1 mCi = 37 MBq). The butyl stoppers of the serum bottles were fitted with plastic centre wells (Kontes, no. K882320) before closure of the bottles. The vials were incubated at 10 °C for 21 h, then the reaction was stopped by the addition of 0.2 ml H₂SO₄ (1.5 mol l⁻¹ solution). The ¹⁴CO₂ produced was trapped in 0.2 ml 2-phenethylamine which had been dispensed into the centre well. The acid and phenethylamine were injected into the bottle through a 25 gauge syringe needle. The vials were left overnight at room temperature to ensure complete trapping of the ¹⁴CO₂, and then 0.1 ml of the phenethylamine was transferred to 10 ml of a dioxan-based fluor as described by Jones *et al.* (1982). The radioactivity in the samples was then measured in a Tracor Analytic Mark III liquid scintillation counter (Nuclear Chicago). Samples were corrected for quench with a ¹³³Ba external standard, by plotting the inverse of the relative pulse height against efficiency. This procedure gave better linearity than the channels ratio method.

RESULTS AND DISCUSSION

Nitrogen flux in the hypolimnion

To determine the relative importance of sulphate reduction and putrefaction in Lake Mendota, Nriagu (1968) calculated the net change in the organic and inorganic sulphur content of the particulate material. Although the processes controlling the distribution of the two elements are not strictly comparable, a similar approach has been adopted here to examine the flux of nitrogen in the hypolimnion of Blelham Tarn (Table 1). The nitrogen content of the seston diminished as it settled out of the water column, as did the content of the bottom sediment with depth. The percentage decrease in nitrogen over 1 cm depth of sediment, equivalent to approximately one year's sedimentation (Pennington, 1974), is double that for carbon. This implied a loss of nitrogen to the water column both from suspended material and from the sediment. The nature of the fluxes are summarized in Table 1(b). Generally speaking the input of nitrogen exceeded output, with a loss from the sediment equivalent to 2.2 g N m⁻² per annum over the first centimetre alone. Given that this would account for about 70% of the ammonia released, without considering diffusion of ammonia from greater depths, it would appear that sediment organic nitrogen (by far the largest pool) was a likely major source of the ammonia. Losses and gains due to nitrogen gas evolution and sedimentation varied considerably from year to year (Jones & Simon, 1981), but nitrate removal and ammonia accumulation in the hypolimnion were more consistent with the latter being the larger. With the above figures borne in mind, we then examined the release of ammonia from lake sediments with particular attention being paid to the turnover of organic nitrogen.

Table 1. *A summary of nitrogen transport in Blelham Tarn during summer stratification*

Data for the construction of this table were taken from Jones (1976), Jones & Simon (1980) and Jones & Simon (1981).

(a) *Elemental composition [$\text{mg (g dry wt)}^{-1}$] of particulate material in the lake**

	C	N
Epilimnetic seston	375 (99)	46 (19.7)
Sedimenting seston		
Below the thermocline	255 (84)	28 (10.1)
Above the sediment	274 (111)	30 (14.9)
Surface sediment	156 (3.9)	14.5 (3.2)
Percentage decrease over depths 1–2 cm	10%	21%

(b) *Fluxes of nitrogen (g N m^{-2}) in the hypolimnion during summer stratification*

Inputs		Outputs	
NO_3^- removed	2.8	NH_4^+ released	3.1
N content of		N_2 gas released†	0.8–2.3
sedimenting seston†	4.4–10.3		
Totals	7.2–13.1		3.9–5.4

Difference in N content of sediment over 1 cm depth (≈ 1 year) = 2.2 g N m^{-2}

(c) *Pool sizes of N in sediment (mg m^{-2})*

Nitrate N	0.4
Protein/amino N	5.5
Total N	7.3

* The values in parentheses are standard deviations ($n \geq 5$).

† These factors were more variable and the data represent the range observed over 3 years.

