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Colorless Sulfur Bacteria, *Beggiatoa* spp. and *Thiovulum* spp., in O_2 and H_2S Microgradients

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The interactions between colorless sulfur bacteria and the chemical microgradients at the oxygen-sulfide interface were studied in *Beggiatoa* mats from marine sediments and in *Thiovulum* veils developing above the sediments. The gradients of O_2, H_2S, and pH were measured by microelectrodes at depth increments of 50 μm. An unstirred boundary layer in the water surrounding the mats and veils prevented microturbulent or convective mixing of O_2 and H_2S. The two substrates reached the bacteria only by molecular diffusion through the boundary layer. The bacteria lived as microaerophiles or anaerobes even under stirred, oxic water. Oxygen and sulfide zones overlapped by 50 μm in the bacterial layers. Both compounds had concentrations in the range of 0 to 10 μmol liter^{-1} and residence times of 0.1 to 0.6 s in the overlapping zone. The sulfide oxidation was purely biological. Diffusion calculations showed that formation of mats on solid substrates or of veils in the water represented optimal strategies for the bacteria to achieve a stable microenvironment, a high substrate supply, and an efficient competition with chemical sulfide oxidation. The continuous gliding movement of *Beggiatoa* cells in mats or the flickering motion of *Thiovulum* cells in veils were important for the availability of both O_2 and H_2S for the individual bacteria.

Beggiatoa and *Thiovulum* species are representatives of the gradient-type colorless sulfur bacteria which live in aquatic environments at the transition between O_2 and H_2S. They are often observed as isolated patches on organic-rich sediments along protected shores of lakes and of the sea. They may also cover the decaying remains of dead animals and plants from which large amounts of H_2S are produced (11, 16). In the presence of oxygen, the bacteria oxidize H_2S to elemental sulfur, S^0, which accumulates as sulfur droplets inside the cell wall. The elemental sulfur can be further oxidized to sulfate by the bacteria (19, 24). Under anaerobic conditions, S^0 may serve as an alternative electron acceptor in *Beggiatoa* species and be reduced back to H_2S (19).

When oxygen and sulfide coexist, they react spontaneously with a half-life in the order of 1 h (1, 6, 8). The sulfur bacteria living at the O_2-H_2S interface must therefore always compete with the autocatalytic oxidation of sulfide. Both *Beggiatoa* and *Thiovulum* species have adapted to this requirement by growing as sheets at the transition between oxygen and sulfide. *Beggiatoa* cells, which are filamentous and gliding organisms, form thin mats over solid substrates from which H_2S is released (11, 16, 24). *Thiovulum* cells, which are very large, spherical bacteria with peritrichous flagella, form fragile veils floating in the water, only loosely attached to the underlying substrate (3, 17, 28). These typical growth forms demonstrate a highly developed chemotactic behavior of the bacteria, which enables them to adjust rapidly to the unstable chemical gradients.

Although the general habitat of the colorless sulfur bacteria has been known for a long time (15, 16), the microenvironmental factors which determine growth are not well understood. Both *Beggiatoa* and *Thiovulum* species excrete slime and form mucilagenous sheets (17, 24, 28). This may perhaps provide a special adaptation to the life in dynamic gradients between two spontaneously reacting substrates. How do these bacteria interact with these gradients, and what is the chemical microenvironment immediately surrounding the bacterial cells? It has not previously been possible to answer these questions due to insufficient resolving power of the available chemical techniques.

Microelectrodes were recently introduced in the study of O_2, H_2S, and pH gradients in microbial communities (13, 22; unpublished data). The electrodes have dimensions which allow chemical measurements to be made with a spatial resolution of 50 μm or even less. They provide an ideal tool for investigating the steep gradients in which colorless sulfur bacteria live. The microelectrodes were used in the present

study to analyze the oxygen and sulfide environment of *Beggiatoa* and *Thiovulum* cells growing on mud cores from coastal sediments. Diffusion fluxes and reaction rates of O_2 and H_2S were calculated from the results to explain the interactions between the gradient bacteria and the dynamic chemocline.

