Denitrification Rates in a Marine Sediment as Measured by the Acetylene Inhibition Technique

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A method has been developed for measurement of denitrification activity in sediments by application of the acetylene inhibition technique. Acetylene-saturated water was injected, at close intervals, into sediment cores which were then incubated for a few hours at the in situ temperature. Frozen segments of the cores were assayed for accumulation of N₂O by a combined gas extraction and detection system. The segments were thawed under a stream of helium from which N₂O (and other gases) was collected in a liquid N₂ trap, and the quantity of N₂O was measured by gas chromatography. The maximum rate of denitrification in a coastal marine sediment was 35 nmol of N per cm² of sediment per day at 2.5°C, and the rate of denitrification for the total sediment was 0.99 mmol of N per m² per day.

Measurements of denitrification rates in sediments are lacking because of the absence of suitable methodology. The most promising methods are short-term assays of the denitrification-accumulation of N₂O by a combined gas extraction and detection system. The segments were thawed under a stream of helium from which N₂O (and other gases) was collected in a liquid N₂ trap, and the quantity of N₂O was measured by gas chromatography. The maximum rate of denitrification in a coastal marine sediment was 35 nmol of N per cm² of sediment per day at 2.5°C, and the rate of denitrification for the total sediment was 0.99 mmol of N per m² per day.

MATERIALS AND METHODS

Sampling and incubations. Randers Fjord is an estuary on the east coast of Jutland, Denmark. A sampling site was chosen near the shore of this estuary where the bottom was sandy and had a deep, oxidized surface layer.

The rates of denitrification in the sediment were measured in the winter (January 1978) when the temperature in situ was 2.5°C. The perturbing activity of the benthic macrofauna was low at this temperature, but some variation between the cores was inevitable due to the burrows of mainly polychaetes and lamellibranchs.

A series of cores was taken in Plexiglas tubes (20 cm long and 2.6 cm wide) from a restricted area of a few square meters. Some cores were frozen on location in liquid N₂ and used for the assays of N₂O concentrations of N₂O-, N₂O, and N₂O. The samples were stored in a freezer at −20°C for a week before they were assayed. Other cores were used for the denitrification assay which was initiated in the laboratory on the day of sampling. The tubing of these cores had a vertical series of silicon rubber inserts that served for a sideward injection of acetylene into the sediment. The inserts were placed at 1-cm intervals, and a quantity of 100 µl of acetylene-saturated, distilled water (1.6 ml of C₂H₂ per ml of water) was injected into the sediment behind each hole. Injections were performed while the needle was slowly withdrawn through the sediment horizontally and in five directions from each insert.

The injections gave about 3% saturation (50 µl of C₂H₂ per ml) of the interstitial water. In a previous study on a different sediment (6), a 0.7% saturation of the interstitial water with acetylene blocked the reduction of N₂O in N₂O-amended samples. A preliminary experiment showed that the inhibition was complete also in the present sediment without N₂O-en-
enrichment when acetylene was applied at 1 to 2% of saturation. This inhibition efficiency was verified from measurements of N\textsubscript{2}O consumption in bottles with mixed sediment samples that were amended with N\textsubscript{2}O (300 \muM) and incubated anaerobically with acetylene (0.5 to 5.0% saturation of the interstitial water). The samples had been preincubated for a day without acetylene and N\textsubscript{2}O to exhaust the native pool of nitrogen oxides.

The overlying water phase was discarded from the cores before incubation to facilitate the supply of oxygen at the sediment surface. The cores were then stoppered, and acetylene gas was added to 5% (vol/vol) in the air space. The incubations were performed at 2.5°C in the dark, and the cores, which were frozen at intervals in liquid N\textsubscript{2}, were stored in the freezer for later analysis. During the incubation, gas samples were frequently taken from the air space above the cores to measure any N\textsubscript{2}O released from the sediment surface. The assays for N\textsubscript{2}O accumulation in the cores were performed within a week.