Nitrogen flux in sediment cores

An examination of ammonia release in anaerobic sediment cores [essentially an extension of Mortimer's (1941) experiments] showed that the absence of nitrate in the overlying water had little effect on the initial rate of ammonia release (Fig. 1). In fact more ammonia was released in the nitrate-free core during the later stages of incubation. This may have been related to changes

Fig. 1. Changes in concentration, with time, of dissolved CO_2 ($\circ$), NO_3^- ($\bullet$) and NH_4^+ ($\square$) in water overlying sediment cores from the profundal zone of Blelham Tarn. The water overlying the sediment was (a) hypolimnetic water, (b) an artificial hypolimnetic water containing oxygen, nitrate and sulphate as electron acceptors, or (c) an artificial hypolimnetic water in which nitrate was omitted.

in sediment E_h ; this is considered later. A similar experimental technique was used to determine the effect of added protein on the exchange of ammonia and carbon dioxide between the sediment and the water. The rates of ammonia release in unamended cores and in the lake were within the range reported for profundal sediments by Kamp-Nielsen & Anderson (1977). Addition of protein stimulated production of carbon dioxide and ammonia in the ratio 1.8 : 1. In the absence of ^{15}N analytical facilities this ratio was used to correct $^{14}\text{CO}_2$ release from labelled protein and amino acids to approximate ammonia equivalents.

Population estimates of ammonia-producing bacteria

The relative population sizes of ammonia-producing bacteria were determined by an MPN technique. The medium of Stanier *et al.* (1966) had been used previously to examine populations of nitrate-reducing and denitrifying bacteria (Horsley, 1979; Jones, 1979; Jones *et al.*, 1980) producing estimates of 10^7 – 10^8 ml^{-1} of the former and 10^4 – 10^5 ml^{-1} of the latter. On this occasion, however, it was necessary to distinguish production of ammonia from nitrate and that from protein or amino acids. The medium was therefore modified by the replacement of yeast extract (which also interfered with the ammonia determination) by vitamins and a selection of minor salts. A variety of carbon sources were tested and glycerol was found to be the most satisfactory. Modification of the E_h of the medium with FeS did not alter the results obtained. Although the use of a more defined medium, of necessity, resulted in a reduction in the MPN estimates obtained, it was hoped that the results would provide some indication of the relative potential of each metabolic group. In practice, the results showed that there were few differences between the estimates of bacteria which produced ammonia from nitrate, arginine or urea (Table 2). Populations of organisms which produced ammonia from nitrate tended to increase with depth in both littoral and profundal sediments, and urea decomposers were more numerous in the latter. Bearing in mind the poor precision of such estimates, it is impossible to draw further conclusions from these data, except to point out that the counts obtained were two to three orders of magnitude lower than those of bacteria which reduced nitrate to nitrite only. Pure culture isolates were obtained from the above MPN bottles. Organisms which reduced nitrate to ammonia included some members of the *Enterobacteriaceae*, but were dominated by the genus *Aeromonas*, regardless of the carbon source used in the isolation medium. Amongst the bacteria which produced ammonia from amino acids, members of the genus *Pseudomonas* were the most frequently encountered.

Table 2. MPN estimates of anaerobic bacteria involved in nitrogen transformations in the sediments of Blelham Tarn during summer stratification

Site	Sediment depth (cm)	$10^{-3} \times \text{MPN bacteria ml}^{-1}*$			
		$\text{NO}_3^- \rightarrow \text{NH}_4^+$	Reaction tested:		
			Arginine $\rightarrow \text{NH}_4^+$	Arginine $\rightarrow$ urea	Urea $\rightarrow \text{NH}_4^+$
Littoral	0–1	4.0	0.9	35	2.0
	1–2	0.5	1.1	–	9.5
	2–3	35.0	3.5	–	3.5
	3–4	13.0	3.3	16	–
	4–5	17.0	7.9	–	–
Profundal	0–1	4.5	7.9	2.2	25.0
	1–2	22.0	1.7	1.0	17.0
	2–3	7.9	2.2	5.4	–
	3–4	26.0	1.1	2.5	–
	4–5	35.0	17.0	1.6	–

–, MPN not calculated because the distribution of positive scores did not conform to a Poissonian series.

