Nitrogen Cycling in Coastal Marine Environments
Edited by T. H. Blackburn and J. Sørensen


CHAPTER 11

# *Nitrate Reduction and Denitrification in Marine Sediments*

ISAO KOIKE AND JAN SØRENSEN

## 11.1 INTRODUCTION

The deposition of organic detritus on the surface layer of marine sediments supports an elevated microbial metabolism and limits the penetration of $O_2$ into the sea bottom. An ideal environment for microbial $NO_3^-$ reduction is thus created where $NO_3^-$ can be in ample supply to substitute for $O_2$ in the processes of organic matter degradation.

The principal pathways of microbial $NO_3^-$ reduction are shown in Figure 11.1 which also indicates the products of the processes, i.e. cellular organic nitrogen, $NO_2^-$, gaseous nitrogen ($N_2O$ and $N_2$), and $NH_4^+$. Cellular nitrogen is the product of assimilatory $NO_3^-$ reduction which is widely distributed among algae and higher plants. Many heterotrophic microbes, including yeast and bacteria, also have a capacity for $NO_3^-$ assimilation, but the inhibitory effect of $NH_4^+$ on $NO_3^-$ assimilation (Payne, 1973) suggests that this type of $NO_3^-$ reduction plays a minor role in marine sediments, at least in the $NH_4^+$-rich, coastal types. It should be mentioned, though, that Koike and Hattori (1978a) reported a significant incorporation of $NO_3^-$ and $NO_2^-$ into particulate nitrogen in coastal, marine sediments and further studies are needed to clarify the role of assimilatory $NO_3^-$ reduction in the sea bottom.

Nitrate reduction coupled to energy metabolism is represented by a number of dissimilatory reactions in the sediments. Here, $NO_3^-$ serves as the terminal electron-acceptor and is first reduced to $NO_2^-$. Except for the $NO_3^-$ reduction in a few autotrophic bacteria, a supply of organic substrate is indispensable for the reaction. Nitrite may further be reduced to $N_2O$ and eventually to $N_2$ in the process of denitrification. Most of the denitrifying bacteria are aerobes which can only utilize nitrogenous oxides if $O_2$ is absent (Payne, 1973). Alternatively, the $NO_3^-$ may be reduced only to $NO_2^-$, as in the $NO_3^-$ respiration by fermentative bacteria, which are either facultative or obligate anaerobes (e.g. Herbert, 1982). At least in some cases, however, the $NO_2^-$ may be further reduced to $NH_4^+$ in a

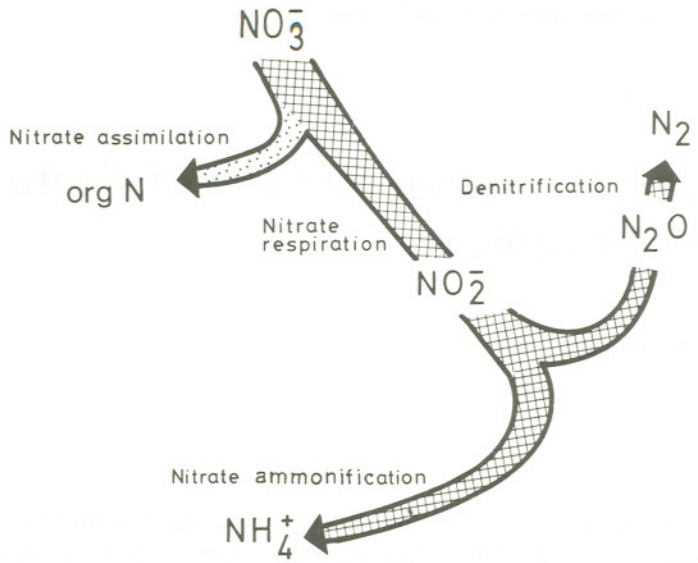


Figure 11.1. Microbial $NO_3^-$ reduction

respiratory process (Steenkamp and Peck, 1981) while in others the $NO_2^-$ seems to serve as an electron sink and is reduced to $NH_4^+$ in a fermentative reaction. Here, reduced NADH is reoxidized by $NO_2^-$ without a direct coupling to ATP production (Cole and Brown, 1980). The overall dissimilatory reduction of $NO_3^-$ to $NH_4^+$ can be referred to as '$NO_3^-$ ammonification' (Figure 11.1).

In the following we shall discuss the significance of the different types of microbial $NO_3^-$ reduction for a variety of marine sediments. We have focused on current, *in situ* assays for the processes, and on some recent observations of temporal and spatial variations of the activities. Finally we have addressed the questions of *in situ* control of the processes, and of composition of $NO_3^-$-reducing populations in the marine sediments.

## 11.2 OXYGEN AND NITRATE PROFILES IN MARINE SEDIMENTS

The zone of dissimilatory $NO_3^-$ reduction is typically located in a layer immediately below the oxic surface layer, although significant heterogeneity may arise around faunal burrows (Sørensen, 1978b; Sørensen *et al.*, 1979). Detailed $O_2$ profiles from marine sediments have not been available until relatively recently. In the pelagic sediments, for instance, some recent determinations have been made either with gas chromatographic techniques (Grundmanis and Murray,

1982; Sørensen and Wilson, 1984) or with polarography using microelectrodes (Reimers *et al.*, 1984). The former approach has mainly been used to illustrate the relatively deeper distribution of $O_2$ in pelagic sediments and to localize the site of $NO_3^-$ reduction where $O_2$ is eventually depleted. The microelectrode technique was originally developed for coastal sediments in which the penetration of $O_2$ is usually limited to a few millimeters below the surface (Revsbech *et al.*, 1980).

Measurements of $NO_3^-$ and $NO_2^-$ profiles in marine sediments may also give important information both on production of $NO_3^-$ (nitrification) and $NO_3^-$ reduction. There are two major sources of $NO_3^-$ (and $NO_2^-$) in the porewaters of marine sediments: one is the supply by diffusion from the overlying water and the other is nitrification at the oxic layer of the sediment. In nearshore environments, such as those of saltmarshes and embayments, the ground water may sometimes constitute a third, important source of $NO_3^-$ (Capone and Bautista, 1985).

Figure 11.2 shows three types of $NO_3^-$ (plus $NO_2^-$) profiles in pelagic sediments from the western Pacific Ocean (I. Koike, unpublished). Profiles of dissolved organic nitrogen and of dissolved primary amines are included to illustrate the availability of organic substrate in the three sediments. In the Sagami Basin (Figure 11.2 top), measurable $NO_3^-$ was found only in the upper few centimeters of the cores, and a sharp decrease of the $NO_3^-$ concentration from the sediment–water interface indicated that $NO_3^-$ reduction was significant in the sediment. Both the total organic nitrogen and the primary amine concentrations were high, and the almost linear increase of $NH_4^+$ concentrations with depth reflected active, anaerobic decomposition of the accumulated organic material. The $NO_3^-$ profile recorded in the Sagami Basin is also typical for many coastal sediments in which the organic content is relatively high (e.g., Nishio *et al.*, 1982).

An example of a second type of $NO_3^-$ profile is shown for the Ogasawara Trench (Figure 11.2, center). This profile has a distinct peak of $NO_3^-$ which is clearly a result of nitrification in the surface layer. A reduction of the $NO_3^-$ occurred at the lower edge of the $NO_3^-$ profile. The type of profile is found in pelagic sediments where local accumulation of organic material takes place (Goloway and Bender, 1982; Christensen and Rowe, 1984). This profile is also found in many coastal sediments, especially in those which have a relatively low content of organic matter, and in those which have a significant faunal community and transport of oxygenated surface water to the deeper layers (e.g. Vanderborght *et al.*, 1977; Grundmanis and Murray, 1977).

A third type of profile, in which the $NO_3^-$ concentration steadily increases with depth, is also seen in the Western Pacific (Figure 11.2, bottom). Nitrification is here a predominant process and $NO_3^-$ reduction is not detectable from the profiles, if the process occurs at all. In general there will be a trend from the latter type of profile found in pelagic waters to the other two, which are commonly found in coastal and estuarine waters (Bender *et al.*, 1977; Christensen and Rowe, 1984).

