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Denitrification in San Francisco Bay Intertidal Sediments

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The acetylene block technique was employed to study denitrification in intertidal estuarine sediments. Addition of nitrate to sediment slurries stimulated denitrification. During the dry season, sediment-slurry denitrification rates displayed Michaelis-Menten kinetics, and ambient NO_3^- + NO_2^- concentrations (≤26 μM) were below the apparent K_m (50 μM) for nitrate. During the rainy season, when ambient NO_3^- + NO_2^- concentrations were higher (37 to 89 μM), an accurate estimate of the K_m could not be obtained. Endogenous denitrification activity was confined to the upper 3 cm of the sediment column. However, the addition of nitrate to deeper sediments demonstrated immediate N_2O production, and potential activity existed at all depths sampled (the deepest was 15 cm). Loss of N_2O in the presence of C_2H_2 was sometimes observed during these short-term sediment incubations. Experiments with sediment slurries and washed cell suspensions of a marine pseudomonad confirmed that this N_2O loss was caused by incomplete blockage of N_2O reductase by C_2H_2 at low nitrate concentrations. Areal estimates of denitrification (in the absence of added nitrate) ranged from 0.8 to 1.2 μmol of N_2 m^{-2} h^{-1} (for undisturbed sediments) to 17 to 280 μmol of N_2 m^{-2} h^{-1} (for shaken sediment slurries).

Denitrification can benefit aquatic environments faced with eutrophication. By removing nitrate from shallow waters, sediment denitrification may impose a nutrient limitation against excessive algal growth. This can be of importance in estuaries, especially those which receive large inputs of nitrogenous compounds derived from agricultural runoff or municipal sewage effluent (or both), such as San Francisco Bay (15). The extensive intertidal mudflats of San Francisco Bay (especially its southern portion) may therefore represent important sinks for nitrogen entering the system.

A number of workers have attempted to measure denitrification in marine or estuarine sediments either by using $^{15}NO_3^-$ (11, 12) or by measuring evolution of dinitrogen (16). These methods may overestimate in situ rates because of either increasing nitrate pool sizes ($^{15}NO_3^-$ method) or by contamination with atmospheric nitrogen. Despite the discovery of the acetylene block assay of nitrous oxide reductase (1, 32) and sensitive chromatographic methods for N_2O analysis (6), there have been relatively few reports of short-term denitrification rates in marine or estuarine sediments (5, 18–20). Recently, Kaspar (5) employed the blockage method to study denitrification in the intertidal sediments of Delaware Inlet, New Zealand. He reported that short-term rates followed zero-order kinetics when ambient nitrate concentrations were high enough (>1 μM) to achieve a successful enzyme block by acetylene. In this paper we report that intertidal San Francisco Bay sediments display Michaelis-Menten kinetics with respect to nitrate concentration when assayed by the acetylene-block technique. However, only a partial blockage of nitrous oxide reductase by acetylene was achieved at low nitrate concentrations.

MATERIALS AND METHODS

Site description. Sediments were taken from an intertidal mudflat located in South San Francisco Bay (3). The site was in a tidal slough, about 300 m downstream from the Palo Alto municipal sewage treatment facility. During low tides the mudflat was exposed to the atmosphere for about 5 h. Water samples were filtered (0.45 μm), analyzed for salinity (refractive index), and stored frozen for subsequent analysis of NO_3^-, NO_2^-, and NH_4^+ by autoanalyzer (15). Nitrite was always a minor constituent (≤5% of NO_3^-), and therefore the nitrate data presented represent the sum of NO_3^- plus NO_2^-. The sediment surface was frequently covered with a film of benthic diatoms, which supported sizeable populations of snails (*Nassarius obseletus*) and clams (*Macoma balthica*). Populations of these organisms were most abundant from May to October. Experiments were started within 2 h of collection of sediment and water samples.

Experiments with sediment slurries. Sediments were collected with short cores (diameter, 3 cm) from the upper 7 cm of the mudflat (3). Cores (volume, 50 cm^3) were extruded under a flow of N_2 (150 cm^3 min^{-1}) into wide-mouthed, 250-ml Erlenmeyer flasks that contained 100 ml of either bay water collected at the site or NO_3^--free artificial bay water (ABW; 13). During the winter rainy season (November through April) the ABW was diluted with distilled water to achieve ambient salinity. Flasks were sealed under N_2 (unless indicated otherwise) with rubber stoppers and then flushed for an additional 15 min with N_2. Selected flasks were supplemented with $NaNO_3$ at the concentrations indicated below. Flasks were incubated in the dark at 20°C with rotary shaking (150 rpm). After a 5-min preincubation, C_2H_2 was added (15 kPa). This amount of C_2H_2 was previously found to be effective at blocking N_2O reductase in these sediments (3). In some experiments higher levels of C_2H_2 were employed, as indicated below. After C_2H_2 addition, flasks were shaken for 3 to 15 min (as indicated below) to facilitate gas exchange between phases before withdrawal of the initial samples. Gas phase samples were withdrawn with glass syringes and either stored in evacuated tubes as described previously (24) or, for short-term (2-h) storage, kept in 0.5-ml glass syringes with their needle ends inserted into rubber bungs. The production of N_2O was quantified by ^{63}Ni electron capture or thermal conductivity detector gas chromatography (3, 24). In some experiments, the concentrations of NO_3^-, NO_2^-, and NH_4^+ were monitored during incubation as follows. Sediment (200 cm^3) was placed in 1-liter flasks containing 400 ml of ABW. Slurry (40 ml) was removed periodically with a syringe (and replaced with 40 cm^3 of N_2) and centrifuged (5,000 × *g* for 15 min), and the

