Impact of Bacterial NO_3^- Transport on Sediment Biogeochemistry

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Experiments demonstrated that *Beggiatoa* could induce a H_2S -depleted suboxic zone of more than 10 mm in marine sediments and cause a divergence in sediment NO_3^- reduction from denitrification to dissimilatory NO_3^- reduction to ammonium. pH, O_2 , and H_2S profiles indicated that the bacteria oxidized H_2S with NO_3^- and transported S^0 to the sediment surface for aerobic oxidation.

Large species of white sulfur bacteria in the family *Beggiatoaceae* may accumulate and transport NO_3^- at intracellular concentrations up to 500 mM (5, 10, 18, 19). Therefore, it has been suggested that anaerobic H_2S oxidation coupled with dissimilatory NO_3^- reduction to ammonium (DNRA) by these bacteria contributes to the formation of a suboxic zone. The suboxic zones are characterized by positive redox potential and only trace concentrations of free H_2S , in spite of high sulfate reduction rates (5–7, 10, 13). Calculations based on NO_3^- content, mobility, and biomass distributions of *Beggiatoa* or *Thioploca* in marine sediments have indicated that bacterial transport and reduction of NO_3^- may indeed match H_2S production and influence distribution of H_2S in the sediment (11, 18). The extent to which this potential is realized within marine sediments is, however, not clear.

Sulfide-depleted surface sediment is essential for survival of benthic infauna, and the pathway of benthic NO_3^- reduction is important for control of eutrophication in nitrogen-limited coastal waters. In this study, we wanted to get more direct measures of the environmental role of *Beggiatoa* spp. by measuring sediment profiles of O_2 , pH, and S and turnover rates of N compounds in the presence and absence of natural *Beggiatoa* populations.

Sediment with *Beggiatoa* filaments was collected from Aarhus Bay, Denmark, in Plexiglas tubes (inside diameter, 54 mm). The effect of different NO_3^- or O_2 concentrations on pore water chemistry was investigated on intact sediment samples, while the impact of *Beggiatoa* filaments was studied in reconstructed samples prepared as follows. The upper 3 cm containing the bulk of *Beggiatoa* filaments was cut off and kept separately in beakers. The remaining sediment was homogenized, transferred to new Plexiglas tubes, and kept with H_2S -enriched anoxic bottom water for 24 h to kill the remaining *Beggiatoa* filaments. The effect was confirmed by microscopical inspection. The sediment cores were hereafter left for 6 h in aerated sulfide-free seawater. *Beggiatoa* filaments from beakers were then added to the surface of 10 cores with broad pipettes,

the remaining 10 cores serving as controls. Filaments with a diameter of approximately 13 μm dominated the *Beggiatoa* community completely. All cores were kept at 16°C in aquariums with seawater renewed daily; *Beggiatoa* cores and control cores were incubated in separate aquariums. The O_2 concentration was maintained at $160 \pm 10 \mu\text{M}$. Half of the cores were incubated at a NO_3^- concentration of $47 \pm 2 \mu\text{M}$, while the other half was incubated at an in situ NO_3^- concentration (2 μM).

Pore water O_2 , $\Sigma\text{H}_2\text{S}$ (H_2S plus HS^- plus S^{2-}), and pH profiles were measured with microsensors (8, 14, 15). Profiles of pore water and cellular NO_3^- were determined as described in reference 18, and NO_3^- was measured according to Braman and Hendrix (1). For measurement of denitrification and DNRA, $^{15}\text{NO}_3^-$ (50 μM , 98 atom%) was added to two aquariums containing reconstructed sediment cores with or without *Beggiatoa* filaments, respectively ($n = 4$ for each treatment). $^{15}\text{NO}_3^-$ was renewed daily, and effluxes of ^{15}N -labeled N_2 and NH_4^+ were measured for 14 days according to Christensen et al. (3). $^{15}\text{N}_2$ and $^{15}\text{NH}_4^+$ were determined according to Risgaard-Petersen et al. (16, 17).