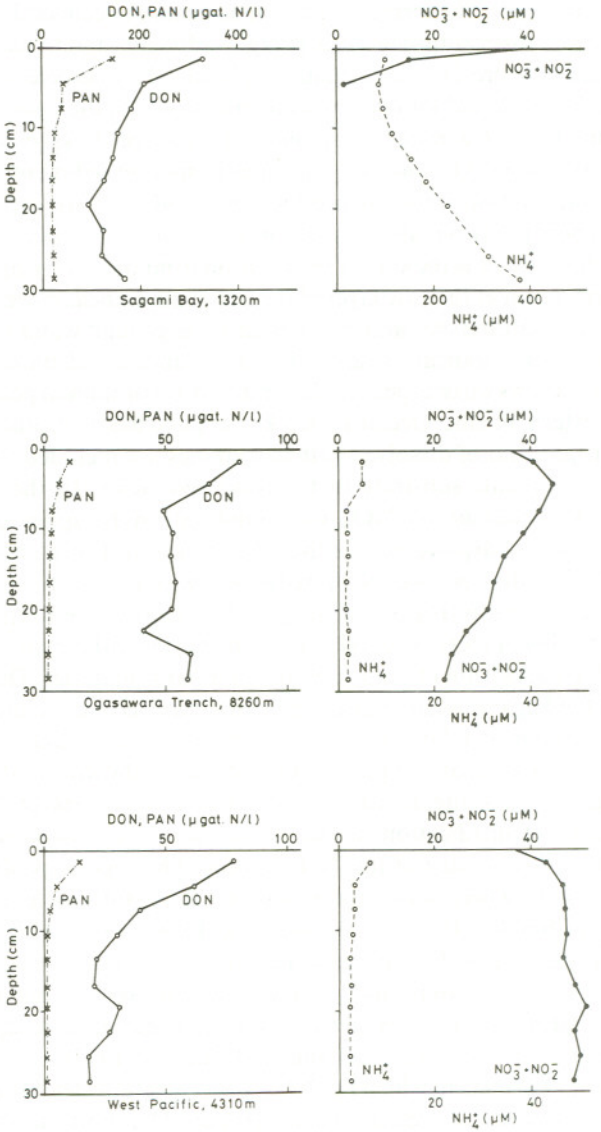


Figure 11.2. Vertical profiles of dissolved organic nitrogen (DON), primary amines (PAN), $NO_3^-$ (plus $NO_2^-$) and $NH_4^+$ in marine sediments from the western Pacific Ocean: Sagami Bay at 34°57′N, 139°15′E (top), Ogasawara Trench at 28°28′N, 143°20′E (center) and western Pacific at 26°57′N, 142°55′E (bottom)

## 11.3 ASSAYS OF NITRATE REDUCTION AND DENITRIFICATION IN MARINE SEDIMENTS

### 11.3.1 Diffusion–advection models

A determination of $NO_3^-$ reduction and denitrification in deep-sea sediments has not been possible until recently, and so far the reported rates are only from a very limited part of the world ocean. Except for the experimental approach by Sørensen *et al.* (1984), all estimates of the $NO_3^-$ reduction and denitrification in deep-sea sediments have been based on models which incorporate $N_2$:Ar ratios or $NO_3^-$ profiles in the sediment porewaters. Several authors (Barnes *et al.*, 1975; Wilson, 1978; Nishio *et al.*, 1981) have thus reported an excess of $N_2$ in the sediments, which probably reflected denitrification activity. Based on the diffusion coefficient for $N_2$ in the porewaters, an estimate of the areal denitrification has been obtained from $N_2$ profiles (Wilson, 1978; Hattori, 1983). In a different approach a one-dimensional, diffusion–advection model for $NO_3^-$ has been developed to incorporate both the nitrification and $NO_3^-$ reduction. The box model of Vanderborght and Billen (1975), which contained an oxic layer with nitrification and an underlying layer with $NO_3^-$ reduction, was originally developed for coastal sediments, but was later used in deep-sea sediments with different types of nitrate profiles (Jahnke *et al.*, 1982; Goloway and Bender, 1982; Christensen and Rowe, 1984).

Direct measurements and diagenetic modeling have given a range for the activity of $NO_3^-$ reduction in deep-sea sediments between 0.17 mmol N $m^{-2}$ $y^{-1}$ (offshore sediment of the North Atlantic) and 30 mmol N $m^{-2}$ $y^{-1}$ (hydrothermal mound near Galapagos) (Table 11.1). There are, of course, several uncertainties about the estimates of $NO_3^-$ reduction and denitrification from the diffusion–advection models. A close coupling between nitrification and $NO_3^-$ reduction has thus been reported for coastal sediments (Koike and Hattori, 1978b; Nishio *et al.*, 1983; Jenkins and Kemp, 1984) and the possible occurrence of $NO_3^-$ reduction in the oxic surface layer may lead to an understimated activity. The use of the Redfield ratio (Jahnke *et al.*, 1982; Goloway and Bender, 1982) presents another uncertainty in the models, since the elemental composition of the detritus being decomposed is unknown. Finally, in most of the diffusion models the rate of $NO_3^-$ reduction is assumed to be equal to that of denitrification. As noted earlier, a considerable portion of the $NO_3^-$ may be reduced to $NH_4^+$ in the coastal sediments, and it is yet unknown if the process can be neglected in the deep sea.

In most of the coastal and estuarine studies the rates of $NO_3^-$ reduction and denitrification have been estimated directly by experimental procedures. Accurate modeling of the $NO_3^-$ profiles would also be impossible in most of the coastal sediments where the gradients are sharper and more frequently disturbed by the faunal activity.

Table 11.1. Rates of nitrate reduction and denitrification in deep-sea sediments

| Site | Depth (m) | Nitrate reduction (mmol N $m^{-2} y^{-1}$) | Denitrification (mmol N $m^{-2} y^{-1}$) | Method | References |
|---|---|---|---|---|---|
| Eastern equatorial Atlantic | 3310–4980 | 1.3 | | $NO_3^-$ diffusion model | Bender *et al.* (1977) |
| | 3310–4980 | 1.3–3.0 | | $NO_3^-$ diffusion model | Goloway and Bender (1982) |
| | 3880–4956 | 0.3–2.2 | | $NO_3^-$ diffusion model | Jahnke *et al.* (1982) |
| | 5100 | | 0.3 | $C_2H_2$ inhibition method | Sørensen *et al.* (1984) |
| Northeast Atlantic | 1334–5275 | | 3.8 | $N_2$ diffusion model | Wilson (1978) |
| Bermuda Rise | 4595–4629 | 0.5–5.9 | | $NO_3^-$ diffusion model | Goloway and Bender (1982) |
| Northwest Atlantic | 1840–3630 | 4–15 | | $NO_3^-$ diffusion model | Christensen and Rowe (1984) |
| | 5105–5120 | 0.2–0.7 | | $NO_3^-$ diffusion model | Christensen and Rowe (1984) |
| | 5090 | 0 | | $NO_3^-$ diffusion model | Christensen and Rowe (1984) |
| Bering Sea Basin | 3650 | 20 | | $NO_3^-$ diffusion model | Tsunogai *et al.* (1979) |
| Santa Barbara Basin | 590 | | 1.5 | $N_2$ diffusion model | Barnes *et al.* (1975); Hattori (1983) |
| Eastern equatorial Pacific | 3568–3638 | 0.8–1.0 | | $NO_3^-$ diffusion model | Goloway and Bender (1982) |
| | 3116–3214 | 14–21 | | $NO_3^-$ diffusion model | Goloway and Bender (1982) |
| | 4368–4394 | 0.6–1.0 | | $NO_3^-$ diffusion model | Goloway and Bender (1982) |
| | 4843–4890 | 0 | | $NO_3^-$ diffusion model | Goloway and Bender (1982) |
| Equatorial Pacific | 3572–5867 | 0 | | $NO_3^-$ diffusion model | Grundamanis and Murray (1982) |
| Galapagos hydrothermal | 2645–2740 | 15–30 | | $^{15}N$ tracer method | Goering and Pamatmat (1970) |

### 11.3.2 Direct estimates of $N_2$ production

Kaplan *et al.* (1979) were the first to determine $N_2$ production *in situ* by placing a bell jar on a marsh sediment and measuring the emission of $N_2$ gas. Seitzinger *et al.* (1980) further developed a similar technique by incubating undisturbed sediment in gas-tight glass chambers under a water phase of lowered $N_2$ content. The technique seems ideal to maintain undisturbed gradients in the sediment, but incorporates a risk of $N_2$ contamination from the atmosphere and requires a rather long incubation time (1–2 weeks) for a detection of steady $N_2$ production.

