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Anaerobic Sulfide Oxidation with Nitrate by a Freshwater *Beggiatoa* Enrichment Culture

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A lithotrophic freshwater *Beggiatoa* strain was enriched in O_2-H_2S gradient tubes to investigate its ability to oxidize sulfide with NO_3^- as an alternative electron acceptor. The gradient tubes contained different NO_3^- concentrations, and the chemotactic response of the *Beggiatoa* mats was observed. The effects of the *Beggiatoa* sp. on vertical gradients of O_2, H_2S, pH, and NO_3^- were determined with microsensors. The more NO_3^- that was added to the agar, the deeper the *Beggiatoa* filaments glided into anoxic agar layers, suggesting that the *Beggiatoa* sp. used NO_3^- to oxidize sulfide at depths below the depth that O_2 penetrated. In the presence of NO_3^- *Beggiatoa* formed thick mats (>8 mm), compared to the thin mats (ca. 0.4 mm) that were formed when no NO_3^- was added. These thick mats spatially separated O_2 and sulfide but not NO_3^- and sulfide, and therefore NO_3^- must have served as the electron acceptor for sulfide oxidation. This interpretation is consistent with a fourfold-lower O_2 flux and a twofold-higher sulfide flux into the NO_3^--exposed mats compared to the fluxes for controls without NO_3^-. Additionally, a pronounced pH maximum was observed within the *Beggiatoa* mat; such a pH maximum is known to occur when sulfide is oxidized to S^0 with NO_3^- as the electron acceptor.

Beggiatoa spp. are gliding, filamentous, colorless sulfur bacteria (22). These multicellular bacteria can occur in dense mats at the surface of sulfide-rich sediments in many freshwater and marine habitats (2, 10, 11, 21). The filaments of bigger marine species of *Beggiatoa* can be more than 120 μm wide (2) and >1 cm long, are white, and are visible with the naked eye; even single filaments of narrow freshwater *Beggiatoa* species whose filaments are ca. 3 μm wide (14, 21) can be observed with a stereomicroscope. *Beggiatoa* spp. are sulfide-oxidizing bacteria that have an important effect on the benthic sulfur cycle (4, 6). The presence of *Beggiatoa* mats at the sediment surface prevents toxic sulfide from diffusing into the water column, because biological sulfide oxidation is much more rapid and efficient than chemical sulfide oxidation (13).

In addition, *Beggiatoa* spp. can have a great effect on the aquatic nitrogen cycle when they use NO_3^- anaerobically as an alternative electron acceptor in place of O_2. The ability of freshwater and marine *Beggiatoa* spp. to oxidize sulfide anaerobically with NO_3^- has been studied for some time (11, 19, 20, 21), especially because large marine species contain a vacuole in which NO_3^- can be stored at concentrations up to 160 mmol/liter (11). This enables the filaments to penetrate into anoxic sediment layers and perform anaerobic sulfide oxidation. However, anaerobic sulfide oxidation by freshwater *Beggiatoa* species has not been unequivocally documented, and the impact of freshwater *Beggiatoa* species on the nitrogen cycle is unclear (5, 11). Therefore, there is significant interest in obtaining more information about possible anaerobic sulfide oxidation with NO_3^- by freshwater *Beggiatoa* species.

The freshwater *Beggiatoa* strain that was used in this study was sustained for more than 2 years in highly enriched O_2-H_2S gradient tubes (12). Using microsensors to measure changes in the O_2 contents, H_2S contents, pH, and NO_3^- contents in these gradient tubes, the position of the *Beggiatoa* filaments in the transparent agar could be optically related to high-resolution chemical gradients. This experimental approach was used to address the following questions. (i) Does the freshwater *Beggiatoa* sp. exhibit a chemotactic response to the presence of different NO_3^- and H_2S concentrations? (ii) Does a *Beggiatoa* mat use NO_3^- as an alternative electron acceptor in place of O_2? (iii) Do the *Beggiatoa* filaments alter the vertical O_2, H_2S, and pH gradients differently when they are exposed to NO_3^- in addition to O_2?

MATERIALS AND METHODS

Sampling site and cultivation. Samples of *Beggiatoa* sp. with a filament width of 3 μm were collected in 2003 from the NO_3^--rich stream Giber Aa, south of Aarhus, Denmark. Here, mats of *Beggiatoa* were found on the mud around outlets for primary treated sewage.