MATERIALS AND METHODS

Colorless sulfur bacteria were obtained from sediment cores collected in shallow water of Danish lagoons (Limfjorden, Aarhus Bay, Kalø Lagoon) during the period July to October 1981. The sediments consisted of organic-rich muds, which at the time of sampling were covered by a whitish film of *Beggiatoa* spp. The in situ temperature was 12 to 18°C, and the salinity was in the range of 20 to 25‰. The cores were kept in the laboratory at 20°C and submerged in slowly circulating, aerated seawater from the biotope. After a few days to a few weeks, the sediments were covered by dense mats of *Beggiatoa* spp. In some cores, *Thiovulum* veils also had developed. The following studies were made either on the whole, preincubated cores or on small samples of the sulfur bacteria collected directly from the enrichments.

Measurements of oxygen, sulfide, and pH were made by the use of microelectrodes (2, 25). Details of the application of these electrodes to the study of microbial mats are described elsewhere (13; unpublished data). The oxygen electrodes had a sensing tip diameter of 1 to 5 μm. The electrode current was read on a picoamperemeter with a calomel electrode as the reference. Linear calibration was made between zero oxygen measured below the bacterial mats and air saturation maintained in the aerated water above the mat. In microaquaria, where the water was stagnant, air saturation was read at the air-water interface. The silver-silver sulfide electrode had a sensing tip 25 μm in diameter, and the electrode potential was read on a millivoltmeter with expanded scale. Calibration was made at a range of sulfide concentrations in seawater buffered at pH 7.0. The pH electrode had a diameter of 50 μm, and the length of the sensitive part was 100 to 200 μm. Calibration was made with standard pH buffers in seawater at pH 7.0 and 9.2. The electrodes were attached to a micromanipulator with which they could be moved at defined increments of 10 to 100 μm.

Measurements of the microgradients in whole cores were done under a dissection microscope. At 25 to 50 times magnification, the exact position of the electrode tip could be determined relative to visible *Beggiatoa* filaments or *Thiovulum* cells. This was important for correlating the zones of sulfur bacteria with the chemical gradients at a spatial accuracy of less than ±25 μm. All measurements were made in semidarkness because of the presence of purple sulfur bacteria below the *Beggiatoa* mats. Even at normal room light these photosynthetic bacteria caused a detectable lowering of the sulfide concentration in the mat. When the electrode position was found in the light, the cores were kept dark for 10 to 15 min until a steady state was reached and a profile could be measured.

Isolated *Beggiatoa* tufts or pieces of *Thiovulum* veil were also studied in a microaquarium made from a glass slide and a cover slip and sealed on three sides with petrolatum. The chemical gradients were measured while observing the bacteria in a light microscope at high magnification.

Measurements of mat topography and spatial distribution of cells were made with the micromanipulator, with the electrode as the pointer.

RESULTS

***Beggiatoa* mats.** The vertical gradients of oxygen, sulfide, and pH were measured in a dense *Beggiatoa* mat covering the black mud of a sediment core. The *Beggiatoa* filaments comprised three size groups, having diameters of 1.5 to 3, 5 to 6, and 15 to 30 μm, respectively. The size groups were mixed within the mat structure, with the smallest filaments concentrated mainly in the denser part of the mat and the larger ones protruding in loops at the surface. The whole *Beggiatoa* layer was 500 to 700 μm thick. Diatoms were present in patches in the uppermost 0 to 100 μm, and filamentous cyanobacteria (*Oscillatoria* sp.) lay scattered on the surface. At 300 to 700 μm depth (i.e., below the O_2-H_2S interface), purple sulfur bacteria formed small colonies of oval cells.

The three chemical gradients were measured consecutively at exactly the same point in the mat (Fig. 1). Oxygen was uniformly distributed in the aerated and slowly circulating seawater above the mat. Between 500 μm (0.5 mm) above the mat and the mat surface the water was unstirred, and the oxygen concentration suddenly dropped linearly from air saturation to near zero. Oxygen penetrated through this unstirred layer by molecular diffusion and reached 100 μm into the bacterial mat. Hydrogen sulfide was present at high concentration, 500 to 1,000 μmol liter^{-1}, in the black mud. In the uppermost 2 mm, it diffused upward along a steep, linear gradient which ended just at the lower boundary of oxygen. In the expanded view in Fig. 1, the overlap between oxygen and hydrogen sulfide is seen to be only about 50 μm. In this narrow zone, all the oxidation of the sulfide evidently took place. Due to heterotrophic processes in the mud, the pH dropped steeply through the boundary layer from the normal value of 8.2 in the seawater to 7.4 in the mud. There was a pH minimum within the *Beggiatoa* mat, probably due to acidic products from the sulfide oxidation.