### 11.3.3 Use of $^{15}N$ isotope techniques

In this section we shall emphasize the experimental protocol for some important $^{15}N$ assays of $NO_3^-$ reduction and denitrification. Goering and Pamatmat (1970) were the first to use the $^{15}N$ isotope for a demonstration of denitrification in marine sediments. An excess of labeled $NO_3^-$ was added to a sediment slurry and the dissolved gases were extracted and analyzed for $^{15}N$ enrichment after a period of incubation. Since this pioneer work several methodological improvements have been made, and more attention is now being paid to the effect of isotope addition on microbial activity, and to the maintenance of a natural microenvironment for the bacteria during the incubations. With current techniques the addition of $^{15}N$-labeled $NO_3^-$ or $NO_2^-$ most often results in increased oxidant concentration and in enhancement of the microbial activity. To evaluate this effect the rate of product formation can be measured at different levels of $^{15}N$-$NO_3^-$ addition and the kinetics of the process determined. By extrapolation to *in situ* concentrations the natural activity may then be approached. Such a technique was adopted in a study of denitrification in Japanese coastal sediments and in a Danish estuary (Koike *et al.*, 1978; Oren and Blackburn, 1979). Unfortunately the use of suspensions also implies a destruction of the gradients of organic substrate distribution in the sediment. Additions of peptone, glucose and amino acids to organic-rich, coastal sediment gave no significant stimulation of the denitrification, however, and the limitation by organic substrate availability may in such cases be of minor importance (I. Koike and A. Hattori, unpublished).

A different $^{15}N$ isotope assay has recently been developed for undisturbed cores of coastal and estuarine sediment. In the experimental setup shown in Figure 11.3, filtered seawater containing $^{15}N$-labeled $NO_3^-$ is passed at a constant flow rate over the cores (Nishio *et al.*, 1982). The $NO_3^-$ diffuses into the sediment and is reduced to, e.g., $^{15}N$-labeled nitrogen gas at the site of denitrification. When the denitrification occurs close to the sediment surface a major fraction of the labeled gas diffuses into the overlying water, and if the water phase is the predominant source of $NO_3^-$ in the cores, the *in situ* rate of denitrification can be determined directly by the isotope enrichment of the

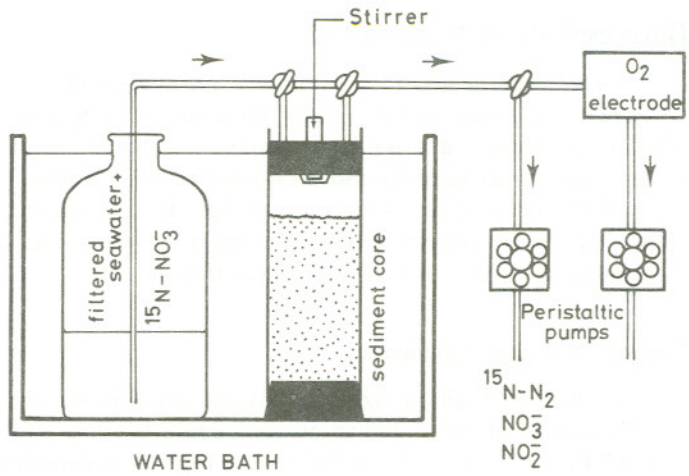


Figure 11.3. Continuous-flow system for determination of inorganic nitrogen cycling in sediments ($^{15}N$ isotope technique)

nitrogen gas in the effluent water. In addition, the net consumptions of $O_2$ and $NO_3^-$ can be determined easily from the change of their concentrations in the effluent compared to the source water.

If nitrification in the sediment is also an important $NO_3^-$ source in the cores, there may be an upward diffusion of unlabeled $NO_3^-$ which causes an underestimation of both the $NO_3^-$ reduction and the denitrification. In this case a separate set of cores may be used to determine the fraction of denitrification which is supported by $NO_3^-$ being produced within the sediment. This particular set of cores should receive a small $^{15}N$ enrichment in the $NH_4^+$ pool of the overlying water, but is otherwise treated as described for the $NO_3^-$-amended cores. The overall denitrification can then be obtained by summation of the activities in the two sets of cores (Nishio *et al.*, 1983).

As an example of simultaneous determination of several processes, a record of the inorganic nitrogen transformations in a coastal sediment is shown in Figure 11.4 (data from Nishio, 1982). In this particular sediment the denitrified nitrogen came from the overlying water and from nitrification within the sediment, in almost equal amounts. The recorded activities provide a useful insight into the sediment nitrogen cycle.

In summary, the $^{15}N$ isotope technique has several advantages: (1) destruction of the microenvironments in the sediment is avoided; (2) net consumptions for both $O_2$ and $NO_3^-$ are obtained, together with the rate of denitrification; (3) the source of the nitrate being denitrified can be identified; (4) the overall activity of several nitrogen transformations in the sediment can be obtained simultaneously. Some disadvantages of the technique are: (1) the incubation time is long (days)

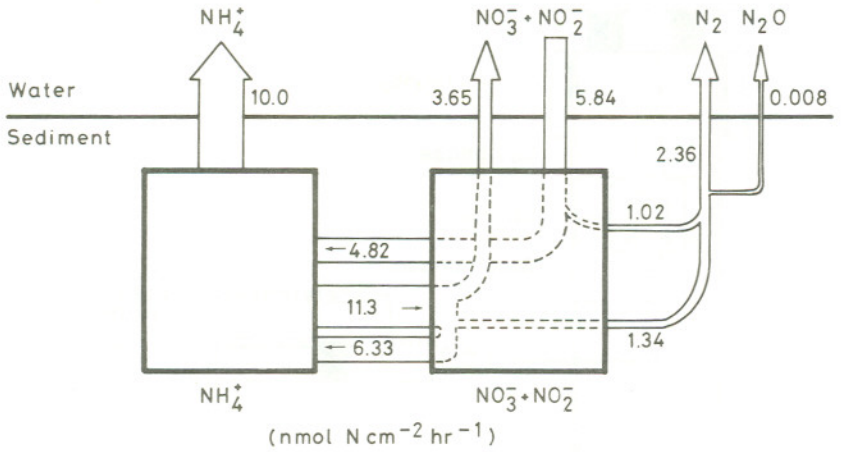


Figure 11.4. Inorganic nitrogen cycling in Odawa Bay sediment (Japan)

when denitrifying sites are located at several centimeters of depth in the cores; (2) the assay is rather tedious and may require a mass spectrometer for a high sensitivity of $^{15}N$ detection.

### 11.3.4 Use of the $C_2H_2$ inhibition technique

As alternative to the $^{15}N$ isotope assays, the 'acetylene inhibition technique' has been used frequently to determine denitrification. Soon after $C_2H_2$ was found to block the reduction of $N_2O$ to $N_2$ in denitrifying bacteria (Balderston *et al.*, 1976; Yoshinari and Knowles, 1976), a simple and sensitive assay for denitrification in marine sediments was developed (Sørensen, 1978b). After injection of dissolved $C_2H_2$ into the cores the spontaneous accumulation of $N_2O$ can be taken as a measure of *in situ* denitrification.

A major difficulty of the technique is to provide a homogeneous and sufficient concentration of the inhibitor in the sediment, immediately after the small aliquots of $C_2H_2$-saturated water have been injected (Figure 11.5). In recent applications of the assay a final $C_2H_2$ concentration in the porewater of 5–10% (v:v) has been adopted (Andersen *et al.*, 1984; Jørgensen and Sørensen, 1985). The $N_2O$ may be extracted from the sediment by either a gas stripping (Sørensen, 1978b) or a headspace extraction procedure as described by Andersen *et al.* (1984).

The highly sensitive detection of $N_2O$ by EC (electron capture) gas chromatography allows a short incubation time (a few hours) and the assay maintains near-*in-situ* profiles of both the $O_2$ and $NO_3^-$ in the cores. The dilution of the porewater and the physical disturbance seems insignificant compared to the variability among different cores. Initial comparisons of the $C_2H_2$ and the $^{15}N$