The *Beggiatoa* filaments were enriched in lithotrophic agar gradient tubes, modified as described by Nelson and Jannasch (12). These gradient tubes contained two layers of agar, a layer of dense bottom agar (1.5% Bacto Agar [Difco Laboratories]) containing a high ΣH_2S concentration ($[\Sigma H_2S] = [H_2S] + [HS^-] + [S^{2-}]$) overlaid by a layer of softer top agar (0.25%) without ΣH_2S, which led to opposing gradients of ΣH_2S and O_2 in the top agar. The composition of the medium is shown in Table 1. The pH was adjusted to approximately 7.0 with NaOH. The gradients were prepared in screw-cap tubes (length, 150 mm; inside diameter, 14 mm). The tubes were filled with 4 ml of autoclaved bottom agar and 8 ml of top agar. Unless indicated otherwise, the bottom agar was prepared with 4 mmol/liter Na_2S. The top agar also contained 150 μl of a sterile vitamin solution (Table 1), 4 mmol/liter $NaHCO_3$, and, unless indicated otherwise, 50 μmol/liter $NaNO_3$, 50 μmol/liter NH_4Cl, and 50 μmol/liter sodium acetate. The screw caps on the tubes were left loose to permit exchange of the headspace gas

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TABLE 1. Compositions of medium, micronutrient solution, and vitamin solution

Medium or solution	Composition
Medium	0.01 g EDTA, 0.12 g $CaSO_4 \cdot 2H_2O$, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.016 g NaCl, 0.14 g Na_2HPO_4, 0.138 g NaH_2PO_4, 0.264 g $CaCl_2 \cdot 2H_2O$, 2 ml $FeCl_3$ solution (0.29 g/liter), 1 ml micronutrient solution, 1,000 ml distilled water
Micronutrient solution	0.5 ml H_2SO_4 (>98%), 2.28 g $MnSO_4 \cdot H_2O$, 0.5 g $ZnSO_4 \cdot 7H_2O$, 0.5 g H_3BO_3, 0.025 g $CuSO_4 \cdot 5H_2O$, 0.025 g $Na_2MoO_4 \cdot 2H_2O$, 0.045 g $CoCl_2 \cdot 6H_2O$, 1,000 ml distilled water
Vitamin stock solution[a]	1 mg vitamin B_{12}, 1 mg inositol, 1 mg biotin, 1 mg folic acid, 10 mg *p*-aminobenzoic acid, 100 mg nicotinic acid, 100 mg D-pantothenate, 200 mg thiamine (each vitamin was dissolved in 10 ml distilled water)

[a] For the final vitamin solution 1 ml of each vitamin stock solution was added to 100 ml (final volume) of distilled water.

with the atmosphere. To allow gradient development, the agar was aged for at least 2 days before inoculation. For the different experiments, *Beggiatoa* filaments were taken from existing gradient tubes, pooled, and mixed, and identical subsamples of enriched *Beggiatoa* biomass were inoculated approximately 5 mm below the agar surface. All cultures were grown at room temperature in the dark.

Vertical position of the *Beggiatoa* mats. For determination of the NO_3^-- and ΣH_2S-dependent vertical positions of the *Beggiatoa* mats, the agar was prepared with 0, 100, 200, 400, and 600 μmol/liter $NaNO_3$ and with 4 and 8 mmol/liter Na_2S, respectively ($n = 3$). The mat positions within the gradient system were determined using the tip of a microsensor dummy as a pointer. The dummy was mounted vertically on a micromanipulator, which was attached to a heavy stand. Via its motor drive, the micromanipulator allowed slow, small-scale insertion of the microsensor dummy into the agar down to the *Beggiatoa* mat, while the tip was viewed through the side of the gradient tube with a stereomicroscope (magnification, ×10 to ×20). The meniscus of the agar surface was defined as a depth of 0 μm, from which the position of the clearly visible upper boundary of the *Beggiatoa* mat was measured. The mat position was determined 1 to 6 days after inoculation.

Chemical microgradients. The O_2 concentrations, H_2S concentrations, pH values, and NO_3^- concentrations in the gradient tubes were measured with microsensors. Agar was prepared with 0 and 600 μmol/liter $NaNO_3$, and profiles were determined 2 and 4 days after inoculation; profiles in uninoculated tubes that were the same age were also determined.