Chemical gradients, such as those presented in Fig. 1, were measured at three different positions of the same mat and with very similar results. The overlap between oxygen and sulfide was less than 100 μm in all three profiles. The data shown in Fig. 1 represent only one set of measurements, as average values would tend to blur the sharpness of the oxygen-sulfide interface. Judging from the shape of the O_2 gradient and from the electrode sensitivity of 2 to 5 μmol

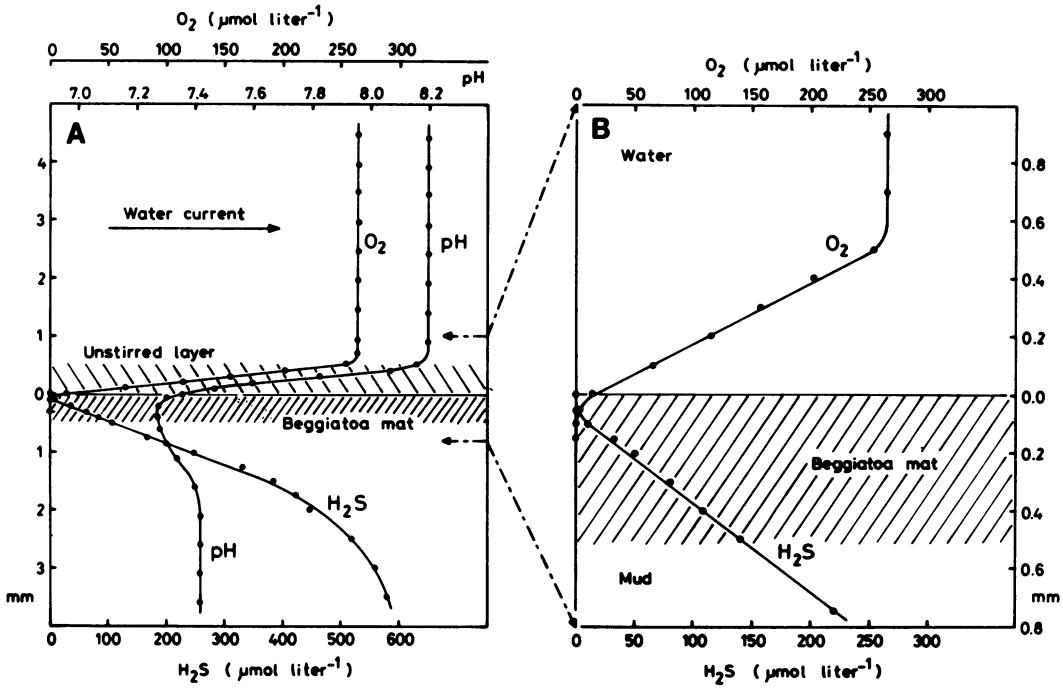

FIG. 1. Vertical distribution of O_2, H_2S, and pH through a *Beggiatoa* mat growing on a mud surface (A). The interface between O_2 and H_2S is shown in expanded scale (B). An unstirred boundary layer in the water at 0.0 to 0.5 mm created a steep diffusion gradient of O_2 from the circulating water above.

of O_2 liter^{-1}, it is unlikely that the oxygen flux penetrated below 100 μm depth, allowing a significant bacterial respiration here at an undetected trace concentration of O_2.

It was an unexpected observation that the bacteria which were exposed to a current of aerated seawater actually lived under anoxic or microoxic conditions. (The word "microoxic" is suggested here as a term for environments of very low oxygen concentration. The microoxic environment is the natural habitat of microaerophilic organisms.) This was due to the unstirred boundary layer, which was therefore studied in more detail in a sequence of oxygen microprofiles. Ten vertical profiles were measured in a row at 1-mm intervals (Fig. 2). The figure clearly illustrates how the unstirred boundary layer covered the *Beggiatoa* mat as a 1-mm-thick blanket, which prevented the bacteria from being exposed to the high oxygen concentrations in the water. As in Fig. 1, oxygen penetrated only 100 μm into the mat where the zero oxygen isopleth was found. Small hollows into the mat were also unstirred as shown in Fig. 2. *Beggiatoa* cells, which were present in the hollows, grew in loose tufts, a structure which was distinctly different from the smooth surface of the surrounding mat.