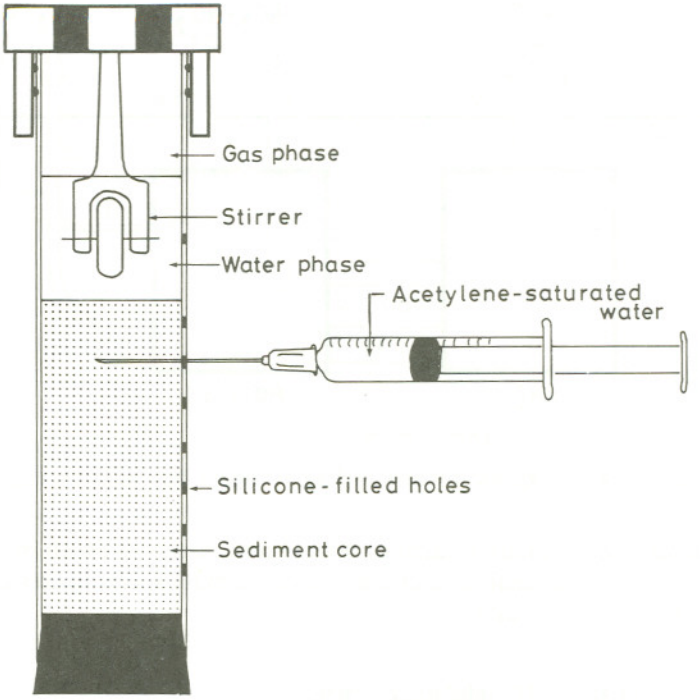


Figure 11.5. Injection of $C_2H_2$ for determination of denitrification activity in sediments (acetylene inhibition technique)

isotope techniques showed similar denitrification rates for $NO_3^-$-amended cores (Sørensen, 1978b), but the efficiency of the $C_2H_2$ blockage may be less than optimal when the denitrification takes place at low $NO_3^-$ concentrations (Kaspar, 1982; Oremland *et al.*, 1984). The problem seems critical when the sediments show concentrations of only a few $\mu$M $NO_3^-$ in the denitrification zone (Kaspar, 1982; Oremland *et al.*, 1984; Jørgensen and Sørensen, 1985). It is not yet clear whether the lack of inhibition is due to particular conditions for $N_2O$ reduction in the bacteria at limited oxidant supply, or if the presence of certain compounds in the $NO_3^-$-limited sediment affects the inhibitory action of $C_2H_2$. It is interesting that inorganic sulfide, which may itself be inhibitory for $N_2O$ reduction in denitrifying bacteria (Sørensen *et al.*, 1980), was shown to release the blockage of the $N_2O$ reduction by $C_2H_2$ (Tam and Knowles, 1979).

A potential difficulty of the technique is also the apparent inhibition of nitrification ($NH_4^+$ oxidation) by $C_2H_2$ which was discovered after the introduction of the denitrification assay (Walter *et al.*, 1979). When sediment cores are incubated with $C_2H_2$ a blockage of the nitrification may result in a progressive depletion of the $NO_3^-$ pool, and the denitrification rate should decrease if the activity is dependent on $NO_3^-$ availability. As a result the incubation time must

often be adjusted according to the rate of $NO_3^-$ depletion in the cores. The inhibition of nitrification may sometimes be used for a simultaneous determination of total $NO_3^-$ reduction and denitrification (Jørgensen and Sørensen, 1985). If nitrification is excluded in the $C_2H_2$-amended cores the disappearance of $NO_3^-$ (plus $NO_2^-$) should give a measure of total $NO_3^-$ reduction. In combination with the direct assay for denitrification the assay therefore provides a measure of the rate at which $NO_3^-$ is being reduced to products other gaseous nitrogen ($NH_4^+$ and organic N).

The inhibitory action of $C_2H_2$ on the dissimilatory $NO_2^-$ reduction to $NH_4^+$ (Kaspar *et al.*, 1981) has not yet been studied in bacterial cultures, and remains uncertain in terms of the actual mechanism involved. Some of the $SO_4^{2-}$ reducers are known to possess the capacity for dissimilatory $NO_3^-$ reduction to $NH_4^+$ (Steenkamp and Peck, 1981), and it has also been shown that the $C_2H_2$ inhibits cell proliferation in certain sulfate-reducing bacteria (Payne and Grant, 1982). However, the actual role of the $SO_4^{2-}$ reducers in the $NO_3^-$ reduction is unknown.

Summarizing the advantages of the 'acetylene inhibition technique', the most apparent ones are: (1) the determination of denitrification is rapid, sensitive and inexpensive; (2) the physical disturbance is kept at a minimum and near-*in-situ* profiles of $NO_3^-$ can be maintained if the incubation time is short; (3) in some sediments the total $NO_3^-$ reduction and the denitrification can be determined simultaneously. The major problems associated with the assay are: (1) the inhibition of $N_2O$ reduction by $C_2H_2$ is sometimes incomplete, especially at low $NO_3^-$ concentrations; (2) the inhibitor may affect other processes in the sediment which are important for the $NO_3^-$ reduction.

## 11.4 VARIATION OF NITRATE REDUCTION AND DENITRIFICATION ACTIVITIES IN MARINE SEDIMENTS

A comparison of the different methodologies presently in use to estimate denitrification *in situ* is urgently needed. Koike and Hattori (1979) compared the denitrification measured by the $^{15}N$ technique and the $NO_3^-$ reduction estimated by a diffusion–advection model in sediments from the Bering Sea Shelf. They obtained almost identical rates by the two methods. Haines *et al.* (1981) later applied the $C_2H_2$ inhibition technique to these sediments and found activities similar to those reported by Koike and Hattori (1979). A new comparison of the $^{15}N$ and $C_2H_2$ methodologies in undisturbed cores would be appropriate now, after the $^{15}N$ isotope technique has been further developed (Nishio *et al.*, 1983).

### 11.4.1 Regional variation of denitrification

Even in coastal sediments the reported rates for denitrification are from a limited number of mostly temperate locations. The available data are listed in Table 11.2, showing the wide application of $^{15}N$ isotope and $C_2H_2$ inhibition

Table 11.2. Rates of nitrate reduction and denitrification in estuarine and coastal sediments

| Site | Depth (m) | Nitrate reduction (mmol N $m^{-2} d^{-1}$) | Denitrification (mmol N $m^{-2} d^{-1}$) | Method | References |
|---|---|---|---|---|---|
| Randers Fjored (estuarry) (Denmark) | 0–1 | | 0.1–1.0 | $C_2H_2$ inhibition method | Sørensen *et al.* (1979) |
| Norsminde Fjord (estuary) (Denmark) | 0–1 | | 0.2 | $^{15}N$ tracer bottle incubation method | Oren and Blackbrun (1979) |
| Norsminde Fjord (estuary) (Denmark) | 0–1 | | 0–3.0 | $C_2H_2$ inhibition method | Sørensen *et al.* (1979) |
| Great Sippewissett Marsh (USA) | 0–1 | | 0–360 | $^{15}N$ tracer in bottle incubation and $N_2$ production measurement in bell jar | Kaplan *et al.* (1979) |
| Bering Sea Shelf | 70–120 | | 0.2 | $^{15}N$ tracer in bottle incubation method | Koike and Hattori (1979) |
| Alaskan Continental Shelf (USA) | 1–300 | | 0.1–0.6 | $C_2H_2$ inhibition technique | Haines *et al.* (1981) |
| Tokyo Bay (Japan) | 20–30 | 0.8–1.7 | 0.4–0.8 | Continuous flow sediment-water system with 15-N tracer | Nishio *et al.* (1982) |

| | | | | | |
|---|---|---|---|---|---|
| Odawa Bay (Japan) | 0.5–2 | 0–2.2 | 0.0–0.9 | Continuous flow sediment–water system with $^{15}N$ tracer | Nishio *et al.* (1982) |
| Tama Estuary (Japan) | 0.5–2 | 6.7–45.6 | 3.1–19.0 | Continuous flow sediment–water system with $^{15}N$ tracer | Nishio *et al.* (1982) |
| Delaware Inlet (New Zealand) | 0–1 | | 0.3 | $C_2H_2$ inhibition technique | Kaspar (1982) |
| Patuxent River Estuary (USA) | 1–5 | | 1.7–2.1 | $^{15}N$ tracer method using intact sediment core | Jenkins and Kemp (1984) |
| Providence River Estuary (USA) | 5–10 | | 0.7–2.8 | $N_2$ production measurement using intact sediment core | Seitzinger *et al.* (1984) |
| Narragansett Bay (USA) | 5–30 | | 0.9–2.7 | $N_2$ production measurement using intact sediment core | |
| San Francisco Bay (USA) | 0–1 | | 0–0.1 | $C_2H_2$ inhibition technique | Oremland *et al.* (1984) |
| Lendrup Strand (Denmark) | 0–1 | | 0.3–5.1 | $C_2H_2$ inhibition technique | Andersen *et al.* (1984) |
| Norsminde Fjord (Denmark) | 0–1 | 2–50 | 0.5–8.0 | $C_2H_2$ inhibition technique | Jørgensen and Sørensen (1985) |

techniques. The sediments show a wide range of *in situ* activities, but most values group around 0.1–0.5 mmol $N\,m^{-2}\,d^{-1}$ for shelf sediment (Koike and Hattori, 1979; Haines *et al.*, 1981), around 0.5–2.0 mmol $N\,m^{-2}\,d^{-1}$ for coastal bays (Nishio *et al.*, 1982; Seitzinger *et al.*, 1984), 0–20 mmol $N\,m^{-2}\,d^{-1}$ in estuarine sediments (Sørensen *et al.*, 1979; Oren and Blackburn, 1979; Nishio *et al.*, 1982; Kaspar, 1982; Jenkins and Kemp, 1984; Andersen *et al.*, 1984; Oremland *et al.*, 1984; Jørgensen and Sørensen, 1985) and 0–350 mmol $N\,m^{-2}\,d^{-1}$ in saltmarsh sediment (Kaplan *et al.*, 1979). There is obviously a trend of higher activity levels in the shallow, nearshore waters where the supplies of organic substrate and $NO_3^-$ are generally higher, although sometimes very irregular. For instance, significant terrestrial inputs in the coastal areas are often observed by elevated $NO_3^-$ concentrations. Tidal water movements and penetration of $NO_3^-$-rich ground water into the sediment may further stimulate denitrification in saltmarshes and other shallow waters (Kaplan *et al.*, 1979; Capone and Bautista, 1985). Here the enhanced transport of $NO_3^-$ into denitrification zones becomes increasingly important for both the regional and seasonal variation of denitrification.