The microsensors were either purchased from Unisense A/S (Aarhus, Denmark) or manufactured at the Max Planck Institute for Marine Microbiology (Bremen, Germany). The O_2 microsensors with a guard cathode (17) had tip diameters of 10 to 15 μm and 90% response times of <5 s. They were calibrated with air- and N_2-flushed medium used for agar preparation (100 and 0% air saturation, respectively). The glass-type pH microsensors (18) had tip diameters of <12 μm and 90% response times of <20 s and were calibrated with commercial buffer solutions (pH 4.0, 7.0, and 9.2; Mettler-Toledo, Switzerland). The pH microsensors were used together with homemade reference electrodes, which consisted of a chlorinated Ag wire (length, 30 mm; diameter, 0.5 mm) that was inserted into one end of a glass capillary. The capillaries (length, 100 mm; inside diameter, 1 mm) were filled with 1% agar prepared in 3-mol/liter KCl and thus served as a salt bridge. The H_2S microsensors (3) had tip diameters of 10 μm and 90% response times of <10 s. They were calibrated with deoxygenated PO_4 buffer (200 mmol/liter K_2HPO_4/KH_2PO_4, pH 7.5) to which Na_2S was added stepwise to obtain final concentrations of approximately 0 to 400 μmol/liter (9). The precise ΣH_2S concentration of each calibration solution was determined spectrophotometrically by the method of Pachmeyer (16). The concentrations of free H_2S in the calibration solutions were calculated as follows:

$$[H_2S] = [\Sigma H_2S]/[1 + (10^{pH}/10^{pK_1})] \quad (1)$$

where $pK_1 = 7.027$ is the negative logarithm of K_1, the first dissociation constant of the sulfide equilibrium system (pK_2 can be neglected at pH <9). From these data, the calibration curve for the H_2S microsensor was plotted. ΣH_2S gradients in the tubes were calculated as follows:

$$[\Sigma H_2S] = [H_2S] \times [1 + (10^{pH}/10^{pK_1})] \quad (2)$$

using the $[H_2S]$ and the pH gradients measured with microsensors.

LIX-type NO_3^- microsensors (1) with tip diameters of 5 to 10 μm and 90% response times of <30 s were prepared on the day before use to improve the signal stability. NO_3^- microsensors were used together with homemade reference electrodes (see above). Calibration was performed using uninoculated gradient tubes in which the $NaNO_3$ concentration was adjusted to 0, 15, 30, 60, 150, 300, or 600 μM. All sensors were calibrated before and after measurement at room temperature. One microsensor at a time was mounted on a motorized micromanipulator that was operated by the software *Profix* (Unisense A/S, Aarhus, Denmark). The microsensor was positioned in the center of the tube cross section and then lowered toward the agar surface (depth, 0 μm [see above]). Starting at this depth, vertical profiles were recorded at increments of 100, 200, or 400 μm down to 30 mm. The O_2, pH, H_2S, and NO_3^- profiles were determined at the same spot of the same tube whenever possible and were related to the position and thickness of the *Beggiatoa* mat in the inoculated enrichment culture (for mat position designations see above). The lower boundary of the mat was defined as the position where filaments were present more than just sporadically.

Flux calculations. The amounts of O_2 and ΣH_2S that flowed across a unit of area per unit of time (flux) were determined for uninoculated controls as well as for the tubes that were inoculated with the *Beggiatoa* enrichment. Assuming steady-state conditions, Fick's first law of diffusion was used:

$$J = -D(\delta C/\delta x) \quad (3)$$

where J is the flux (in nmol cm^{-2} s^{-1}), D is the diffusion coefficient (in cm^2 s^{-1}), C is the concentration (in nmol cm^{-3}), and x is the depth (in cm). The diffusion coefficients for O_2 and ΣH_2S (in agar at room temperature) were 2.03×10^{-5} and 1.57×10^{-5} cm^2 s^{-1}, respectively (13). For the uninoculated controls, the linear regions of the concentration gradients above and below the O_2-ΣH_2S overlap zone were used for $\delta C/\delta x$ (13); for the *Beggiatoa*-containing gradient tubes, the linear regions above and below the *Beggiatoa* mat were used.

