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# Estimation of Sediment Denitrification Rates at In Situ Nitrate Concentrations

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The denitrification rates in a marine sediment, estimated by using $^{15}N$-nitrate, $V_{max}$, $K_m$, and sediment nitrate concentrations, were 12.5 and 2.0 nmol of $N_2$-N $cm^{-3}$ $day^{-1}$ at 0 to 1 and 1 to 3 cm, respectively, at 12°C. The total rate was 165 nmol of $N_2$-N $m^{-2}$ $day^{-1}$.

There are many reports on denitrification in sediments (3). The relevance of the reported rates may, in many cases, be questioned, since nitrate is generally added at concentrations much greater than those found naturally. This would increase the rate significantly if the nitrate concentration limits denitrification in situ. Moreover, in longer incubations, the added nitrate may cause enrichment of denitrifying bacteria, so that denitrification rates are again overestimated. In this study an attempt was made to estimate the actual denitrification rate in a marine sediment.

Sediment cores from Kysing Fjord (Denmark) were taken in October 1977. Figure 1A shows the redox profile of the sediment, measured according to Fenchel (2). Sediment samples of approximately 3 $cm^3$ from different depths were placed in weighed 14-ml serum bottles, which were then completely filled with aerated water from the sampling station (containing 3.4 μM nitrate), stopped with butyl rubber stoppers, and sealed with a metal cap after the addition of $^{15}N$-nitrate (VEB Berlin Chemie, 96.3% $^{15}N$). The $^{15}N$-$NO_3^-$ was added to a final concentration of 50 to 500 μM (knowing the total water content of the bottle). A bubble of air (270 μl) was then injected into each bottle, and an equal volume of water was withdrawn. The bottles were incubated in the dark at the in situ temperature of the sediment, 12°C. The $^{15}N$ content in replicate 15-μl samples of the 270-μl gas phase was determined as described by Sørensen (6). Four repetitive scans of standards (0.37 to 2.10% $^{15}N$) in the optical emission spectrometer had standard deviations of <0.02. The standard deviation between replicate analyses was of the same order of magnitude. An error of 0.02% excess atompercent $^{15}N$ in the assay would give an error of only 2.4 nmol of $NO_3^-$-N denitrified per $cm^3$ of sediment. No correction was made for the $^{15}N_2$ removed in the previous sampling, or for the change in bubble volume due to sampling, $O_2$ utilization, and $N_2$ production. At 18 h there would be a potential 20% change in bubble volume due to oxygen disappearance but less than a 2% change due to $N_2$ production. Dinitrogen was distributed approximately equally between gas and liquid phases; therefore, the maximum error in the rate calculation due to a decrease in bubble volume would have been 10%.

Nitrate was determined colorimetrically after its reduction to nitrite by a micro-copper-cadmium column and by assaying the nitrate (10). Correction was made for the nitrite already present in the sample. Pore water was extracted by direct centrifugation (the upper 2 cm) or after extraction of the sediment with an equal volume of 1.0 M KCl solution. The nitrate concentration profile of the sediment is shown in Fig. 1B.

The quantity of $N_2$ evolution (nanomoles per cubic centimeter) was calculated from the excess over natural abundance of $^{15}N_2$ in the total $N_2$ pool, bubble plus dissolved, the latter being calculated from the solubility of nitrogen in water (11.7 ml of $N_2$ per liter of water; 25‰ salinity for an atmosphere of air at 12°C) (5). It was assumed that there was an equilibrium between $^{15}N_2$ in the gas and liquid phases. The excess percent $^{15}N$ in the $N_2$ pool was used to calculate the quantity of 96.3% $^{15}N$-$NO_3^-$ which would have produced it.

Figure 2A shows a plot of $N_2$ evolution from the upper 1 cm of sediment, with time. The substrate had decreased to almost zero after 4 days, which was reflected in decreasing rates. Initial rates were determined from tangents to the slopes. Assuming that Michaelis-Menten kinetics were followed, a double-reciprocal plot of these rates against $NO_3^-$ concentration (Fig. 3) gave the following kinetic constants: $K_m$ = 344 μM nitrate; $V_{max}$ = 422 nmol of $N_2$-N $cm^{-3}$ of sediment $day^{-1}$ (correlation coefficient of slope,

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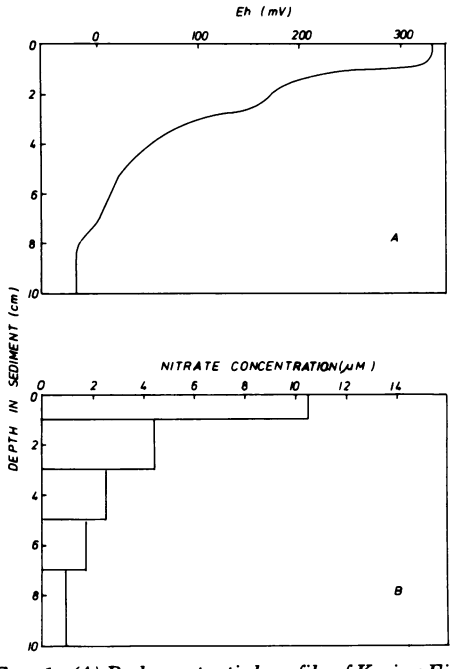


FIG. 1. *(A) Redox potential profile of Kysing Fjord sediment. (B) Nitrate concentration profile of Kysing Fjord sediment.*

0.99). Since the rate measurement with a 500 μM nitrate concentration fitted on the reciprocal plot, it seemed likely that sufficient reductant was present to saturate the denitrification enzymes, even at this nigh nitrate concentration. The 1- to 3-cm fraction (data not illustrated) was similar, with lower rates, but below 3 cm no activity was demonstrated (e.g., 5 to 7 cm in Fig. 2B). Below 3 cm there was a long lag period before $N_2$ was evolved, presumably due to an increase in a small initial population of denitrifying bacteria. This illustrates the problem caused by long incubations.