It has long been recognized that oxidation of H_2S with O_2 in *Beggiatoa* mats on the sediment surface acts as an efficient filter and a final barrier to the release of the toxic H_2S to the water phase (4, 9). Previous descriptive studies have further indicated that oxidation of H_2S with NO_3^- in *Beggiatoa*- or *Thioploca*-colonized sediments can act in a similar manner (5, 11, 18). Here we provide experimental evidence for this hypothesis. Our data show directly that *Beggiatoa* can induce rapid movement of the $\Sigma\text{H}_2\text{S}$ front ($\Sigma\text{H}_2\text{S} > 0.5 \mu\text{M}$) deeper into the sediment and that this is coupled to NO_3^- transport (Fig. 1). In contrast to pure chemical immobilization of H_2S (e.g., see reference 2), this process is independent of sediment resuspension and fauna-mediated bioturbation and is able to proceed as long as sufficient NO_3^- is available for the oxidation of H_2S . In the absence of infauna and with the next resuspension event possibly not occurring for a long time, the migrating *Beggiatoa* may thus serve to prepare the sediment for infaunal recolonization by creating a less hostile (i.e., H_2S free) environment.

In the presence of *Beggiatoa*, DNRA was the predominant benthic NO_3^- reduction pathway. In contrast, denitrification was the only detectable pathway in the absence of *Beggiatoa*

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FIG. 1. Profiles of $\Sigma\text{H}_2\text{S}$ (circles), cellular pool of NO_3^- (squares), and pH (triangles). (A to C) Profiles in reconstructed sediment cores inoculated with *Beggiatoa* (open symbols) and control cores without *Beggiatoa* filaments (closed symbols) after 1 and 4 days of incubation. (D to F) Profiles in natural *Beggiatoa*-colonized sediments exposed to an in situ NO_3^- concentration of 1 μM (closed symbols) and with NO_3^- -enriched bottom water (50 μM , open symbols). Error bars = standard error ($n = 3$ for $\Sigma\text{H}_2\text{S}$ and $n = 2$ for NO_3^-). The pH profiles are averaged from three profiles. Oxygen penetrated less than 0.2 mm in all cores and is not shown.

(Fig. 2). In theory, other, smaller bacteria that accompanied the transposed *Beggiatoa* tufts might be involved, but the instant and persistent DNRA activity strongly suggests that the large biomass of *Beggiatoa* was the primary agent.

Beggiatoa performs H_2S oxidation in two steps (12, 20). H_2S is first oxidized:

Then S^0 is oxidized:

The first step increases pH, while the second step decrease pH, and together the two processes are pH neutral. The distinct sigmoid pH profile in the *Beggiatoa*-inhabited sediment cores (Fig. 1) thus indicates a spatial separation of the processes. The pH maximum right at the H_2S front clearly indicates the

FIG. 2. Production of $^{15}\text{N}_2$ (circles) and $^{15}\text{NH}_4^+$ (squares) during a 14-day time course from reconstructed sediment cores in the presence (open symbols) and absence (i.e., control cores) of *Beggiatoa* (closed symbols). Error bars indicate standard error ($n = 4$).

location of the first step (reaction 1). Further, the distinct pH minimum at the oxic-anoxic interface strongly suggests that the *Beggiatoa* filaments prefer the energetically more favorable and much more acidogenic aerobic oxidation of S^0 :

FIG. 3. Change in pH profiles in *Beggiatoa*-colonized sediment cores following an immediate oxygen reduction in the water column from 266 μM to 30 μM . The time zero pH profile (closed circles) was measured at 266 μM O_2 and is averaged from pH measurements at four independent sites. pH was subsequently measured at the same site 6, 16, and 27 min after the O_2 concentration was reduced to 30 μM (open symbols). O_2 penetrated to 0.25 ± 0.03 mm ($n = 7$, broken line) at the high O_2 concentration. A few minutes after O_2 was reduced to 30 μM in the bottom water, no O_2 was detectable in the sediment. $\Sigma\text{H}_2\text{S}$ was detectable from a depth of approximately 5 mm.

A rapid disappearance of the subsurface pH minimum after reducing the O₂ concentration in the overlying water of *Beggiatoa*-colonized sediment (Fig. 3) further supported the proposed combined anaerobic-aerobic H₂S oxidation mediated by NO₃⁻ and S⁰ transport (the combination of reactions 1 and 3). This mechanism also implies a fourfold better utilization of NO₃⁻, as the first step only accounts for one-fourth of the complete H₂S oxidation to SO₄²⁻.

In conclusion, this study has demonstrated that bacterial nitrate transport is yet another complex mechanism that really has to be taken into account to understand how sulfide-depleted sediment environments are sustained.