* Counts are based on a 10-fold dilution series, with 5 replicates at each dilution; 95% confidence limits are obtained by multiplying and dividing the MPN by 3.3.

Nitrate dissimulation

Nitrate dissimulation to ammonia has been the subject of several recent studies with pure cultures of bacteria (Cole & Brown, 1980; Dunn *et al.*, 1979; Pope & Cole, 1982) and has been shown to occur in relatively homogeneous environments such as digested sludge (Kaspar *et al.*, 1981). However, its importance in stratified environments such as sediments remains to be demonstrated. Although injected [^{15}N]nitrate may be converted to ammonia in deeper sediments (Sørensen, 1978) such zones are often depleted of the electron acceptor (Molongoski & Klug, 1980) and therefore an inaccurate assessment of the natural situation may be obtained. Stewart *et al.* (1982) calculated that the quantity of nitrate converted to ammonia was an order of magnitude less than that involved in denitrification in the Blelham Tarn experimental tubes. These estimates were based on results from an *in situ* ^{15}N experiment, in which the ^{15}N was assimilated and entered the sediment as algal biomass. Therefore mineralization and nitrification had to take place before the above calculation could be made. To obtain a realistic estimate of the importance of denitrification and dissimulation to ammonia in lakes, it would be necessary to introduce [^{15}N]nitrate into the hypolimnion at the onset of summer stratification. Only in this way could the supply and downward transport of electron acceptor be accurately simulated. On the other hand, bacteria in the surface sediments may receive a supply of nitrate, either at the beginning of summer stratification (profundal zone) or throughout summer (littoral zone). Addition of nitrate to such sediments resulted in an almost stoichiometric conversion to nitrogen gas, with no significant increase in the amount of ammonia released (Fig. 2). These results are in general agreement with the findings of Chen *et al.* (1972) who added [^{15}N]nitrate to water overlying intact sediment cores.

Fig. 2

Fig. 2. The fate of nitrate (2 mmol l⁻¹) added to surface littoral and profundal sediments of Blelham Tarn. (a) Production of N₂ gas in littoral sediments with (○) and without (●) added nitrate, and in profundal sediments with (□) and without (■) added nitrate. (b) Production of NH₄⁺ in these sediments; symbols as in (a).

Fig. 3

Fig. 3. Effect of temperature on the release of NH₄⁺ from profundal sediments of Blelham Tarn. (a) Release from sediment in serum vials, 25 June 1981, diluted in overlying core water (●), or in hypolimnetic water (○). (b) Release from whole sediment cores, 14 July 1981 into overlying core water (●) and into sediment interstitial water (○).

Mechanisms of ammonia release

One of the problems associated with experiments such as the one described above, where net changes in inorganic nitrogen species in the aqueous phase are being measured, is that no distinction is made between chemical and biological mechanisms of ammonia release. It is known that, under oxidizing conditions, ammonia may be chemically bound in soils and sediments, release into the aqueous phase occurring only upon waterlogging or reduction in E_h . Brock (1978) proposed that one method of distinguishing biological and chemical mechanisms in the natural environment might be to look for the temperature optimum of the former. Sediments which had been resuspended in ammonia-free water showed clearly that such an optimum (about 30 °C) existed (Fig. 3a). Therefore the release of ammonia from sediment particles was largely due to a biological mechanism. If, however, whole sediment cores were incubated over the same temperature range, the results implied that there was a significant chemical component to the release (Fig. 3b). Although the core experiment took place more than 2 weeks later in the summer, when the E_h was lower in the profundal zone, it was clear that the release of ammonia from intact sediment was, to a large degree, a chemical process. Experiments in which known quantities of NH_4Cl were added to surface sediments showed that reducing profundal sediments did not adsorb ammonia. However, additions made before the onset of summer stratification, when these and littoral sediments were in the oxidized state, showed a capacity to adsorb 550–740 and 160–250 $\mu\text{mol NH}_4^+$ (g dry wt) $^{-1}$, respectively. The process was rapid (2–5 min) and completely reversed by the addition of KCl to a final concentration of 1 mol l $^{-1}$. Such adsorption may, therefore, contribute to a considerable lag between the biological production of ammonia in the sediment, and its release into the water column. The extent of the sediment 'memory' could be measured in the field or in sediment cores by the introduction of ^{15}N -labelled organic matter in the form of decomposing algae. Long-term measurements of the site used by Stewart *et al.* (1982) should therefore provide valuable information on this aspect of nitrogen cycling.