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supernatant was filtered (0.45 μm; Nucleopore Corp.) and frozen for subsequent analysis. Estimates of dissolved N_2O were calculated by applying N_2O solubility coefficients (28) to the equations of Flett et al. (4). Areal estimates of sediment slurry denitrification rates were extrapolated to a 1-m^2 basis by multiplying the rates obtained from individual cores (circular area, 7.1 cm^2) by 1,414.

The occurrence of denitrification activity with depth in the sediments was studied. Samples were taken using suction cores (35-cm length by 5.1-cm outer diameter) fitted with tape-covered side holes (diameter, 1.6 cm) spaced at 2-cm intervals. Cores were subsampled at each depth interval by removing 5 cm^3 of sediment with subcores fashioned out of 5-ml plastic syringes (hub end removed). Subcores were divided into two 2.5-cm^3 subsamples and added to 25-ml test tubes (18 by 150 mm; Bellco Glass, Inc.) that contained 5 ml of either NO_3^--free or NO_3^--supplemented (1 mM) ABW. All manipulations took place under a flow of N_2. Tubes were sealed with butyl rubber stoppers and flushed with N_2 (75 cm^3 min^{-1}) for 2 min. Tubes were next vortexed (to disperse sediment), injected with C_2H_2 (30 or 60 kPa as indicated below), and incubated at 18°C with reciprocal shaking (100 rpm) while lying in a horizontal position. N_2O was determined as outlined above.

Chamber experiments. Sediment samples from the upper 3 cm of the intertidal mudflats were recovered with minimal disturbance and assayed for denitrification by using cylindrical Plexiglas chambers. The chambers (wall thickness, 0.6 cm; height, 160 cm; inner diameter, 7.6 cm; total volume, 600 cm^3) were molded to a circular piece of Plexiglas (diameter 9.8 cm) at their top. The circular section had a hole in its center for insertion of a serum stopper. Sediment was recovered intact (150 to 200 cm^3) by inserting the tapered, open bottom coring end of the chamber into the sediment to a depth of 3 cm and then maneuvering a large, black rubber stopper (no. 14) up underneath the captured material to seal the chamber bottom. The large stoppers were taped in place, and the chambers were returned to the laboratory (within 1 h of collection). Bay water (25 ml) was added to wet the sediments, and some samples were supplemented with $NaNO_3$ (0.1 or 1.0 mM). The chambers were sealed with serum stoppers either under air or N_2 (flushed for 10 min with an N_2 flow of 200 cm^3 min^{-1}). Selected chambers received C_2H_2 (15 kPa). Chambers were incubated statically at 18 to 20°C, and N_2O was sampled and analyzed as described above. Areal estimates of chamber denitrification rates were extrapolated to a 1-m^2 basis by multiplying the rates obtained in the chambers (circular area, 44.2 cm^2) by 226.2.

Experiments with washed bacterial cell suspensions. A culture of a denitrifying *Pseudomonas* sp. isolated from a tropical marine seagrass rhizome was kindly provided by J. Martin. The organism was grown in anaerobic batch culture by using half-strength basal synthetic seawater medium (23) supplemented with sodium succinate (20 mM) and $NaNO_3$ (10 mM). Cultures were grown in 500-ml Erlenmeyer flasks containing 250 ml of medium (under N_2) at 29°C with rotary shaking (150 rpm). Cells were harvested during late log phase by centrifugation (12,000 × *g* for 15 min). The pellet was suspended in 140 ml of half-strength basal synthetic seawater medium that lacked nitrate, succinate, and yeast extract. Cells were centrifuged and suspended an additional two times to assure removal of residual nitrate ions. After these repeated washings, cells were suspended in 140 ml of half-strength basal synthetic seawater medium in a 250-ml Erlenmeyer flask. The flask was sealed with a rubber stopper penetrated by a steel gassing canula which bubbled N_2 (flow, 200 cm^3 min^{-1}) through the cell suspension. The flask was vented with a syringe needle passing through the rubber stopper. Cells were flushed with N_2 for 0.5 h before subsamples (15 ml) of the cell suspension (126 to 190 μg of protein per ml) were transferred (by glass syringe) into 50-ml Erlenmeyer flasks arranged in an N_2 flushing train (~150 cm^3 min^{-1}) sealed with recessed, butyl rubber stoppers (no. 1). Flasks were flushed for 0.5 h with N_2 after receiving cell suspensions. Sodium succinate (final concentration, 10 mM) and $NaNO_3$ (various concentrations) were added by syringe from anaerobic stock solutions. Acetylene was added at the concentrations indicated below, and flasks were incubated at 29°C with rotary shaking (150 rpm). Gas phase samples were withdrawn and stored in 0.5-ml Glaspak syringes (Becton Dickinson & Co.) by inserting their needle ends into large rubber bungs. Samples were analyzed for N_2O within 2 h of collection. Protein concentrations of the washed cell suspensions were determined by the procedure of Lowry et al. (10).