***Thiovulum* veils.** *Thiovulum* cells developed abundantly in some of the sediment cores studied. Often the cells were swimming freely among the *Beggiatoa* filaments, but in a few cores, coherent veils formed floating just above the sediment surface. It was possible to measure the oxygen gradient just above the veils. Precise measurements of the sulfide gradient below the veil was more difficult because the veil tended to adhere to the electrode tip. A weak current of aerated seawater flowed above the veils. They were very fragile but often redeveloped within a few hours after they had been disturbed. Small pieces of a veil were transferred to a petri dish with oxic seawater. Here the *Thiovulum* cells rapidly formed spheres of veils with diameters from a few hundred micrometers to a few millimeters. The smaller spheres floated freely in the water, whereas the larger ones adhered to the glass wall. The oxygen gradient above one of the spheres was measured.

The results are shown in Fig. 3. The sphere was 2 mm in diameter and the cells were 8 to 16 μm wide. The oxygen concentration was uniform in the air-saturated water around the sphere. About 600 μm from the surface of the *Thiovulum* veil the oxygen concentration decreased linearly and reached zero in the middle of the veil, which was about 100 μm thick. The *Thiovulum* sphere thus enclosed a small volume

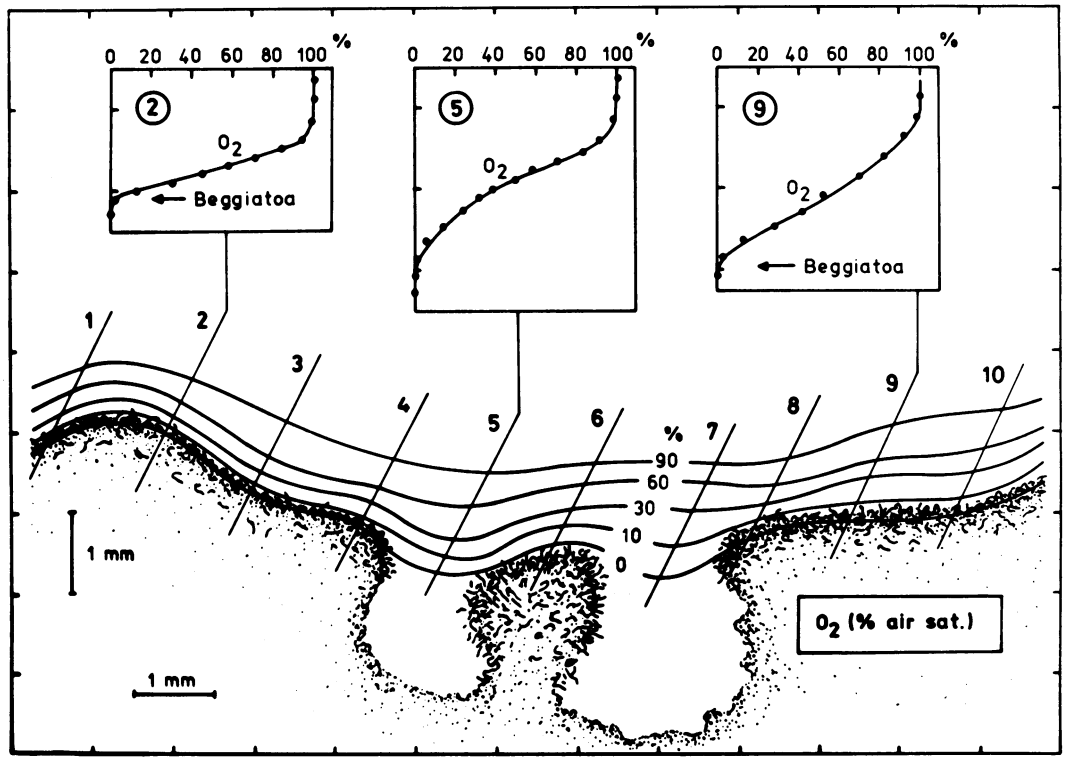

FIG. 2. Vertical section through a 0.5-mm-thick *Beggiatoa* mat on a mud surface. Beggiatoa formed a tuft within the anoxic water in the central hollow. Isopleths of oxygen concentration expressed as the percentage of air saturation show how the unstirred boundary layer covered the mat like a blanket and created microoxic conditions at the mat surface. The three insets show actual oxygen profiles from which isopleths were constructed. Arrows indicate the surface of the *Beggiatoa* mat.

of anoxic water. The anoxic conditions were created and maintained by the high oxygen consumption of the veil. Since sulfide could not be detected inside the sphere, the bacteria may have used internal storage material such as S^0 or organic reserves as substrate. The *Thiovulum* cells experienced only a small percentage of the oxygen concentration of the surrounding water. This was again due to the unstirred boundary layer of water which surrounded the veil and through which oxygen was transported by diffusion.