Table 3. Turnover of ^{14}C -labelled protein, amino acids and urea in littoral and profundal sediments of Blelham Tarn during July 1981

Site	Sediment depth (cm)	Turnover time (h)					
		Protein		Amino acids		Urea	
		500 $\mu\text{g l}^{-1}$	2.5 $\mu\text{g l}^{-1}$	25 $\mu\text{g l}^{-1}$	2.5 $\mu\text{g l}^{-1}$	540 $\mu\text{g l}^{-1}$	5.4 $\mu\text{g l}^{-1}$
Profundal	0–1	236	191	87	95	43	41
	1–2	301	159	111	82	45	38
	2–3	238	222	90	94	50	38
Littoral	0–1	165	130	101	105	49	35
	1–2	181	148	105	148	42	34
	2–3	220	134	105	119	53	37

Table 4. Pool sizes of organic nitrogen in lake sediments and estimated rates of ammonia production

Sediment	Substrate	Pool size* ($\mu\text{mol l}^{-1}$)	Estimated rate of ammonia production [$\text{nmol (g dry wt)}^{-1} \text{d}^{-1}$]
Littoral	Urea	3.4–5.8	280–640
	Amino acids		
	Fluorescamine	1.1–2.6	8.4–35
	Interstitial protein	3.7	1.3
Profundal	Urea	2.4–4.6	500–720
	Amino acids		
	Fluorescamine	4.6–13.0	84–159
	Ninhydrin (whole sediment)	506	490
	Interstitial protein	30.4	14.7

* Amino acids and proteins are expressed in glycine equivalents.

Production of ammonia from amino and urea nitrogen

The release of ammonia into the water column may be divided into two phases, the initial phase, when nitrate is still present in the overlying water column, and a second phase when the nitrate has been utilized. This may be seen in Fig. 1 and in field data presented by Jones & Simon (1980). The remainder of this paper is concerned with data collected during the second phase, when nitrate dissimilation could be discounted as a mechanism of ammonia generation. Sediment samples were incubated with ^{14}C -labelled protein, amino acids and urea, added both as trace quantities and at somewhat higher concentration. The calculated turnover times (Table 3), showed clearly that a greater potential existed for urea metabolism than for amino acid decomposition, the longest times being recorded with the denatured protein. These results provide an interesting parallel to the studies of Floodgate *et al.* (1981) who demonstrated rapid metabolism of urea in the marine water column. In contrast, although Satoh (1980) was able to demonstrate the release of urea associated with the decomposition of freshwater algae, there was no apparent decomposition by the planktonic bacteria (Satoh, 1981). However, turnover times by themselves may only indicate potential metabolism; estimates of substrate pool sizes are required to determine rates of ammonia production. The results, which showed that rates were generally higher in the profundal sediment, are summarized in Table 4. They also showed that more ammonia was produced from urea than from free amino acids and interstitial protein at both sites. If the total protein concentration, estimated by hydrolysis, or from the total nitrogen content of the sediment, is considered, then rates of ammonia production equivalent to $10^4 \text{ nmol g}^{-1} \text{ d}^{-1}$ would be obtained.

Such estimates cannot be considered until methods exist to measure the true availability of protein in sediment. The use of ninhydrin reagent with whole sediment appears to provide the best estimate at present. In the meantime it must be recognized that the slow turnover of protein suggests that this is the rate-limiting step in the process and that urea may be a significant intermediate in the generation of ammonia in the benthos of lakes.

We wish to thank N. and L. Hetherington for their technical assistance, C. M. Turley for valuable discussions on the determination of urea, T. Furness who drew the figures and Mrs J. Hawksford who typed the script. This research was financed by the Department of the Environment (Contract no. DGR 480/509) and the Natural Environment Research Council.