RESULTS

Sediment slurries. Production of N_2O occurred when sediments were mixed with bay water and incubated anaerobically in the presence of C_2H_2 (Fig. 1). Production was never observed when slurries were anaerobically incubated without C_2H_2, and incubation under air plus C_2H_2 caused a 5-h lag before N_2O production became apparent (data not shown). In an experiment conducted during the dry season (1 October 1981) at ambient NO_3^- concentrations, N_2O

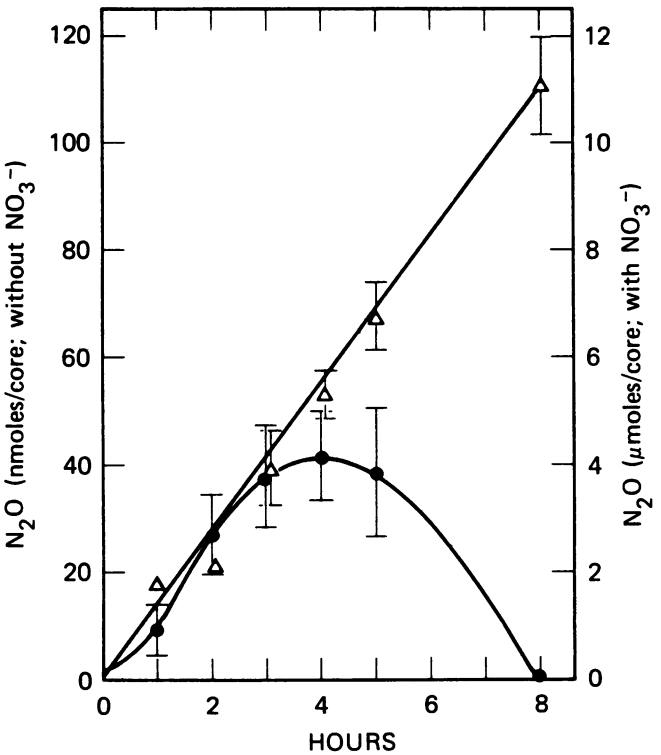

FIG. 1. N_2O production by sediment slurries incubated in bay water (ambient NO_3^-, 4.6 μM) with 15 kPa of C_2H_2 on 1 October 1981. Flasks were incubated at ambient nitrate concentrations (●) or supplemented with 1 mM $NaNO_3$ (100 μmol $flask^{-1}$) (△). Each point represents the mean of three flasks, and each bar indicates 1 standard deviation.

production ceased after 3 h and was followed by a decline and eventually disappearance of N_2O from the gas phase (Fig. 1). The mean rate of N_2O production for these unsupplemented slurries was 17 μmol m^{-2} h^{-1} (Table 1) for the first 3 h. The addition of NO_3^- (1 mM) to these slurries enhanced N_2O production 100-fold, and rates were linear over the 8-h incubation (Fig. 1). In another experiment conducted during the dry season (30 September 1981), the addition of 0.1 mM $NaNO_3$ to slurries enhanced N_2O production by about 8.5-fold (Table 1). By contrast, in an experiment conducted during the rainy season (16 January 1982) at higher ambient levels of NO_3^-, the linear rates of N_2O production by replicate, unsupplemented slurries were both 57 μmol m^{-2} h^{-1} (Table 1). The addition of $NaNO_3$ (0.1 mM) to these slurries caused only a twofold enhancement of N_2O production. Thus, the stimulation of denitrification rates by nitrate addition was more pronounced during the dry season as opposed to the rainy season (Table 1).

Incubation of sediments in ABW with increasing amounts of NO_3^- demonstrated saturation kinetics (Michaelis-Menten) with respect to rates of N_2O formation from added NO_3^- (Table 2). Double-reciprocal plots of these data revealed an apparent K_m for nitrate of 50 μM for the experiment on 19 October 1981. This K_m value lies above the ambient NO_3^- levels (4.6 to 26 μM) evident before the rainy season (Table 1). During the experiment on 5 April 1982 (Table 2), relatively high rates of denitrification were evident in flasks incubated either without added nitrate (34 nmol $core^{-1}$ h^{-1}) or below 5 μM nitrate (42 to 84 nmol $core^{-1}$ h^{-1}). This may have been caused by nitrate carryover upon sampling during the rainy season when ambient levels were higher (Table 1). Thus, an accurate estimate of the apparent K_m for nitrate could not be obtained during the rainy season.