RESULTS

Mat position experiments. The experiments showed that the mat position depended on three factors: the concentrations of NO_3^- and ΣH_2S and the length of incubation (Fig. 1). Generally, the mat position was deeper when the NO_3^- concentration was higher. This effect was less pronounced when 8 mmol/liter Na_2S was used instead of 4 mmol/liter Na_2S. In all treatments *Beggiatoa* mats moved upward with time (12). Three-way analysis of variance with NO_3^- and ΣH_2S concentrations as between-subject factors and with time as a within-subject factor revealed that the dependence of the mat position on all three factors (for NO_3^-, $F_{4,19} = 478$ and $P < 0.001$; for ΣH_2S, $F_{1,19} = 529$ and $P < 0.001$; and for time, $F = 1{,}229$, $df = 5$, and $P < 0.001$) was highly significant.

O_2 and ΣH_2S microgradients. Without NO_3^- addition, the vertical O_2 and ΣH_2S gradients were steeper in the *Beggiatoa* gradient tubes than they were in the uninoculated controls (Fig. 2A to D). Correspondingly, the O_2 and ΣH_2S fluxes into the *Beggiatoa* mats were greater than those into the O_2-ΣH_2S overlap zone (Table 2). Furthermore, the O_2 and ΣH_2S gradients became steeper with time, which resulted in upward movement of both the O_2-ΣH_2S overlap zone (uninoculated controls) and the *Beggiatoa* mat (Fig. 2A to D; cf. Fig. 1). The *Beggiatoa* mat in the experiment without added NO_3^- was approximately 0.4 mm thick and was slightly above the O_2-ΣH_2S

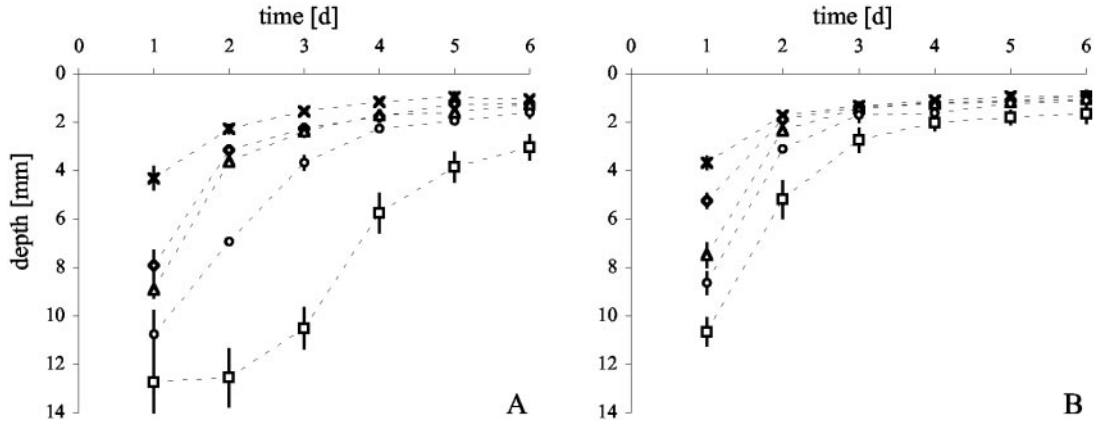

FIG. 1. Mean depth (in mm) of the upper boundary of the *Beggiatoa* mat, depending on the NO_3^- and ΣH_2S concentrations in the gradient tubes over time (days [d]). (A) Bottom agar prepared with 4 mmol/liter Na_2S. (B) Bottom agar prepared with 8 mmol/liter Na_2S. Symbols: ×, no NO_3^-; ◇, 100 μmol/liter NO_3^-; △, 200 μmol/liter NO_3^-; ○, 400 μmol/liter NO_3^-; □, 600 μmol/liter NO_3^-. Some of the error bars, which indicate standard deviations ($n = 3$), are smaller than the symbols.

overlap zone. NO_3^- addition to *Beggiatoa* tubes had a strong effect on the O_2 and ΣH_2S microgradients, on the mat position, and on the thickness of the mat, which increased to >8 mm (Fig. 2E and F). The NO_3^- effect was most pronounced 2 days after inoculation. An approximately 4-mm gap appeared between the O_2 and ΣH_2S profiles (Fig. 2E). Additionally, the corresponding O_2 microgradient was considerably less steep, resulting in a flux of 3.6 pmol cm^{-2} s^{-1}, which was only one-half the value obtained for the uninoculated control and less than one-fourth the value obtained for the treatment without