REFERENCES

- BILLEN, G., JOIRIS, C. & WIJNANT, J. (1980). Concentration and microbiological utilization of small organic molecules in the Scheldt estuary, the Belgian coastal zone of the North Sea and the English Channel. *Estuarine and Coastal Marine Science* **11**, 279–294.
- BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry* **72**, 248–254.
- BROCK, T. D. (1978). The poisoned control of biogeochemical investigations. In *Environmental Biogeochemistry and Geomicrobiology* 3, pp. 717–725. Edited by W. E. Krumbein. Michigan: Ann Arbor Science Publishers.
- BURESH, R. J. & PATRICK, W. H. (1981). Nitrate reduction to ammonium and organic nitrogen in an estuarine sediment. *Soil Biology and Biochemistry* **13**, 279–283.
- CHAN, Y. K. & CAMPBELL, N. E. R. (1980). Denitrification in Lake 227 during summer stratification. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 506–512.
- CHANEY, A. L. & MARBACH, E. P. (1962). Modified reagents for the determination of urea and ammonia. *Clinical Chemistry* **8**, 130–132.
- 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**, 158–161.
- COLE, J. A. & BROWN, C. M. (1980). Nitrite reduction to ammonia by fermentative bacteria: a short circuit in the biological nitrogen cycle. *FEMS Microbiology Letters* **7**, 65–72.
- CUNNINGHAM, C. R. & DAVISON, W. (1980). An optoelectronic sediment detector and its use in the chemical micro-profiling of lakes. *Freshwater Biology* **10**, 413–418.
- DOWNES, M. J. (1978). Improved hydrazine reduction method for the automated determination of low nitrate levels in freshwater. *Water Research* **12**, 673–675.
- DUNN, G. M., HERBERT, R. A. & BROWN, C. M. (1979). Influence of oxygen tension on nitrate reduction by a *Klebsiella* sp. growing in chemostat culture. *Journal of General Microbiology* **112**, 379–383.