The actual denitrification rates were calculated from:

$$v = \frac{V_{max}C}{K_m + C}$$

The rate for the top 1 cm at a nitrate concentration of 10.5 μM was 12.5 nmol of $N_2$-N $cm^{-3}$ $day^{-1}$, and for 1 to 3 cm it was 2.0 nmol of $N_2$-N $cm^{-3}$ $day^{-1}$. The total rate, on an area basis, was 165 μmol of $N_2$-N $m^{-2}$ $day^{-1}$. These calculations are based on the validity of Michaelis-Menten kinetics at the low nitrate concentrations found in the sediment. If, however, different bacteria in the sediment have different uptake constants, a reciprocal plot of the effect of saturation will approach linearity, but the extrapolation back to the true rate may be in considerable error (9).

The highest rates of denitrification were found in the upper 1 cm of the sediment, where the concentrations of nitrate, and possibly inhibitory oxygens, were highest. Denitrification under seemingly oxic conditions has, however, been observed in many situations (3).

It seems that the present method has not previously been used to estimate in situ denitrification rates. van Kessel (8) and Nedwell (4) published saturation curves for denitrification in sediments, but in general Michaelis-Menten kinetics have not been applied to measure natural rates of denitrification. Michaelis-Menten kinetics were not proven in this investigation. A more sophisticated analysis (7) of more data points would help in elucidating this point.

We believe that this method can be useful in estimating denitrification rates in natural systems. The advantage of the method lies in its relative simplicity; its main disadvantages are the necessity of using a small bubble volume and inability to measure possible changes in this volume. These changes, however, would be expected to be minimal during day 1, when the most reliable data are obtained. The use of an air-saturated system is justified by assuming that oxygen would be rapidly removed, by reaction with FeS in the anoxic sediment, and that oxygen would persist for at least 1 day in the

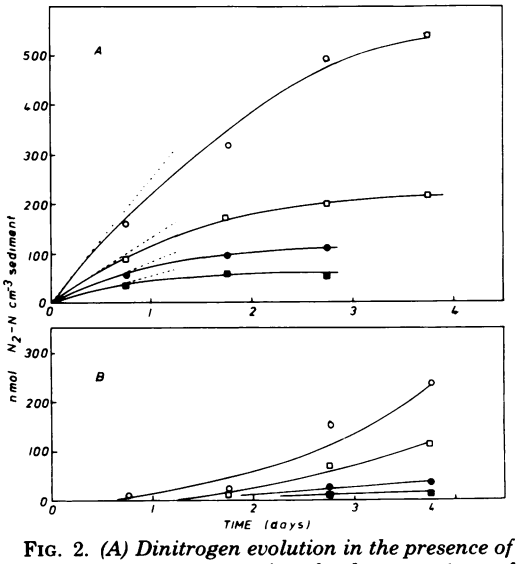


FIG. 2. *(A) Dinitrogen evolution in the presence of different nitrate concentrations by the upper 1 cm of Kysing Fjord sediment. Symbols: ○, 500 μM nitrate; □, 200 μM nitrate; ●, 100 μM nitrate; ■, 50 μM nitrate. (B) Dinitrogen evolution in the presence of different nitrate concentrations at a depth of 5 to 7 cm in Kysing Fjord sediment. Symbols as in (A).*

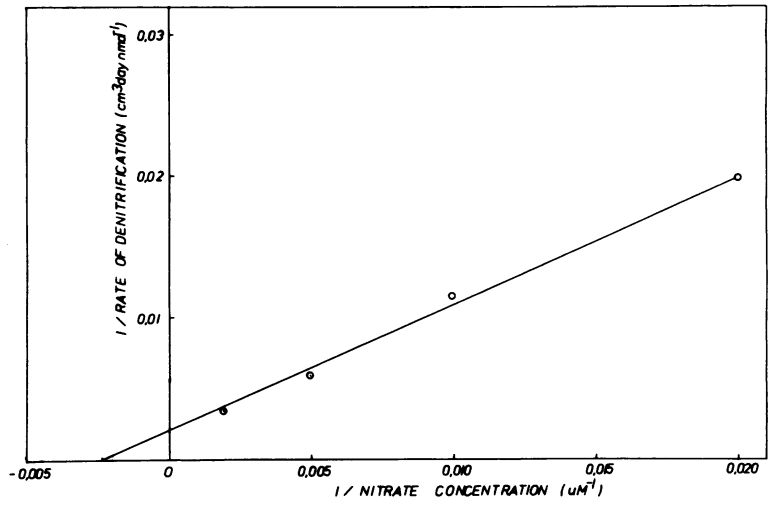


FIG. 3. *Lineweaver-Burk plot of denitrification rate as a function of nitrate concentration for the upper 1 cm of Kysing Fjord sediment.*

upper, oxic sediment segments. This oxygen could have the important effect of continuing the repression of nitrate reductase in potential, but not actual, denitrification bacteria.

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