The first indications of a significant reduction of $NO_3^-$ to $NH_4^+$ in the coastal sediments came from additions of $^{15}N$-labeled $NO_3^-$ to the uppermost centimeters which contained the natural zone of $NO_3^-$ reduction. In most cases the results gave a slight dominance of denitrification (30–80%) over $NO_3^-$ reduction to $NH_4^+$ (7–52%) (Koike and Hattori, 1978a; Sørensen, 1978a). The $NO_3^-$-stimulated (potential) activities were obviously higher than those occurring *in situ*, and the activities at low and natural $NO_3^-$ concentrations must be determined by one of the direct assays. Such a determination of both pathways has only been performed in a few cases, either directly by the $^{15}N$ isotope technique (Nishio *et al.*, 1982) or as the difference between total $NO_3^-$ reduction and denitrification (Jørgensen and Sørensen, 1985). As shown in Table 11.2, the range of activities recorded for denitrification *in situ* is about half of that for overall $NO_3^-$ reduction, although the actual proportioning between the two major pathways of $NO_3^-$ reduction varies considerably in single measurements. The direct determinations of $NO_3^-$ reduction to $NH_4^+$ are at present too few to evaluate its role in the marine sediments.

### 11.4.2 Seasonal variation of denitrification

All the data shown in Table 11.2 are from temperature regions where large seasonal fluctuations of the activities may be expected. Such a variation was already indicated from early *in-situ* studies (Kaplan *et al.*, 1979; Sørensen *et al.*, 1979), but complete seasonal records of the denitrification in coastal and estuarine sediments have not been available until recently (Sørensen, 1984; Jørgensen and Sørensen, 1985; Smith *et al.*, 1985). A correlation between the temperature and the overall denitrification pattern was observed in the

temperature marsh sediments (Kaplan *et al.*, 1979; Smith *et al.*, 1985) and may be expected in areas with a substantial $NO_3^-$ supply throughout the year. In the estuaries, however, the input of $NO_3^-$ often varies dramatically; the rapid depletion of $NO_3^-$, which can occur after a bloom of phytoplankton productivity in the spring and early summar, causes an immediate decline in the denitrification activity (Sørensen, 1984; Jørgensen and Sørensen, 1985; Smith *et al.*, 1985) and may result in a maximum of activity already in the spring or early summer when the temperature is increasing and the $NO_3^-$ is still abundant. Figure 11.6 shows such a seasonal pattern for both the $NO_3^-$ availability and the activities of $NO_3^-$ reduction and denitrification from the inner part of a Danish estuary (redrawn

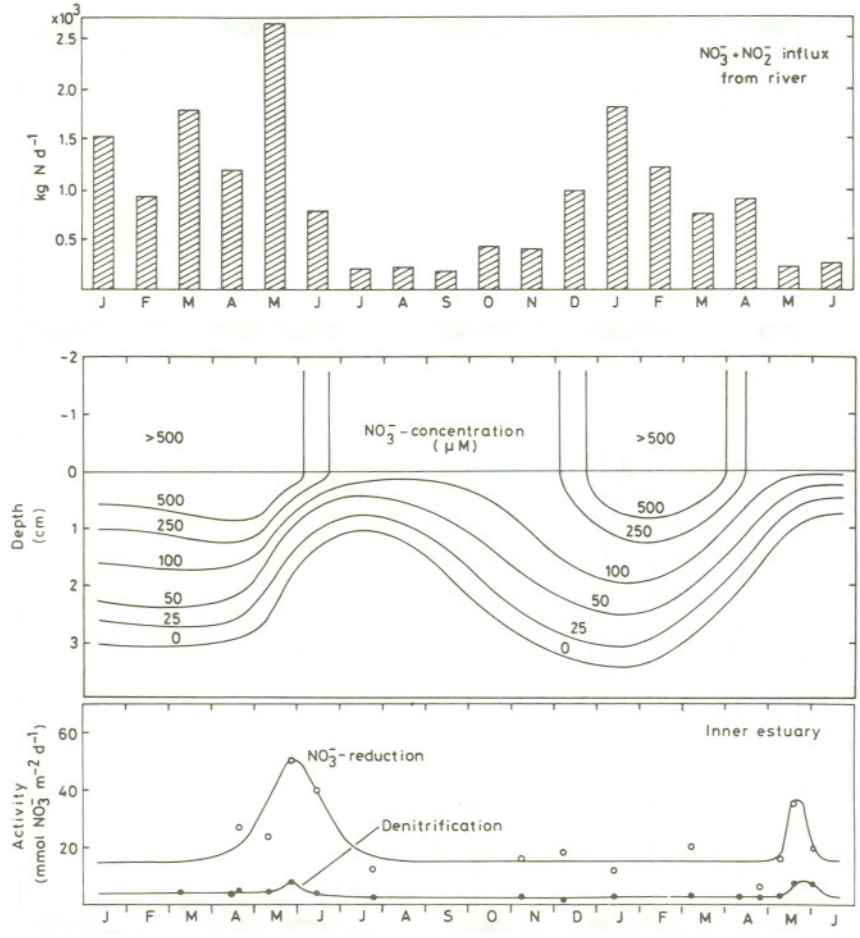


Figure 11.6. Seasonal variation of $NO_3^-$ availability and activities of $NO_3^-$ reduction and denitrification in Norsminde Fjord sediment (Denmark)

from Jørgensen and Sørensen, 1985). During the fall, when the temperature is still rather high, the return of a $NO_3^-$ input with the river water may sometimes support a secondary maximum of denitrification (K. S. Jørgensen and J. Sørensen, unpublished). The seasonal variations are smaller offshore, like in the deeper waters of coastal bays, etc., where denitrification remains high throughout the summer (Seitzinger *et al.*, 1984).

### 11.4.3 Diel variation of denitrification

In estuaries, sunlight can support a considerable productivity of the microalgae at the sediment surface, and since the oxic–anoxic boundary is located just a