- FLOODGATE, G. D., FOGG, G. E., JONES, D. A., LOCHTE, K. & TURLEY, C. M. (1981). Micro-biological and zooplankton activity at a front in Liverpool Bay. *Nature, London* **290**, 133–136.
- GÖCKE, K. (1977). Comparison of methods for determining the turnover times of dissolved organic compounds. *Marine Biology* **42**, 131–141.
- HOPPE, H.-G. (1976). Determination and properties of actively metabolizing heterotrophic bacteria in the sea, investigated by means of micro-autoradiography. *Marine Biology* **36**, 291–302.
- HORSLEY, R. W. (1979). The heterotrophic, nitrate-reducing bacterial flora of Grasmere, English Lake District. *Journal of Applied Bacteriology* **46**, 507–520.
- JONES, J. G. (1971). Studies in freshwater bacteria: factors which influence the population and its activity. *Journal of Ecology* **59**, 593–613.
- 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 nitrate reduction in freshwater sediments. *Journal of General Microbiology* **115**, 27–35.
- JONES, J. G. & SIMON, B. M. (1980). Decomposition processes in the profundal region of Blelham Tarn and the Lund tubes. *Journal of Ecology* **68**, 493–512.
- JONES, J. G. & SIMON, B. M. (1981). Differences in microbial decomposition processes in profundal and littoral lake sediments, with particular reference to the nitrogen cycle. *Journal of General Microbiology* **123**, 297–312.
- JONES, J. G., DOWNES, M. T. & TALLING, I. B. (1980). The effect of sewage effluent on denitrification in Grasmere (English Lake District). *Freshwater Biology* **10**, 341–359.
- JONES, J. G., SIMON, B. M. & GARDENER, S. (1982). Factors affecting methanogenesis and associated processes in the sediments of a stratified eutrophic lake. *Journal of General Microbiology* **128**, 1–11.
- KAMP-NIELSEN, L. & ANDERSON, J. M. (1977). A review of the literature on sediment: water exchange of nitrogen compounds. *Progress in Water Technology* **8**, 393–418.
- KASPAR, H. F., TIEDJE, J. M. & FIRESTONE, R. B. (1981). Denitrification and dissimilatory nitrate reduction to ammonium in digested sludge. *Canadian Journal of Microbiology* **27**, 878–885.
- KOCHERT, G. (1978). Protein determination by dye binding. In *Handbook of Phycological Methods: Physiological and Biochemical Methods*, pp. 92–93. Edited by J. A. Helebust & J. S. Craigie. Cambridge: Cambridge University Press.
- LATHAM, M. J. & WOLIN, M. J. (1978). Use of a serum bottle technique to study interactions between strict anaerobes in mixed culture. In *Techniques for the Study of Mixed Populations*, pp. 113–124. Edited by D. W. Lovelock & R. Davies. London: Academic Press.
- MOLONGOSKI, J. J. & KLUG, M. J. (1980). Anaerobic metabolism of particulate organic matter in the sediments of a hypereutrophic lake. *Freshwater Biology* **10**, 507–518.
- 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.
- NEWELL, B. S., MORGAN, B. & CUNDY, J. (1967). The determination of urea in seawater. *Journal of Marine Research* **25**, 201–202.
- NORTH, B. H. (1975). Primary amines in California coastal waters: utilization by phytoplankton. *Limnology and Oceanography* **20**, 20–27.
- NRIAGU, J. O. (1968). Sulfur metabolism and sedimentary environment: Lake Mendota, Wisconsin. *Limnology and Oceanography* **13**, 430–439.
- PATEL, G. B., KHAN, A. W. & ROTH, L. A. (1978). Optimum levels of sulphate and iron for the cultivation of pure cultures of methanogens in synthetic media. *Journal of Applied Bacteriology* **45**, 347–356.
- PENNINGTON, W. (1974). Seston and sediment formation in five Lake District lakes. *Journal of Ecology* **62**, 215–251.
- POPE, N. R. & COLE, J. A. (1982). Generation of a membrane potential by one of two independent pathways for nitrite reduction by *Escherichia coli*. *Journal of General Microbiology* **128**, 219–222.
- SATOH, Y. (1980). Production of urea by bacterial decomposition of organic matter including phytoplankton. *Internationale Revue der gesamten Hydrobiologie* **65**, 295–301.
- SATOH, Y. (1981). Decomposition of urea by a size-fractionated planktonic community in a eutrophic reservoir in Japan. *Hydrobiologia* **83**, 153–160.
- SEPPERS, A. B. J. (1981). Diversity of ammonifying bacteria. *Hydrobiologia* **83**, 343–350.
- SIMON, B. M. & JONES, J. G. (1982). Gas chromatographic determination of total amino acids and protein in sediments using the ninhydrin-CO₂ reaction. *Hydrobiologia* (in the Press).
- SØRENSEN, J. (1978). Capacity for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. *Applied and Environmental Microbiology* **35**, 301–305.
- STAINTON, M. P. (1973). A syringe gas-stripping procedure for gas-chromatographic determination of dissolved inorganic and organic carbon in fresh water and carbonates in sediments. *Journal of the Fisheries Research Board of Canada* **30**, 1441–1445.
- STANIER, R. Y., PALLERONI, N. J. & DOUDOROFF, M. (1966). The aerobic pseudomonads: a taxonomic study. *Journal of General Microbiology* **43**, 159–271.
- STEWART, W. D. P., PRESTON, T., PETERSON, H. G. & CHRISTOFF, N. (1982). Nitrogen cycling in eutrophic freshwaters. *Philosophical Transactions of the Royal Society* **B296**, 491–509.
- UDENFRIEND, S., STEIN, S., BÖHLEN, P., DAIRMAN, W., LEIMGRUBER, W. & WEIGLE, M. (1972). Fluorescamine: a reagent for assay of amino acids, peptides, proteins and primary amines in the picomole range. *Science* **178**, 871–872.
- WOLIN, E. A., WOLIN, M. J. & WOLFE, R. S. (1963). Formation of methane by bacterial extracts. *Journal of Biological Chemistry* **238**, 2882–2886.