The effect of increased C_2H_2 upon N_2O reductase at various NO_3^- concentrations is shown in Fig. 2. N_2O levels in flasks containing slurries incubated with low NO_3^- (≤5 μM) and 15 kPa of C_2H_2 decreased after 0.5 h (Fig. 2A). This effect was countered by increased amounts of C_2H_2 (Fig. 2B and C). In addition, N_2O loss was not observed at higher NO_3^- concentrations (50 μM); however, the total amount of N_2O recovered increased with higher levels of C_2H_2. Initial rates of N_2O production were roughly equivalent for any given NO_3^- concentration at various levels of C_2H_2.

Most of the endogenous denitrification activity was found in the upper 3 cm of the sediment column (Fig. 3A).

TABLE 1. Estimates of mean areal denitrification rates made from incubation of sediment cores as shaken slurries[a]

Date	Ambient NO_3^- (μM)	Denitrification rate (μmol of N_2O m^{-2} h^{-1})	
		Ambient NO_3^-	Plus NO_3^-
30 September 1981	13	79	665[b]
1 October 1981	4.6	17[c]	1,944[d]
16 October 1981	25	238	980[d]
4 November 1981	63	280	310[b]
20 November 1981	84	181	403[b]
16 January 1982	89	57[e]	113[b,e]

[a] Sediments were either incubated at ambient nitrate or with the addition of nitrate at the concentrations indicated. Rates represent the means of three samples unless indicated otherwise.
[b] Supplemented with 100 μM $NaNO_3$.
[c] Single flask determination.
[d] Supplemented with 1,000 μM $NaNO_3$.
[e] Average of duplicate flasks.

TABLE 2. Rates of N_2O production by sediment slurries incubated in ABW under an atmosphere of N_2 plus 15 kPa of C_2H_2 with supplements of various concentrations of nitrate

Added nitrate concn (μM)	Denitrification rate (nmol of N_2O $core^{-1}$ h^{-1})	
	19 October 1981[a]	5 April 1982[b]
0	1.0; 1.0	34
1	4.5; 7.0	58
2.5		42
5	38; 65	84
7.5		150
10	92; 123	102
15		138
25		114
50	311; 324	208
75		234
100	332; 458	492
250		164
500		341
1,000	424; 462	419

[a] Replicate flasks incubated at seven different NO_3^- concentrations; ambient salinity, 25‰; ambient NO_3^-, 26 μM.
[b] Single flasks incubated at 14 different NO_3^- concentrations; ambient salinity, 8‰; ambient NO_3^-, 56 μM.

However, denitrification potential was present at all depths sampled. Sediments supplemented with 1 mM NO_3^- demonstrated linear rates of N_2O production, and rates were comparable for all depth intervals sampled (Fig. 3B). Similar results were obtained when the experiment was repeated (1 month later) with 60 kPa of C_2H_2 instead of 30 kPa, and potential activity was found down to 15 cm (deepest sample).

Denitrification could account for only about 8.5 to 18% of the NO_3^- consumed by incubated sediment slurries (Table 3). No significant accumulation of NO_2^- occurred during these experiments. In the experiment on 18 May 1982, loss of NO_3^- over the 4-h incubation was accompanied by increases in NH_4^+ (from 130 μM at the start to 160 μM at 4

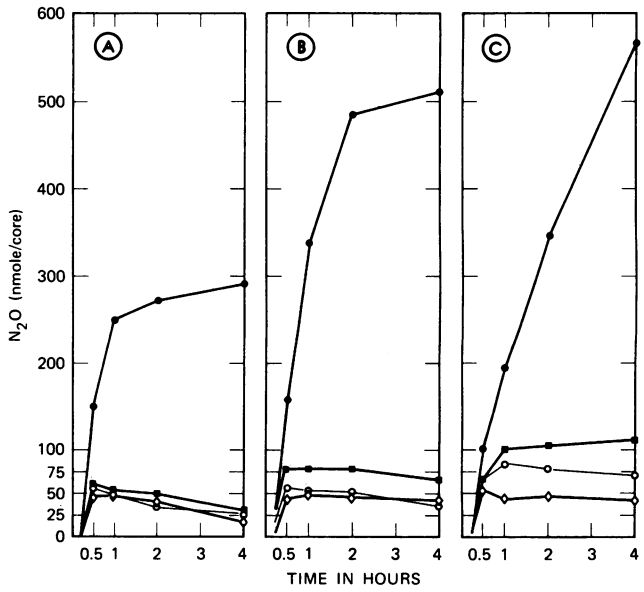

FIG. 2. Effect of nitrate and acetylene levels on the blockage of N_2O reductase in sediment slurries incubated in ABW with no additions (◇) or 0.1 (○), 0.5 (■), or 5.0 (●) μmol of $NaNO_3$ per core. Slurries were incubated with 15 (A), 30 (B), or 45 (C) kPa of C_2H_2.