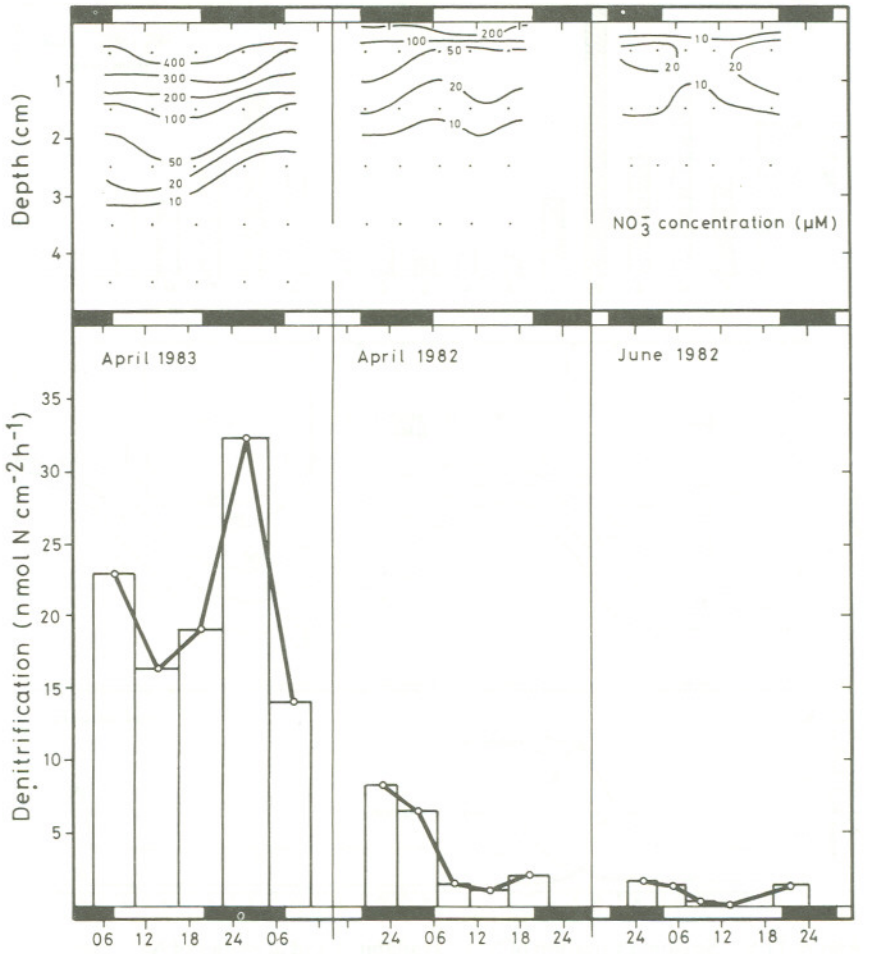


Figure 11.7. Diel variation of $NO_3^-$ availability and activity of denitrification in Lendrup Strand sediment (Denmark)

few millimeters below the sediment surface (Revsbech *et al.*, 1980), the algal $O_2$ production may have a profound influence on the dissimilatory $NO_3^-$ reduction. Figure 11.7 shows a diel pattern for the denitrification in such a shallow estuarine environment (redrawn from Andersen *et al.*, 1984). For the spring season the activity was about 2–5-fold higher in the dark than in the light. As the activity in the uppermost centimeter may account for up to 70–90% of the total denitrification in these sediments (Andersen *et al.*, 1984), it is advisable to look for diel variations of the process before an assessment of the estuarine nitrogen loss by denitrification is made. Direct determinations of such losses have so far only been based on dark incubations (Jørgensen and Sørensen, 1985; Smith *et al.*, 1985).

## 11.5 POPULATIONS OF NITRATE-REDUCING AND DENITRIFYING BACTERIA IN MARINE SEDIMENTS

As opposed to the significant effort which has been made to determine the significance of $NO_3^-$ reduction and denitrification activities in marine sediments, there is only little information on, e.g., the compositions and sizes of $NO_3^-$-reducing and denitrifying populations, their physiological responses to the changing environment, and their patterns of growth and extinction in the sediment. In the following we shall discuss some of the recent work on the dynamics and structure of the $NO_3^-$-reducing and denitrifying populations.

### 11.5.1 Most-probable-number and chemostat enrichment techniques

Enumerations of the nitrate-reducing and denitrifying bacteria in marine sediments have been few and the medium selections are probably still immature for a useful application of the most-probable-number technique. Also, the large diversity within $NO_3^-$-reducing and denitrifying bacteria may well have discouraged a study of their taxonomy in detail. Furthmore, it may be expected that many of the organisms are 'born' aerobes at the sediment surface and thereafter utilize their capacity for $NO_3^-$ respiration by chance. In coastal sediments, which are physically disturbed by the burrowing fauna, the $NO_3^-$-reducing bacteria could include both an endogenous population of $NO_3^-$ reducers and a population of aerobes which happen to possess the genetics for $NO_3^-$ respiration. Finally, the $NO_3^-$-reducing population may also include some of the strict anaerobes like the $SO_4^{2-}$ reducers which are capable of dissimilating the $NO_3^-$ to $NH_4^+$, but are unlikely to utilize the activity unless $NO_3^-$ is introduced into the deeper sediment layers.

Plating methods often select for fast-growing organisms which are not necessarily important in the $NO_3^-$ reduction and denitrification *in situ* (Dunn *et al.*, 1980). For instance, both the organic substrate composition and the carbon:$NO_3^-$ ratio of the media may be important in order to determine if the predominant organisms are denitrifiers or the type which reduces $NO_3^-$ to $NH_4^+$. As an example of a selection on carbon-rich medium, the use of Difco nutrient

broth with $NO_3^-$ gave a predominance of the *Aeromonas–Vibrio* group and, in smaller fractions, other enteric organisms and pseudomonads (Dunn *et al.*, 1980; MacFarlane and Herbert, 1982, 1984).

As an extension of this work, continuous culture enrichments may be used to find the organisms which are best adapted for particular conditions. The sediment from an estuarine mudflat was shown to produce mainly fermenting, enteric organisms when glycerol was used in a carbon-limited chemostat (Dunn *et al.*, 1980). In contrast, a predominance of oxidative pseudomonads was seen only when acetate was used as the carbon source. As a further complication, the product of $NO_3^-$ dissimilation by fermenting bacteria may be either $NO_2^-$ or $NH_4^+$ depending on the carbon:$NO_3^-$ ratio of the medium. Using isolates of both the *Aeromonas–Vibrio* group (MacFarlane and Herbert, 1982) and other enteric organisms (Herbert *et al.*, 1980), there was a complete dissimilation to $NH_4^+$ only when the nitrogen was limiting growth. In contrast, the $NO_2^-$-reducing activity was not induced and $NO_2^-$ accumulated in the medium when the carbon source was in short supply. Thus, even if the fermenting bacteria can dominate numerically, and contribute to the $NO_3^-$ reduction in the sediments, they do not always express their $NO_3^-$-reducing capacity.

### 11.5.2 'Potential' for nitrate reduction and denitrification

Both denitrification and reduction of $NO_3^-$ to $NH_4^+$ were shown to occur immediately after the addition of excess $NO_3^-$ to oxidized surface sediment (Sørensen, 1978a). However, in the underlying sediment denitrification showed a time lag which was probably related to the absence of $NO_3^-$ *in situ* in the deeper layers. The spontaneous reduction of $NO_3^-$ to $NH_4^+$ in the deeper layers of the sediment demonstrated that the organisms involved in this reaction were probably fermenters or sulfate reducers, which had an active metabolism *in situ* and possessed a constitutive enzyme system for $NO_3^-$ reduction.

As the reduction of $NO_3^-$ may occur along two pathways, it would be of considerable interest to compare the kinetics of $NO_3^-$ and $NO_2^-$ reductions in the organisms which are likely to be involved *in situ*. Smith *et al.* (1982) showed that the $NO_3^-$ reduction to $NH_4^+$ in marsh sediment decreased from 52% to 4% of the total when the $NO_3^-$ availability increased. King and Nedwell (1985) showed that as the $NO_3^-$ concentration was increased in an estuarine sediment, denitrification became dominant at the expense of the $NO_3^-$ reduction to $NH_4^+$. It seems unlikely that the shifts were due to kinetic competition for $NO_3^-$, since the concentrations were rather high (0.2–2 mM) in the slurries, but a likely explanation was that the fermenters decreased their demand for the electron sink ($NO_2^-$ reduction to $NH_4^+$) as they shifted towards the energy-favorable respiration with $NO_3^-$. The partitioning between the two pathways apparently depends not only on the composition of populations and their actual enzyme contents, but also on the reaction kinetics and the preference for a maximum of energy yield during $NO_3^-$ reduction.

The availability of organic substrate is not usually considered to be important in the regulation of $NO_3^-$ reduction in coastal sediments. In short-term experiments, where significant cell proliferation is avoided, a stimulation of the $NO_3^-$ reduction by substrate applications would seem to be a useful measure of the regulation by organic matter *in situ*. Such an assay was introduced by Haines *et al.* (1981), who measured denitrification in a number of substrate-amended shelf sediments. Certain sediments were almost unaffected, while others were stimulated by a factor of 3–4 and were apparently under true kinetic control. Similar results have recently been obtained in organic-rich, coastal sediments (K. S. Jørgensen, unpublished; I. Koike and A. Hattori, unpublished).