NO\textsubscript{3}\textsuperscript{-} and NO\textsubscript{2}\textsuperscript{-} assays. The methods of Strickland and Parsons (7) were applied for the determinations of the in situ concentrations of NO\textsubscript{3}\textsuperscript{-} and NO\textsubscript{2}\textsuperscript{-} in the sediment. The frozen cores were cut into 1-cm segments, and samples of about 5 g were thawed in centrifuge tubes containing 2 ml of distilled water and 1 ml of saturated HgCl\textsubscript{2} solution to inhibit microbial activity during the assay. Centrifugation at 2,000 \times g for 15 min cleared the supernatant, and samples of 2 ml were assayed by an automated analysis system (Svend Schræder, Aps).

N\textsubscript{2}O assays. Nitrous oxide was extracted from thawing 1-cm segments in a helium stream and recovered in a liquid N\textsubscript{2} trap. The contents of N\textsubscript{2}O were quantitated by gas chromatography.

The combined gas extraction and detection system is shown in Fig. 1. The helium carrier flowing to the measuring side of the gas chromatographic detection system was split into two lines. Line A went to the detection system through the eight-way valve (Fig. 1, a). Line B purged the flask with the sediment sample (Fig. 1, c) and passed through a liquid N\textsubscript{2}-cooled trap (Fig. 1, e) attached to the eight-way valve, where any N\textsubscript{2}O liberated from the sample was trapped. The addition of HgCl\textsubscript{2} solution to the flask (0.1 ml/g of sediment) prevented microbial activity during the gas extraction; mixing was facilitated by a magnetic stirring bar inside the flask. The sediment segments thawed soon after a beaker of hot water was placed under the flask, and N\textsubscript{2}O was completely recovered from the samples after 30 min of purge. Ultimately, the N\textsubscript{2}O was transferred from the trap to the gas chromatograph by means of the valve when gas line A was switched through the loop and the liquid N\textsubscript{2} trap was removed. The needle valve (Fig. 1, b) served to regulate the flow (50 ml/min) through gas line B. The absorption trap (Fig. 1, d) contained a mixture of 1 g of Ascarite (20/30 mesh) to remove CO\textsubscript{2} and 1 g of Drierite (ground) for removal of water vapor. On the measuring side of the gas chromatograph, a Porapak Q column, 2 m long and 0.32 cm in diameter, was used at ambient temperature in a carrier flow of 20 ml/min. The detection system was a Packard Becker model 417 with a thermal conductivity detector operated at 140°C and used in combination with a Hewlett Packard 7100 BM recorder.

Other assays. Density and porosity of the sediment were determined by weighing known volumes of sediment segments after drying at 110°C for 24 h. These measurements were necessary as bases for conversion of rates and concentrations into appropriate dimensions.

RESULTS AND DISCUSSION

Concentrations of NO\textsubscript{3}\textsuperscript{-}, NO\textsubscript{2}\textsuperscript{-}, and N\textsubscript{2}O in situ. No alteration of the gradients of the nitrogen oxides was observed during storage, indicating that biological activity was sup-

![Fig. 1. Gas extraction and detection system. (A and B) Helium gas lines; (a) eight-way valve, (b) needle valve, (c) sample flask, (d) CO\textsubscript{2} and water absorption trap, (e) liquid N\textsubscript{2} trap, (f) gas chromatograph.](http://aem.asm.org/DownloadedFrom/fig1.jpg)
pressed and diffusion was negligible in the frozen cores. The addition of HgCl₂ proved necessary to inhibit microbial activity in the core segments when these were thawed and assayed.

The in situ concentrations of NO₃⁻, NO₂⁻, and N₂O with depth in the sediment are shown in Fig. 2. The presence of an NO₃⁻ maximum of about 160 μM in the upper 1 to 2 cm suggested that nitrification took place in this zone and was the source of NO₃⁻ in the present sediment. The concentration of NO₃⁻ decreased rapidly with depth, with little below 6 cm. Traces of NO₃⁻, detectable below 6 cm, were possibly due to transport of surface water to deeper layers by the activity of the burrowing macrofauna.