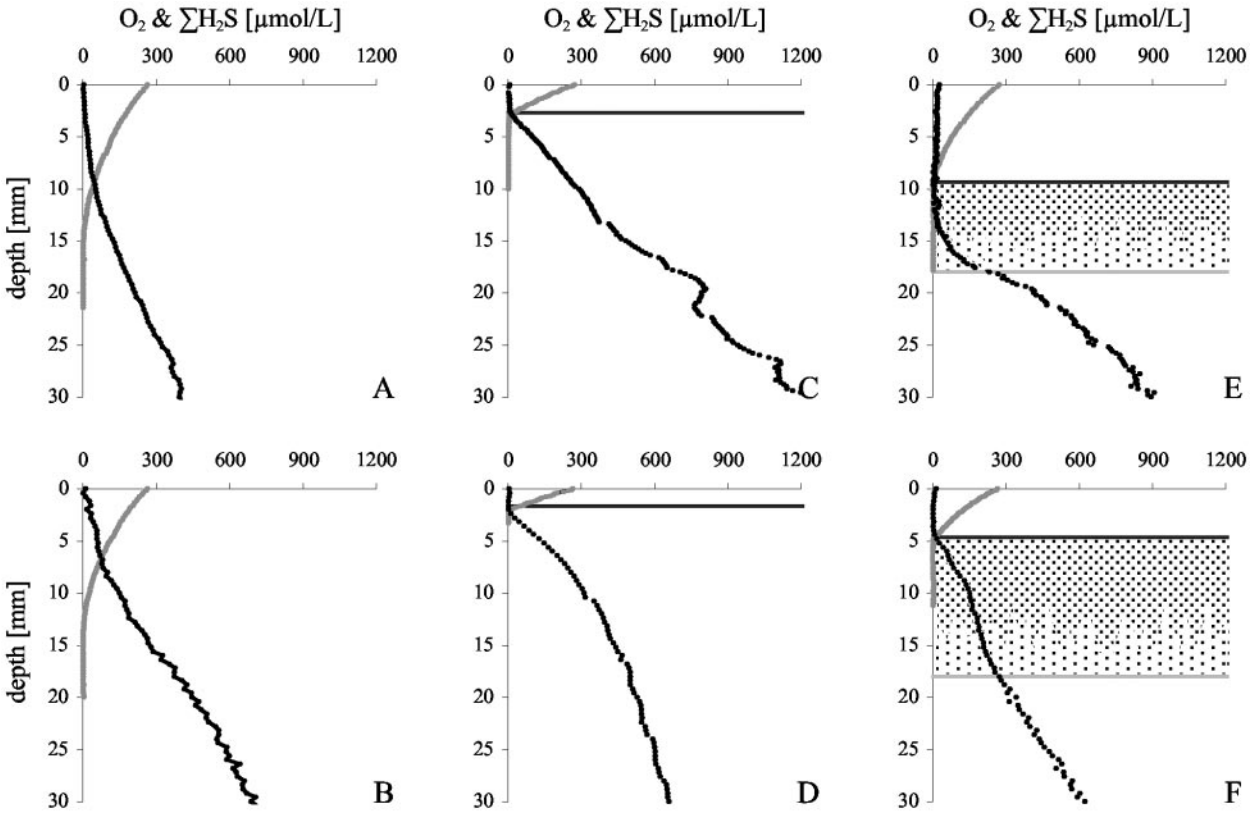

FIG. 2. Microprofiles of O_2 (gray circles) and ΣH_2S (black circles) and positions of the upper (dark gray lines) and, where applicable, lower (light gray lines) boundaries of the *Beggiatoa* mats. (A and B) Uninoculated gradient tubes. (C and D) *Beggiatoa* gradient tubes without NO_3^-. (E and F) *Beggiatoa* gradient tubes with an initial NO_3^- concentration of 600 μM. The incubation times were 2 days (A, C, and E) and 4 days (B, D, and F) after inoculation. The shaded areas within the boundaries of the *Beggiatoa* mats (E and F) indicate that filaments were more abundant in the upper mat regions. Gray and black circles overlap in some panels.