Little is known about the temporal changes of $NO_3^-$-reducing populations in the sediments. Data from the estuary, which were presented by the seasonal activity pattern in Figure 11.6, may serve to illustrate such a dynamic change in the $NO_3^-$-reducing community. Figure 11.8 shows a seasonal record for the 'potential' activities of $NO_3^-$ reduction and denitrification in the sediment as measured in $NO_3^-$-amended slurries (J. Sørensen and F. Bertelsen, unpublished). Throughout the year the sampling depth was adjusted to include the whole $NO_3^-$-containing surface zone in which the bacteria were assumed to be active *in situ*. Constant temperature (about 20 °C) and application of $NO_3^-$ (1 mM) were used each time. The results indicate a rapid decrease of the content of denitrifying enzymes in the summer when both the $NO_3^-$ availability and the *in situ* activity

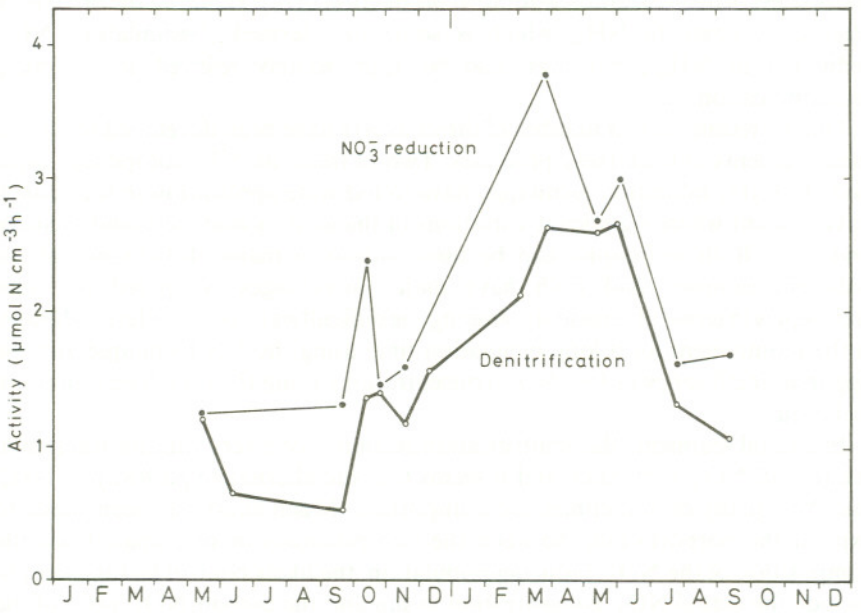


Figure 11.8. Seasonal variation of 'potential' activity of $NO_3^-$ reduction and denitrification in Norsminde Fjord sediment (Denmark)

were low (Figure 11.6) and an increase during the subsequent fall and winter. The 'potential' for denitrification constituted a large fraction of the overall 'potential' for $NO_3^-$ reduction. Since the assay was run at room temperature there could be a difficulty of interpreting the data if the sediment also contained a population of denitrifiers with a low-temperature optimum. Such a cold-adapted population could not be demonstrated in the sediment, however. Even at the end of the winter there was a steady increase of the 'potential' activity in the temperature range from 5 to 30 °C (J. Sørensen, unpublished).

The pattern may be different if the $NO_3^-$ supply remains significant throughout the season. For the upper decimeter of the saltmarsh, which had a high *in situ* activity (Kaplan *et al.*, 1979), the 'potential' denitrification measured at room temperature (20 °C) was also highest in the summer (Kaplan *et al.*, 1977). However, when measured at low temperature (5 °C), the 'potential' was relatively low, independent of the season. The results indicated the presence of both a cold-adapted population, which dominated the modest denitrification in the marsh in the winter, and a population with a higher temperature optimum, which was responsible for the high activity during the summer.

## 11.6 CONCLUSIONS

In marine sediments, which are anoxic a few millimeters or centimeters the surface, the microbial $NO_3^-$ reduction occurs along two major pathways: one is the process of denitrification leading to production of $N_2O$ and $N_2$, the other is an alternative route to $NH_4^+$ which is sometimes termed 'dissimilatory $NO_3^-$ reduction to $NH_4^+$', but may also be appropriately referred to as '$NO_3^-$ ammonification'.

During recent years a number of direct assays have been developed for *in situ* measurements of both these processes. Two of them, an $^{15}N$ isotope technique and the $C_2H_2$ inhibition technique, have found wide application in the coastal environment where significant variations of the activity may be found in space and time. Both techniques can be used without a major disturbance of the sediment samples, and both have major advantages compared to other techniques in terms of sensitivity. Among the difficulties to be considered, though, is the requirement for a long incubation time using the $^{15}N$ technique and the apparent inefficiency at low $NO_3^-$ concentrations using the acetylene inhibition technique.

In coastal sediments the denitrification accounts for a very variable fraction of the overall $NO_3^-$ reduction, and it seems that the alternative pathway to $NH_4^+$ may be equally or sometimes more important. Much effort has been made to explain the porportioning between the two processes *in situ*; apart from the composition of the $NO_3^-$-reducing population, the likely control factors seem to include the actual $NO_3^-$ concentration *in situ* and the carbon:$NO_3^-$ ratio of the sediment. The change of $NO_3^-$ availability seems to be a key factor for the

seasonal control of $NO_3^-$ reduction in the coastal sediments. In shallow waters the diel denitrification pattern may be controlled by a release of $O_2$ in benthic algae at the sediment–water interface.

A structural description of the $NO_3^-$-reducing community may be obtained by an assay of the 'potential' activity (total enzyme content) for $NO_3^-$ reduction and denitrification in the sediments. The seasonal fluctuations of the 'potential' denitrification observed in estuarine sediments are probably related to similar changes in $NO_3^-$ availability and activity *in situ*.

## REFERENCES

Andersen, T. K., Jensen, M. H., and Sørensen, J. (1984). Diurnal variation of nitrogen cycling in coastal marine sediments. I. Denitrification. *Mar. Biol.*, **83**, 171–6.

Balderston, W. L., Sherr, B., and Payne, W. J. (1976). Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectomarinus*. *Appl. Environ. Microbiol.*, **31**, 504–8.

Barnes, R. O., Bertine, K. K., and Goldberg, E. D. (1975). $N_2$:Ar, nitrification and denitrification in southern California borderland basin sediments. *Limnol. Oceanogr.*, **20**, 962–70.

Bender, M. L., Ganning, K. A., Froelich, P. N., Heath, G. R., and Maynard, V. (1977). Interstitial nitrate profiles and oxidation of sedimentary organic matter in the eastern equatorial Atlantic. *Science*, **198**, 605–9.

Capone, D. G., and Bautista, M. (1985). Direct evidence for a groundwater source for nitrate in nearshore marine sediments. *Nature*, **313**, 143–7.

Christensen, J. P., and Rowe, G. T. (1984). Nitrification and oxygen consumption in northwest Atlantic deep-sea sediments. *J. Mar. Res.*, **42**, 1099–1116.

Cole, J. A., and Brown, C. M. (1980). Nitrite reduction to ammonia by fermentative bacteria: a short circuit in the biological nitrogen cycle. *FEMS Microbiol. Lett.*, **7**, 65–72.

Dunn, G. M., Wardell, J. N., Herbert, R. A., and Brown, C. M. (1980). Enrichment, enumeration and characterization of nitrate-reducing bacteria present in sediments of the River Tay Estuary. *Proc. R. Soc. Edin.*, **78B**, 47–56.

Goering, G. G., and Pamatmat, M. M. (1970). Denitrification in sediments of the sea off Peru. *Invest. Pesq.*, **35**, 233–42.

Goloway, F., and Bender, M. (1982). Diagenetic models of interstitial nitrate profiles in deep sea suboxic sediments. *Limnol. Oceanogr.*, **27**, 624–38.

Grundmanis, V., and Murray, J. W. (1977). Nitrification and denitrification in marine sediments from Puget Sound. *Limnol. Oceanogr.*, **22**, 804–13.

Grundmanis, V., and Murray, J. W. (1982). Aerobic respiration in pelagic marine sediments. *Geochim. Cosmochim. Acta*, **46**, 1101–20.

Haines, J. R., Atlas, R. M., Griffiths, R. P., and Morita, R. Y. (1981). Denitrification and nitrogen fixation in Alaskan continental shelf sediments. *Appl. Environ. Microbiol.*, **41**, 412–21.

Hattori, A. (1983). Denitrification and dissimilatory nitrate reduction. In: Carpenter, E. J., and Capone, D. G. (eds), *Nitrogen in the Marine Environment*, pp. 191–232. Academic Press, New York.

Herbert, R. A. (1982). Nitrate dissimilation in marine and estuarine sediments. In: Nedwell, D. B., and Brown, C. M. (eds), *Sediment Microbiology*, pp. 53–71, Academic Press, London.

Herbert, R. A., Dunn, G. M., and Brown, C. M. (1980). The physiology of nitrate dissimilating bacteria from the Tay Estuary. *Proc. R. Soc. Edin.*, **78B**, 79–87.

Jahnke, R. A., Emerson, S. R., and Murray, J. W. (1982). A model of oxygen reduction, denitrification, and organic matter mineralization in marine sediments. *Limnol. Oceanogr.*, **27**, 610–23.