Some NO₃⁻ and N₂O was also present in the deeper layers, but, again, the highest concentrations were found within the upper 6 cm. It is of interest that the maximum concentrations of about 15 and 1.5 μM, respectively, were found below the NO₃⁻ peak, indicating that NO₂⁻ did not accumulate during nitrification.

**Denitrification assay.** The oxygenation of the sediment in situ was approximated in the laboratory by discarding the water phase from the cores and thus exposing the sediment surface directly to air. No method was available to measure oxygen concentrations in the sediment, but the in situ and laboratory conditions could be compared on the basis of the NO₃⁻ and NO₂⁻ concentration profiles. These did not change significantly when cores were stored for a few hours in the laboratory as described above.

The injections of acetylene were done from the side of the cores to facilitate a rapid and even distribution of the inhibitor. It was anticipated that a homogenous distribution was obtained by the water displacement during the injections and, in addition, by subsequent diffusion in the cores. The dilution of the interstitial water was minimal and caused only local distortion of the solute concentration near the injection point because no alteration of the overall gradients was observed in the cores.

**Rates of denitrification.** The incubation with acetylene caused a progressive accumulation of N₂O in the cores. The production of N₂O with depth in the sediment is shown for various incubation times in Fig. 3. Any loss of N₂O by diffusion from the sediment surface was negligible because no N₂O was detected in the gas space above the sediment. Denitrification took place in the upper 6 cm where NO₃⁻ was present in significant concentration. Nitrification and denitrification appeared to occur together in the upper segments, as indicated by Fig. 2 and 3. The occurrence of these two apparently mutually exclusive processes may be similar to the coincidence of sulfate reduction and sulfide oxidation (4).

![Fig. 2. Concentrations of NO₃⁻, NO₂⁻, and N₂O in situ with depth in the sediment. Standard deviations are indicated for duplicate analyses.](http://aem.asm.org/)
**Fig. 3.** Concentration of $N_2O$ with depth in sediment cores incubated for 1 h ($\bullet$), 2 h ($\Delta$), 4.5 h ($\bigcirc$), and 5.5 h ($\blacksquare$) with acetylene (3% $C_2H_2$ saturation or 50 µl of $C_2H_2$ per ml of interstitial water).

**Fig. 4.** Cumulative $N_2O$ production with time in acetylene-incubated sediment cores (3% $C_2H_2$ saturation or 50 µl of $C_2H_2$ per ml of interstitial water).
Most denitrification took place, however, where the highest concentrations of \( \text{NO}_2^- \) and \( \text{N}_2\text{O} \) were found. The profile of \( \text{N}_2\text{O} \) in situ did not show any distinct maximum in this sediment as was the case for cores from other coastal areas (Sørensen, unpublished data). Peak activity of denitrification was observed at 2 to 3 cm, with a maximal rate of 35 nmol of N per cm\(^2\) of sediment per day. In some cores the maximal production took place above or below this depth, and this variation apparently resulted from a general sediment heterogeneity induced by macrofaunal activity.

Denitrification on an area basis is shown with time of incubation in Fig. 4. A least-squares regression process was employed to estimate the slope, i.e., the rate of denitrification for the total sediment. The calculated value was 0.99 mmol of N per m\(^2\) per day. The cumulative production of \( \text{N}_2\text{O} \) in the cores was linear with time, with a correlation coefficient of 0.97, but the cumulative figures also reveal the variation between individual cores.

The lack of previous reports on rates of denitrification in marine sediments restricts interpretations on the general validity of the measured rate, but the result is in accordance with rates that I have measured in other coastal marine sediments during the winter season.

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**LITERATURE CITED**