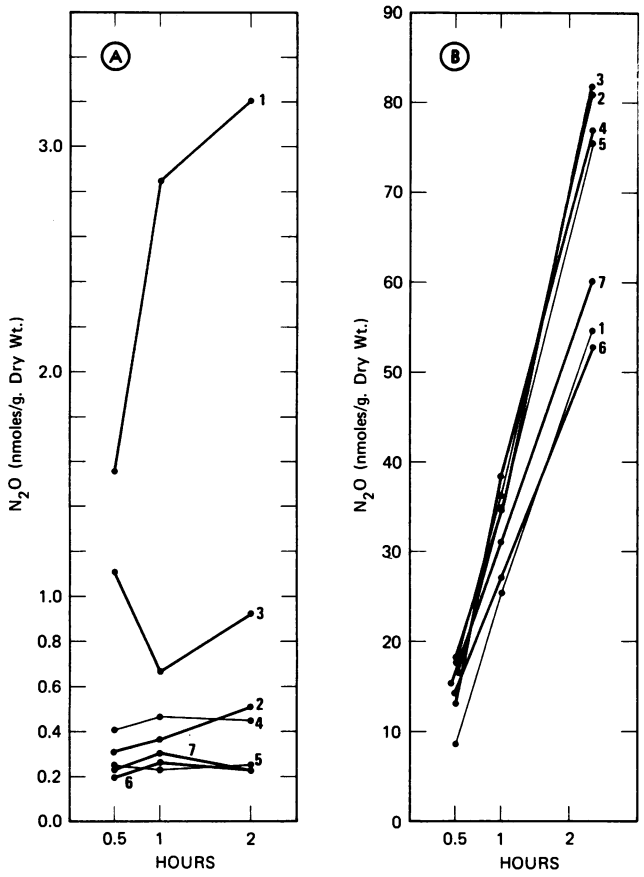

FIG. 3. Denitrification by sediment samples from various depths of a core taken on 29 April 1982 (salinity, 8‰; ambient NO_3^-, 56 μM). Subcore samples were incubated in NO_3^--free ABW (A) or in ABW with 5 μmol of 1 mM $NaNO_3$ per tube (B). Depth intervals (centimeters) were as follows: 1, 0 to 1.3; 2, 1.7 to 3.3; 3, 3.5 to 5.2; 4, 5.6 to 7.1; 5, 7.8 to 9.3; 6, 9.6 to 11.1; 7, 11.8 to 13.5. Samples were incubated for 0.5 h with 30 kPa of C_2H_2 before sample removal.

h). However, a control slurry incubated without added NO_3^- (starting NO_3^- + NO_2^- concentration, 0.8 μM) also had increased NH_4^+ levels by an equivalent amount during the 4-h incubation (95 to 125 μM). During the experiment on 12 November 1982, NH_4^+ levels decreased from 87 μM at the start to 76 μM at the end of the the 6-h incubation.

Chamber experiments. Chambers containing intact, unshaken sediments demonstrated linear rates of N_2O production during a 9-h incubation period under either N_2 + C_2H_2 or air + C_2H_2 atmospheres (data not shown). Rates of N_2O production in N_2 + C_2H_2 chambers (1.2 ± 0.6 μmol of N_2O m^{-2} h^{-1}; mean of 3 ± 1 standard deviation; Table 4; experiment on 28 October 1981) were equivalent to rates observed in the air + C_2H_2 chambers (1.0 ± 0.5 μmol of N_2O m^{-2} h^{-1}). Air + C_2H_2 chambers exhibited a 1- to 2-h lag before linear N_2O production became apparent. However, this lag was not evident when the experiment was repeated. No lag was observed in the N_2 + C_2H_2 chambers. No N_2O production was observed under air or N_2 atmospheres in the absence of C_2H_2. In other experiments, the addition of 1 mM $NaNO_3$ (40 μmol per chamber) to chambers incubated under N_2 + C_2H_2 stimulated N_2O production by 100-fold (Table 4). However, lag phases of about 0.5 h (experiment on 2 October 1981) to 2 h (experiment on 16 December 1981) were evident in NO_3^--amended chambers before attaining these linear rates. Replicate NO_3^--supplemented chambers produced N_2O at a linear rate equivalent to 101 μmol of N_2O m^{-2} h^{-1} (Table 4; experiment on 2 October 1981) for 10 h. In the experiment on 16 December 1981 (Table 4), NO_3^--supplemented chambers had an initial linear N_2O production rate of 15.1 ± 1.2 μmol of N_2O m^{-2} h^{-1} for the first 2 h of incubation, after which rates increased nearly ninefold.