TABLE 2. O_2 and ΣH_2S fluxes in uninoculated controls and in *Beggiatoa*-enriched gradient tubes without NO_3^- and with an initial NO_3^- concentration of 600 μmol/liter[a]

Time (days)	Flux (pmol cm^{-2} s^{-1})					
	Controls		*Beggiatoa* enrichments without NO_3^-		*Beggiatoa* enrichments with 600 μmol/liter NO_3^-	
	O_2	ΣH_2S	O_2	ΣH_2S	O_2	ΣH_2S
2	7.2	2.8	16.7	5.9	3.6	11.9
4	7.8	3.9	23.4	7.4	7.7	4.3[b]

[a] The data correspond to profiles shown in Fig. 2.
[b] The flux may have been underestimated because there were no long-term steady-state conditions for ΣH_2S.

NO_3^- (Table 2). In contrast, the ΣH_2S flux was about twofold higher than that in the *Beggiatoa* gradient tube without NO_3^- and about fourfold higher than that in the uninoculated control (Table 2). The NO_3^- effect was less pronounced after 4 days; the O_2 profile in the NO_3^--containing *Beggiatoa* enrichment culture became steeper, and the ΣH_2S profile became less steep (Fig. 2F).

NO_3^- microgradients. The NO_3^- microsensor measurements for the uninoculated control (Fig. 3A) and the *Beggiatoa* enrichment culture after 2 and 4 days (Fig. 3B and C) illustrate that the NO_3^- concentrations decreased in the presence of *Beggiatoa* sp. during incubation. The mean NO_3^- concentration in the upper 30-mm agar layer decreased from the initial concentration (600 μmol/liter) to 86 μmol/liter after 2 days and to 54 μmol/liter after 4 days. Furthermore, the profiles show that all of the NO_3^- diffused from the small upper agar volume into the mat, whereas some NO_3^- was still diffusing upward from the much larger volume of agar below the mat that also contained a larger total amount of NO_3^-. In contrast to O_2 and ΣH_2S, which were spatially separated after 2 days in the NO_3^--containing treatment, NO_3^- and ΣH_2S overlapped in the *Beggiatoa* mat (Fig. 2E and 3B).

pH microgradients. In the uninoculated control, the pH was 7.8 at the agar surface and increased to 8.3 at a depth of 30 mm due to the increasing ΣH_2S concentration (Fig. 4A). In the *Beggiatoa* enrichment culture without NO_3^-, the pH profile showed that the minimum pH was close to the *Beggiatoa* mat (Fig. 4B). In contrast, in the *Beggiatoa* enrichment culture with NO_3^- the pH profile had a completely different shape and there was a pronounced maximum pH in the *Beggiatoa* mat (Fig. 4C).

DISCUSSION

The hypothesis that the freshwater *Beggiatoa* strain investigated is able to oxidize ΣH_2S anaerobically with the alternative electron acceptor NO_3^- originated from observations made during the mat position experiments; at higher NO_3^- concentrations the *Beggiatoa* mats moved deeper into the agar toward the electron donor ΣH_2S (Fig. 1). This hypothesis was supported by microsensor profiles and flux calculations, which demonstrated that the *Beggiatoa* filaments indeed moved into anoxic, NO_3^--rich agar layers and could oxidize even more ΣH_2S if NO_3 was available (Fig. 2C to F and Table 2). Furthermore, the O_2 flux into the *Beggiatoa* mat exposed to NO_3^- was much lower than the O_2 fluxes in the tubes without NO_3^- and the uninoculated control tubes after 2 days (Table 2). This finding can be explained by the missing O_2-ΣH_2S overlap zone in the NO_3^--amended *Beggiatoa* tubes (Fig. 2E). Because of the spatial separation of O_2 and ΣH_2S, neither chemical nor biological ΣH_2S oxidation with O_2 could take place. The effect of the initial NO_3^- concentration on *Beggiatoa* sp. became less pronounced over time (Fig. 1 and 2C to F), which is explained by the finding that NO_3^- limitation occurred as incubation progressed (Fig. 3). It is likely that not all NO_3^- was immediately used for anaerobic ΣH_2S oxidation and that an unknown