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REFERENCES

1. Brame, R. S., and S. A. Hendrix. 1989. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemiluminescence detection. *Anal. Chem.* **61**:2715–2718.
2. Canfield, D. E., B. B. Jørgensen, H. Fossing, R. Glud, J. Gundersen, N. B. Ramsing, B. Thamdrup, J. W. Hansen, and P. O. J. Hall. 1993. Pathways of organic carbon oxidation in three continental margin sediments. *Mar. Geol.* **113**:27–40.
3. Christensen, P. B., S. Rysgaard, N. P. Sloth, T. Dalsgaard, and S. Schwaerter. 2000. Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. *Aquat. Microb. Ecol.* **21**:73–84.
4. Fenchel, T., and C. Bernard. 1995. Mats of colourless sulphur bacteria. 1. Major microbial processes. *Mar. Ecol. Prog. Ser.* **128**:161–170.
5. Fossing, H., V. A. Gallardo, B. B. Jørgensen, M. Huttel, L. P. Nielsen, H. Schulz, D. E. Canfield, S. Forster, R. N. Glud, J. K. Gundersen, J. Kuver, N. B. Ramsing, A. Teske, B. Thamdrup, and O. Ulloa. 1995. Concentration and transport of nitrate by the mat-forming sulphur bacterium *Thioploca*. *Nature* **374**:713–715.
6. Huettel, M., S. Forster, S. Klöser, and H. Fossing. 1996. Vertical migration in the sediment-dwelling sulfur bacteria *Thioploca* spp. in overcoming diffusion limitations. *Appl. Environ. Microbiol.* **62**:1863–1872.
7. Jannasch, H. W. 1995. Life at the sea floor. *Nature* **374**:676–677.
8. Jeroschewski, P., C. Steuckart, and M. Köhl. 1996. An amperometric microsensor for the determination of H₂S in aquatic environments. *Anal. Chem.* **68**:4351–4357.
9. Jørgensen, B. B. 1977. Distribution of colorless sulfur bacteria (*Beggiatoa*-spp) in a coastal marine sediment. *Mar. Biol.* **41**:19–28.
10. McHatton, S. C., J. P. Barry, H. W. Jannasch, and D. C. Nelson. 1996. High nitrate concentrations in vacuolate, autotrophic marine *Beggiatoa* spp. *Appl. Environ. Microbiol.* **62**:954–958.
11. Musmann, M., H. N. Schulz, B. Strotmann, T. Kjaer, L. P. Nielsen, R. A. Rossello-Mora, R. I. Amann, and B. B. Jørgensen. 2003. Phylogeny and distribution of nitrate-storing *Beggiatoa* spp. in coastal marine sediments. *Environ. Microbiol.* **5**:523–533.
12. Nelson, D. C. 1992. The genus *Beggiatoa*, p. 3171–3180. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), *The prokaryotes*, vol. 4. Springer-Verlag, New York, N.Y.
13. Otte, S., J. G. Kuenen, L. P. Nielsen, H. W. Paerl, J. Zopfi, H. D. Schulz, A. Teske, B. Strotmann, V. A. Gallardo, and B. B. Jørgensen. 1999. Nitrogen, carbon, and sulfur metabolism in natural *Thioploca* samples. *Appl. Environ. Microbiol.* **65**:3148–3157.
14. Revsbech, N. P. 1989. An oxygen microelectrode with a guard cathode. *Limnol. Oceanogr.* **34**:474–478.
15. Revsbech, N. P., and B. B. Jørgensen. 1986. Microelectrodes: their use in microbial ecology. *Adv. Microb. Ecol.* **9**:293–351.
16. Risgaard-Petersen, N., and S. Rysgaard. 1995. Nitrate reduction in sediments and waterlogged soils measured by ¹⁵N techniques, p. 287–296. In K. Alef and P. Nannipieri (ed.), *Methods in applied soil microbiology*. Academic Press Inc., London, United Kingdom.
17. Risgaard-Petersen, N., S. Rysgaard, and N. P. Revsbech. 1995. Combined microdiffusion-hypobromite oxidation technique for determination of the ¹⁵N atom% of NH₄⁺. *Soil Sci. Soc. Am. J.* **59**:1077–1080.
18. Sayama, M. 2001. Presence of nitrate-accumulating sulfur bacteria and their influence on nitrogen cycling in a shallow coastal marine sediment. *Appl. Environ. Microbiol.* **67**:3481–3487.
19. Schulz, H. N., T. Brinkhoff, T. G. Ferdelman, M. H. Marine, A. Teske, and B. B. Jørgensen. 1999. Dense populations of a giant sulfur bacterium in Namibian shelf sediments. *Science* **284**:493–495.
20. Winogradsky, S. 1887. Über Schwefelbakterien. *Bot. Zeitung* **45**:489–610.