Experiments with washed cell suspensions. The results of the experiments with washed cell suspensions are shown in Fig 4. Cells produced low levels of N_2O in the absence of added nitrate (Fig. 4A). The nitrate, therefore, probably came from intracellular supplies. The addition of C_2H_2 to cells incubated without NO_3^- caused accumulations of N_2O; however, N_2O levels declined greatly in flasks with 3.75, 7.5, and 15 kPa of C_2H_2, but leveled off in flasks with 30 and 60 kPa of C_2H_2. More N_2O was recovered with higher amounts of added C_2H_2 (30 and 60 kPa). This trend was also evident for cells incubated with 10 μM NO_3^- (Fig. 4B). N_2O recovered in the flask with the highest amount of C_2H_2 (7.5 kPa) represented a conversion of about 72% of the NO_3^- to N_2O by 90 min. The loss of N_2O was most evident in the flask having the least (0.5 kPa) C_2H_2. At high (50 μM) NO_3^- concentrations (Fig. 4C) the acetylene block appeared to be effective (100% recovery) at all the C_2H_2 concentrations employed (within the range of experimental variability). High levels of C_2H_2 (30 to 60 kPa) were required to achieve a successful block of N_2O reductase in the absence of added nitrate (Fig. 4A). Progressively lower C_2H_2 concentrations were required to achieve this block as nitrate concentrations increased to 10 μM (7.5 kPa; Fig. 4B) and to 50 μM (0.5 to 1.0 kPa; Fig. 4C).

DISCUSSION

Denitrification in the intertidal sediments of South San Francisco Bay was primarily limited by the availability of nitrate ions. The addition of nitrate to slurries and chambers (Table 2; Fig. 1 and 2) always stimulated activity. This was most evident during the dry season when ambient nitrate levels were low (≤26 μM; Table 1) and less than the sediment K_m for nitrate (50 μM; Table 2). The observation that these sediments display saturation kinetics with respect to nitrate concentrations contrasts with the results obtained by Kaspar (5) for intertidal sediments in New Zealand. This discrepancy cannot be explained by differences in ambient nitrate concentrations because the range of values observed in South San Francisco Bay (4.6 to 89 μM; Table 2) were

TABLE 3. Percentage of added nitrate entering denitrification pathways during incubation of sediment slurries[a]

Date	Salinity (‰)	NO_3^- added (μmol $flask^{-1}$)	NO_3^- lost (μmol $flask^{-1}$)	N_2O formed[b] (μmol $flask^{-1}$)	% NO_3^- to N_2O[c]
18 May 1982[d]	6	100	91	8.0	17.6
12 November 1982[e]	18	400	94	4.0	8.5

[a] Slurries were incubated in ABW under 30 kPa of C_2H_2 and supplemented with $NaNO_3$. Carry-over of interstitial NO_3^- + NO_2^- was determined from analyses of unsupplemented slurries and was found to be of minor significance (≤1 μmol $flask^{-1}$).

[b] Includes dissolved N_2O, calculated to be ~30% of gas phase values.

[c] Based on the following stoichiometry: 2 NO_3^- → 1 N_2O.

[d] Incubation of 4 h (no further N_2O production or NO_3^- loss).

[e] Incubation of 6 h (no further N_2O production or NO_3^- loss).

TABLE 4. Estimates of areal denitrification rates made from incubation of undisturbed sediments in Plexiglas chambers[a]

Date	Ambient NO_3^- (μM)	Denitrification rate[b] (μmol of N_2O m^{-2} h^{-1})	
		Ambient NO_3^-	Plus NO_3^-[c]
2 October 1981	ND[d]	1.1 ± 0.3	101[e]
28 October 1981	22	1.2 ± 0.6	ND
16 December 1981	37	0.8 ± 0.1	135 ± 24

[a] All chambers were incubated under an atmosphere of N_2 plus 15 kPa of C_2H_2.
[b] Mean of three or four chambers ± 1 standard deviation.
[c] Supplemented with 25 ml of bay water containing 1 mM $NaNO_3$ (40 μmol $chamber^{-1}$).
[d] ND, Not determined.
[e] Identical rates were obtained for replicate chambers.

similar to those reported by Kaspar (5) in interstitial waters (10 to 59 μM). A possible explanation, therefore, is that the New Zealand sediments may have been carbon limited rather than nitrate limited.