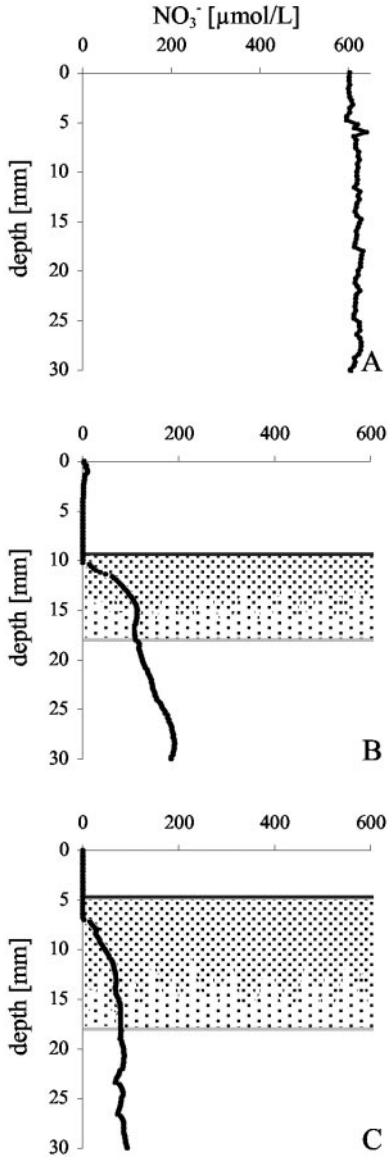

FIG. 3. Microprofiles of NO_3^- (circles) and positions of the upper (dark gray lines) and lower (light gray lines) boundaries of the *Beggiatoa* mats. (A) Uninoculated gradient tube. (B and C) *Beggiatoa* gradient tubes 2 days (B) and 4 days (C) after inoculation. The initial NO_3^- concentration was 600 μM. The shaded areas within the boundaries of the *Beggiatoa* mats (B and C) indicate that filaments were more abundant in the upper mat regions. Circles overlap in some panels.

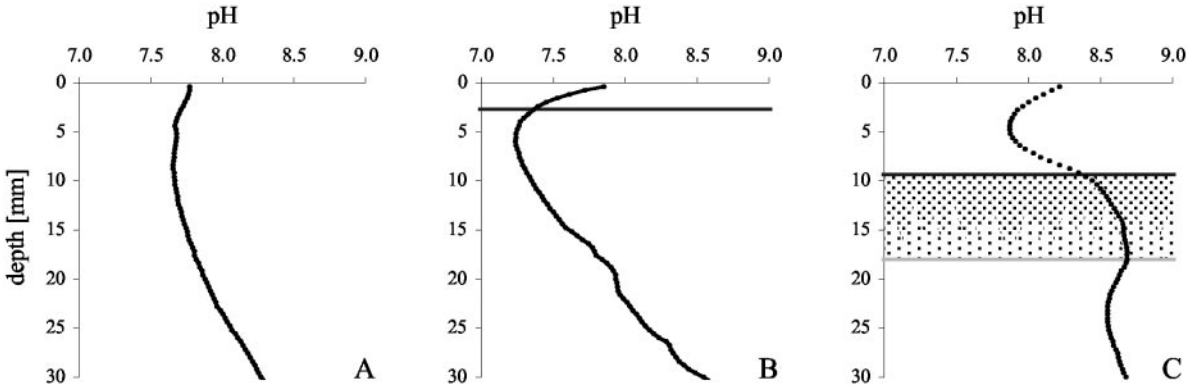

FIG. 4. Microprofiles of pH (circles) and positions of the upper (dark gray line) and, where applicable, lower (light gray line) boundaries of the *Beggiatoa* mats. (A) Uninoculated gradient tube. (B and C) *Beggiatoa* gradient tubes without NO_3^- (B) and with an initial NO_3^- concentration of 600 μM (C). The incubation time was 2 days. The shaded area within the boundaries of the *Beggiatoa* mat (C) indicates that filaments were more abundant in the upper mat regions. Circles overlap in some panels.

fraction of NO_3^- was assimilated or stored intracellularly (11, 23). Vacuoles in freshwater *Beggiatoa* have not been detected so far (22), but cytoplasmic storage of NO_3^- is another possibility. This could explain the finding that more NO_3^- was taken up during the first 2 days of incubation than during the second 2 days (Fig. 3).