Jenkins, M. C., and Kemp, W. M. (1984). The coupling of nitrification and denitrification in two estuarine sediments. *Limnol. Oceanogr.*, **29**, 609–19.

Jørgensen, B. B., and Sørensen, J. (1985). Seasonal cycles of $O_2$, $NO_3^-$ and $SO_4^{2-}$ reduction in estuarine sediments: the significance of an $NO_3$ reduction maximum in spring. *Mar. Ecol. Prog. Ser.*, **24**, 65–74.

Kaplan, W. A., Teal, J. M., and Valiela, I. (1977). Denitrification in saltmarsh sediments: evidence for seasonal temperature selection among populations of denitrifiers. *Microbiol Ecol.*, **3**, 193–204.

Kaplan, W. A., Valiela, I., and Teal, J. M. (1979). Denitrification in a marsh ecosystem. *Limnol. Oceanogr.*, **24**, 726–34.

Kaspar, H. F. (1982). Denitrification in marine sediment: measurement of capacity and estimate of in situ rate. *Appl. Environ. Microbiol.*, **43**, 522–527.

Kaspar, H. F., Tiedje, J. M., and Firestone, R. B. (1981). Denitrification and dissimilatory nitrate reduction to ammonium by digested sludge. *Can. J. Microbiol.*, **27**, 878–85.

King, D., and Nedwell, D. B. (1985). The influence of nitrate concentration upon the end-products of nitrate dissimilation by bacteria in anaerobic salt marsh sediment. *FEMS Microbiol. Lett.*, **31**, 23–8.

Koike, I., and Hattori, A. (1978a). Denitrification and ammonia formation in anaerobic coastal sediments. *Appl. Environ. Microbiol.*, **35**, 278–82.

Koike, I., and Hattori, A. (1978b). Simultaneous determinations of nitrification and nitrate reduction in coastal sediments by a 15-N dilution technique. *App. Environ. Microbiol.*, **35**, 853–7.

Koike, I., and Hattori, A. (1979). Estimates of denitrification in sediments of the Bering Sea shelf. *Deep-Sea Res.*, **26**, 409–15.

Koike, I., Hattori, A., and Goering, J. J. (1978). Controlled ecosystem pollution experiment: effect of mercury on enclosed water column. VI. Denitrification by marine bacteria. *Mar. Sci. Commun.*, **4**, 1–12.

MacFarlane, G. T., and Herbert, R. A. (1982). Nitrate dissimilation by *Vibrio* spp. isolated from estuarine sediments. *J. Gen. Microbiol.*, **128**, 2463–8.

MacFarlane, G. T., and Herbert, R. A. (1984). Dissimilatory nitrate reduction and nitrification in estuarine sediments. *J. Gen. Microbiol.*, **130**, 2301–8.

Nishio, T. (1982). Nitrogen cycling in coastal and estuarine sediments with special reference to nitrate reduction, denitrification and nitrification. Doctoral dissertation, University of Tokyo, Tokyo. pp. 115.

Nishio, T., Koike, I., and Hattori, A. (1981). $N_2$/Ar and denitrification in Tama estuary sediments. *Geomicrobiol. J.*, **2**, 193–209.

Nishio, T., Koike, I., and Hattori, A. (1982). Denitrification, nitrate reduction, and oxygen consumption in coastal and estuarine sediments. *Appl. Environ. Microboil.*, **43**, 648–53.

Nishio, T., Koike, I., and Hattori, A. (1983). Estimates of denitrification and nitrification in coastal and estuarine sediments. *Appl. Environ. Microboil.*, **45**, 444–50.

Oremland, R. S., Umberger, C., Culbertson, C. W., and Smith, R. L. (1984). Denitrification in San Francisco Bay sediments. *Appl. Environ. Microbiol.*, **47**, 1106–12.

Oren, A., and Blackburn, T. H. (1979). Estimation of sediment denitrification rates at *in situ* nitrate concentration. *Appl. Environ. Microbiol.*, **37**, 174–6.

Payne, W. J. (1973). Reduction of nitrogenous oxides by micro-organisms. *Bacteriol. Rev.*, **37**, 409–52.

Payne, W. J., and Grant, M. A. (1982). Influence of acetylene on growth of sulfate-reducing bacteria. *Appl. Environ. Microbiol.*, **43**, 727–30.

Reimers, C. E., Kalhorn, S., Emerson, S. R., and Nealson, K. H. (1984). Oxygen consumption rates in pelagic sediments from the Central Pacific: first estimates from microelectrode profiles. *Geochim. Cosmochim. Acta*, **48**, 903–10.

Revsbech, N. P., Sørensen, J., and Blackburn, T. H. (1980). Distribution of oxygen in marine sediments measured with microelectrodes. *Limnol. Oceanogr.*, **25**, 403–11.

Seitzinger, S., Nixon, S. W., Pilson, M. E., and Burke, S. (1980). Denitrification and $N_2O$ production in nearshore marine sediments. *Geochim. Cosmochim. Acta*, **44**, 1853–60.

Seitzinger, S. P., Nixon, S. W., and Pilson, M. E. (1984). Denitrification and nitrous oxide production in a coastal marine ecosystem. *Limnol. Oceanogr.*, **29**, 73–83.

Smith, C. J., De Laune, R. D., and Patrick, W. H. Jr (1982). Nitrate reduction in *Spartina alterniflora* marsh soil. *Soil. Sci. Soc. Am. J.*, **46**, 748–50.

Smith, C. J., De Laune, R. D., and Patrick, W. H. Jr (1985). Fate of riverine nitrate entering an estuary: I. Denitrification and nitrogen burial. *Estuarine*, **8**, 15–21.

Steenkamp, D. J., and Peck, H. D. Jr (1981). Proton translocation associated with nitrite respiration in *Desulfovibrio desulfuricans*. *J. Biol. Chem.*, **256**, 5450–8.

Sørensen, J. (1978a). Capacity for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. *Appl. Environ. Microbiol.*, **35**, 301–5.

Sørensen, J. (1978b). Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. *Appl. Environ. Microbiol.*, **36**, 139–43.

Sørensen, J. (1984). Seasonal variation and control of oxygen, nitrate ans sulfate respiration in coastal marine sediments. In: Klug, M. S., and Reddy, C. A. (eds), *Current Perspectives in Microbial Ecology*, pp. 447–53. Am. Soc. Microbiol., Washington, DC.

Sørensen, J., and Wilson, T. R. S. (1984). A headspace technique for oxygen measurements in deep-sea sediment cores. *Limnol. Oceanogr.*, **29**, 650–2.

Sørensen, J., Jørgensen, B. B., and Revsbech, N. P. (1979). A comparison of oxygen, nitrate, and sulfate respiration in a coastal marine sediment. *Microbial Ecol.*, **5**, 105–15.

Sørensen, J., Tiedje, V. M., and Firestone, R. B. (1980). Inhibition by sulfide of nitric and nitrous oxide reduction by denitrifying *Pseudomonas fluorescens*. *Appl. Environ. Microbiol.*, **39**, 105–8.

Sørensen, J., Hydes, D. J., and Wilson, T. R. S. (1984). Denitrification in a deep-sea sediment core from the eastern Equatorial Atlantic. *Limnol. Oceanogr.*, **29**, 653–7.

Tam, T. Y., and Knowles, R. (1979). Effects of sulfide and acetylene on nitrous oxide reduction by soil and *Pseudomonas aeruginosa*. *Can. J. Microbiol.*, **25**, 1133–8.

Tsunogai, S., Kusakabe, M., Iizimi, H., Koike, I., and Hattori, A. (1979). Hydrographic features of the deep-water of the Bering Sea: the sea of silica. *Deep-Sea Res.*, **26**, 641–59.

Vanderborght, J.-P., and Billen, G. (1975). Vertical distribution of nitrate concentration in interstitial water of marine sediments with nitrification and denitrification. *Limnol. Oceanogr.*, **20**, 953–61.

Vanderborght, J.-P., Wollast, R., and Billen, G. (1977). Kinetic models of diagenesis in disturbed sediments. Part 2. Nitrogen diagenesis. *Limnol. Oceanogr.*, **22**, 794–803.

Walter, H. M., Keeney, D. R., and Fillery, I. R. (1979). Inhibition of nitrification by acetylene. *Soil Sci. Soc. Am. J.*, **43**, 195–6.

Wilson, T. R. S. (1978). Evidence for denitrification in aerobic pelagic sediments. *Nature (Lond.)*, **274**, 354–6.

Yoshinari, T., and Knowles, R. (1976). Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. *Biochem. Biophys. Res. Commun.*, **69**, 705–10.