Sediment depth profiles indicate that most of the endogenous activity occurs within the upper few centimeters of the sediment column (Fig. 3A). Because addition of nitrate caused immediate stimulation (>50-fold) of denitrification rates by these deeper sediments, it appears that nitrate limits the vertical distribution of denitrification (Fig. 3B). (This agrees with the observation that interstitial NO_3^- + NO_2^- concentrations are detectable only in the upper few centimeters of South San Francisco Bay sediments [C. Fuller and D. Hammond, personal communication].) In addition, Sørensen et al. (20) similarly reported that denitrification was confined to the upper few centimeters of coastal sediments where nitrate was present. It is of interest, however, that deeper sediment samples (e.g., below 10 cm) that displayed little or no endogenous denitrification activity were capable of immediate denitrification when provided with nitrate (Fig. 3). This implies that a population of denitrifying bacteria exists within the deeper sediments that has the same potential activity as the near-surface flora (where nitrate is present). The absence of a lag phase implies that these organisms were physiologically active at the time of sampling. Although bioturbation and high sedimentation rates may redistribute and mix the near surface bacterial flora with the deeper layers, the ability of the deeper flora to use an electron acceptor (i.e., nitrate) not usually encountered was an unexpected result. Therefore, these organisms may be capable of fermentative metabolism or may utilize other electron acceptors (e.g., fumarate or trimethylamine oxide). This explanation was offered by Kaspar et al. (8), who observed an immediate production of $^{13}N_2$ from added $^{13}NO_3^-$ by a 2.5-year-old methanogenic enrichment culture grown in the absence of inorganic nitrogen oxides. We attempted to grow the *Pseudomonas* sp. under anaerobic conditions with trimethylamine oxide substituted for sodium nitrate, but the organism did not grow under these conditions. However, this does not eliminate the possibility that growth could be obtained with either different electron acceptors or other strains of denitrifiers.

The highest areal rates of denitrification were evident in nitrate-supplemented slurries and ranged from 113 to 665 μmol of N_2 m^{-2} h^{-1} (Table 1). This compares favorably with the nitrate-supplemented denitrification potential estimate of Kaspar (5), which when converted equals 429 μmol of N_2 m^{-2} h^{-1}. The values we obtained for unsupplemented, shaken slurries (17 to 280 μmol of N_2 m^{-2} h^{-1}) are comparable to denitrification estimates made by measuring N_2

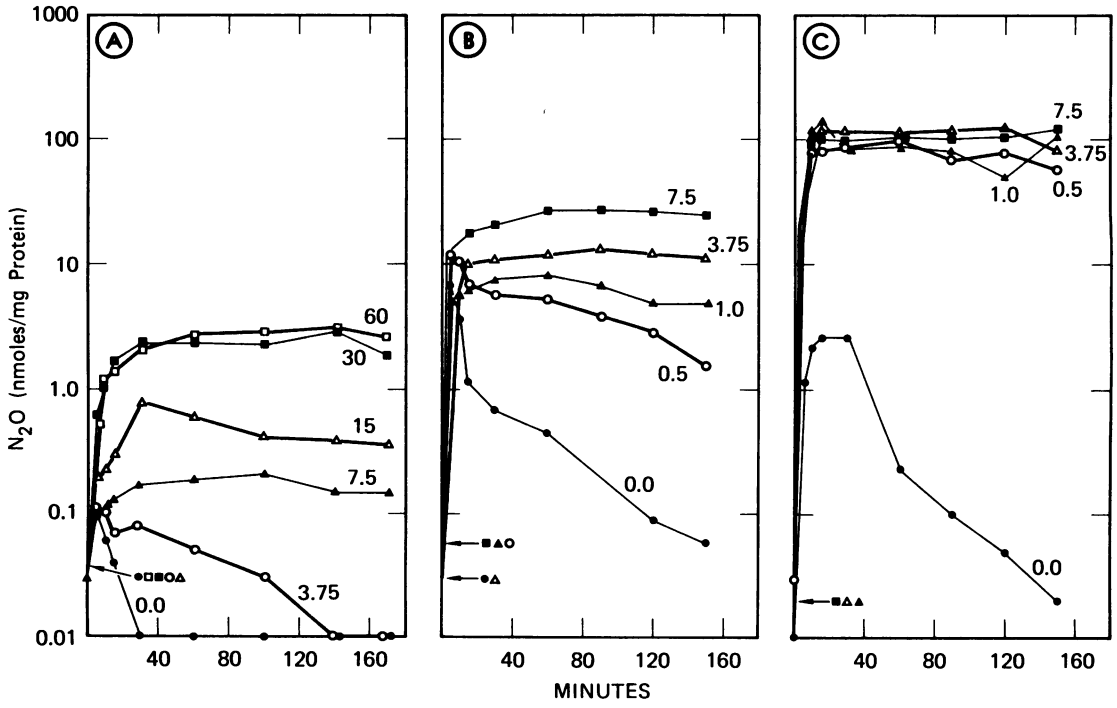

FIG. 4. Efficacy of the C_2H_2 blockage of N_2O reductase in washed cell suspensions of a marine pseudomonad. (A) Cells suspended without nitrate. (B) Cells suspended with 10 μM $NaNO_3$ (150 nmol $flask^{-1}$). (C) Cells suspended with 50 μM $NaNO_3$ (750 nmol $flask^{-1}$). The levels of C_2H_2 (kilopascals) added to flasks are indicated.