Beggiatoa oxidizes ΣH_2S first to S^0, which can be stored as intracellular globules, and subsequently to SO_4^{2-} (22, 24). When O_2 is used as the electron acceptor, the oxidation of H_2S to S^0 is pH neutral (if HS^- is used as the electron donor, its oxidation to S^0 is moderately alkaline; S^{2-} can be neglected at pH <9), whereas the oxidation of S^0 to SO_4^{2-} is acidogenic. In total, the aerobic oxidation of ΣH_2S to SO_4^{2-} is acidogenic, which explains the pH profile found in the *Beggiatoa* enrichment culture without NO_3^-, in which the minimum pH largely coincided with the position of the *Beggiatoa* mat (Fig. 4B) (7, 13). When NO_3^- is used as the electron acceptor, the oxidation of ΣH_2S to S^0 increases the pH, while the oxidation of S^0 to SO_4^{2-} decreases the pH (20). This was visible in the pH profiles that were determined for the NO_3^--containing treatments; after 2 days of incubation, the maximum pH was 8.7 in the lower region of the *Beggiatoa* mat (Fig. 4C), which must have resulted from the oxidation of ΣH_2S to S^0 with NO_3^-. Toward the upper region of the *Beggiatoa* mat, where less ΣH_2S was available, the pH decreased. However, the pH in this layer did not decrease to values lower than those in the uninoculated control (Fig. 4A and C). Therefore, there was no indication that oxidation of S^0 to SO_4^{2-} took place in the upper region of the *Beggiatoa* mat. However, if oxidation of S^0 to SO_4^{2-} occurred at all, NO_3^- rather than O_2 must have been used as the electron acceptor, because the O_2 flux into the *Beggiatoa* mat was extremely low. The measured pH profiles are consistent with the results of a recent study of Sayama et al. (20), in which these authors found similar pH profiles in marine sediment colonized with *Beggiatoa* spp. It was hypothesized that the oxidation of H_2S to S^0 occurred with NO_3^- and was not necessarily spatially coupled to the oxidation of S^0 to SO_4^{2-}.

Furthermore, Sayama et al. (20) demonstrated that the marine *Beggiatoa* spp. investigated reduce NO_3^- to NH_4^+ under anoxic conditions (dissimilatory nitrate reduction to ammonium). This metabolic pathway was also hypothesized to occur in other marine sulfur bacteria (19) and is known to occur in large marine *Thioploca* spp. (15) that are close relatives of large marine *Beggiatoa* spp. (22). Another possibility for anaerobic ΣH_2S oxidation with NO_3^- is denitrification, which was discussed by Sweerts at al. (21) for freshwater *Beggiatoa* spp. To date, this study is the only study in which anaerobic ΣH_2S oxidation with NO_3^- was postulated for freshwater *Beggiatoa* spp., but questions about contamination of the *Beggiatoa* filaments with unicellular denitrifying bacteria have been raised by other authors (5, 11). The *Beggiatoa* enrichment culture used in our study also contained unicellular bacteria. Despite numerous trials, a pure culture could not be obtained, suggesting that this *Beggiatoa* strain is not able to grow without associated bacteria, which is a well-known phenomenon for other bacteria (8). However, the visibility of the *Beggiatoa* filaments in the transparent agar can be used. Using a stereomicroscope, it was observed that NO_3^- had an effect on the filaments because the *Beggiatoa* mat position and thus the chemotactic response of the filaments to O_2 and ΣH_2S were indeed changed. Alternatively, the movement of the *Beggiatoa* filaments may have resulted from an intimate association with unicellular NO_3^- reducers, which were directly responsible for the ΣH_2S oxidation, and because of an absolute dependence of the *Beggiatoa* sp. on these reducers, the *Beggiatoa* sp. followed the movement of the NO_3^- reducers in the gradient tubes. However, this seems unlikely because in this case the *Beggiatoa* sp. would have had to disassociate from the energetically favorable electron acceptor O_2. Hence, the changed chemotactic response of the *Beggiatoa* sp. strongly suggests that the freshwater *Beggiatoa* filaments themselves were chiefly responsible for the anaerobic ΣH_2S oxidation with NO_3^-.

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