evolved in Narragansett Bay sediments (50 μmol of N_2 m^{-2} h^{-1}; 16) and for ^{15}N studies done in the Tama Estuary (68 μmol N_2 m^{-2} h^{-1}; 11). Rates extrapolated for nitrate-supplemented chambers (101 to 135 μmol of N_2 m^{-2} h^{-1}) were about 2 orders of magnitude greater than estimates made with unsupplemented chambers (0.8 to 2.1 μmol of N_2 m^{-2} h^{-1}). Unsupplemented chamber rates probably underestimate denitrification because of inhibition of nitrification by C_2H_2 (26) restricting the nitrate supply to the denitrifiers and reliance upon unfacilitated exchange of gases entering (C_2H_2) and exiting (N_2O) the sediments. Because most of the endogenous activity takes place within the upper 1 cm of the sediment column (Fig. 4A), gas exchange with active sites of denitrification is probably rapid. However, the short time lags evident during some chamber experiments imply a degree of limitation by gas diffusion. By contrast, areal rate estimates for unsupplemented shaken sediment slurries probably overestimate in situ rates because denitrification is severely limited by nitrate, and shaking forces nitrate into zones in which it would not normally be present. Thus, actual denitrification rates probably lie somewhere between those evident in unsupplemented chambers and those displayed by the unsupplemented slurries. These reported values, however, are not meant to be estimates of in situ rates because experiments were conducted in the laboratory (20°C) rather than in the field or at field temperatures.

The quantity of nitrate ions channeled into denitrification pathways by these sediments appears to be relatively low (8.5 to 18%) and may be seasonally variable (Table 3). In the experiment on 18 May 1982, nearly all of the added nitrate was lost within the first 2 h and could not be accounted for by recovery as NO_2^-, N_2O, or NH_4^+. Because an unsupplemented control produced similar levels of NH_4^+ and because a slight decrease of NH_4^+ was noted during the experiment on 12 November 1982, dissimilatory reduction of NO_3^- to NH_4^+ (7, 8, 18) did not appear to account for the nitrate loss. However, our NH_4^+ analyses accounted for only the dissolved pools and did not quantify the adsorbed or nonfree portion. Thus, the possibilities exist that (i) the acetylene block was incomplete, (ii) dissimilatory reduction converted most of the NO_3^- to sediment-bound NH_4^+, or (iii) that nitrate was rapidly consumed by other reduction pathways.

Significant N_2O loss in the presence of acetylene was occasionally observed during our short-term sediment incubations (Fig. 1 and 3) and has been reported to occur during prolonged incubation (1 to 10 days) of soils (14, 29) and sediments (9, 25). Because this phenomenon imposes a limitation upon the use of the acetylene-block assay, the mechanism(s) for its occurrence must first be understood to devise methods to eliminate the problem. Thus far, evidence indicates that several different mechanisms may each be responsible for N_2O loss during application of the acetylene-block assay.

Prolonged (e.g., 3- to 5-day) anaerobic incubation of soils or sediments runs the risk of inducing bacterial consumption of acetylene (3, 27, 31), resulting in subsequent N_2O consumption by the unblocked denitrifiers in the sample. Because acetylene adversely affects ^{63}Ni detectors, it is usually vented and therefore not quantified during the course of electron-capture N_2O analysis (2). Thus, an investigator may be aware of the disappearance of N_2O, but unaware that the cause was due to C_2H_2 removal. Another mechanism for N_2O loss during prolonged incubation periods may be due to reversal of the acetylene block by reduced sulfur compounds (22, 30) formed by the non-denitrifying components of the bacterial flora (e.g., sulfate reducers). Sulfide was shown to relieve the acetylene block in *Pseudomonas aeruginosa*; however the mechanism by which this proceeds was not described (22). This is further complicated by the report that sulfide inhibits N_2O reduction by *Pseudomonas fluorescens* (21). Thus, the efficiency of the acetylene block over prolonged incubations may be limited by components of the bacterial flora which may either produce interfering products (e.g., sulfide) or remove the acetylene.

The acetylene block may also be incomplete during short-term experiments (<2 h), presumably before the non-denitrifying flora can interfere with the assay. Kaspar et al. (8) demonstrated an inverse relationship between ambient nitrate concentration and the partial pressure of acetylene required to achieve a successful block of N_2O reductase in sludge. Similar results were obtained with soils (17) and marine sediments (5). Our experiments with estuarine sediments confirm these results (Fig. 2) and also draw attention to the actual loss of N_2O from the gas phase at low levels of both nitrate and acetylene (Fig. 2A). Results obtained with the washed cell suspensions of the marine pseudomonad demonstrate that these effects (incomplete blockage and N_2O loss) may be explained by a physiological response of the denitrifier population to ambient concentrations of nitrate and acetylene (Fig. 4).

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