A similar situation was found in the sheets of veil floating on the sediment surface (Fig. 3B). The boundary layer was only 400 μm thick due to the water current above, and yet the *Thiovulum* cells were living as microaerophiles. The veil very sharply separated the oxic and anoxic regions of the water column.

Measurements of pH and H_2S concentration around the *Thiovulum* cells were made only in one case in which *Thiovulum* cells were swarming freely in a 200-μm-thick horizon within a hollow of a *Beggiatoa* mat (Fig. 4). The cells were swimming rapidly around the depth where the oxygen concentration just reached zero. About one-third of the cells were on the oxic side and two-thirds were on the anoxic side at any time. Sulfide was detectable only up to 200 μm above the oxic-anoxic interface. The concentration was about 30 μmol liter^{-1} just at the interface and about 75 μmol liter^{-1} at 200 μm below. The H_2S concentrations were, however, fluctuating, as there was no veil to stabilize the gradients, and the numbers are therefore not accurate. The data do show that the swarming *Thiovulum* cells were positioned just at the transition between oxygen and sulfide. The pH decreased over the same depth intervals from 7.62 to 7.40. This corresponded to a pH gradient of 0.5 pH unit per mm in the water.

Microaquaria. The diameter of the oxygen microelectrodes was a few micrometers at the sensing tip, i.e., of the same dimensions as the filaments of *Beggiatoa* or *Thiovulum* cells. It should therefore be possible to study the concentrations of oxygen immediately surrounding individual sulfur bacteria, provided they can be observed at sufficient magnification. This would allow more detailed investigations of their che-

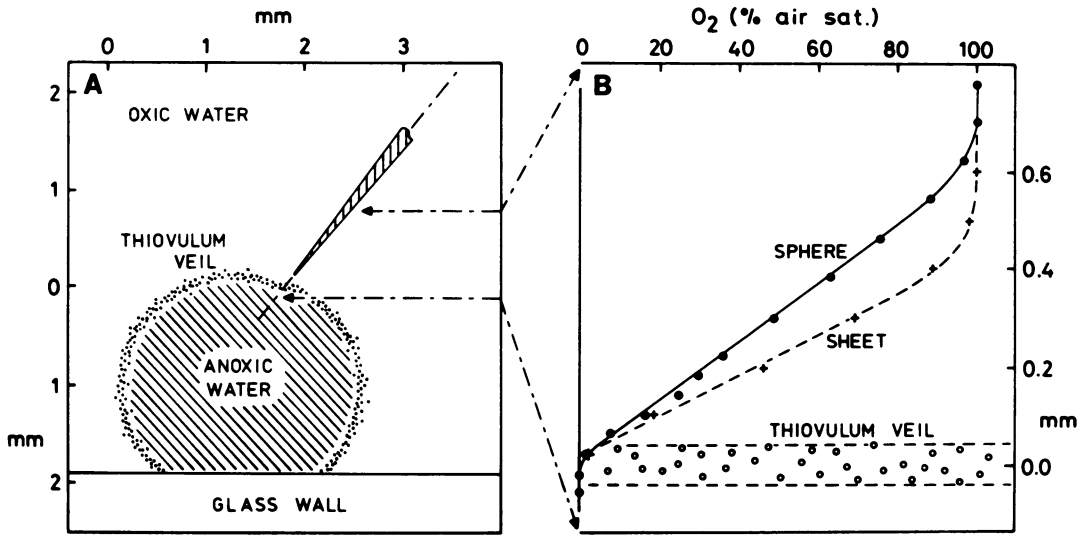

FIG. 3. Spherical *Thiovulum* veil which in oxic seawater enclosed an anoxic pocket. The bacterial oxygen uptake created a steep diffusion gradient in the 0.6-mm-thick unstirred layer surrounding the veil (A). The oxygen profile over a flat *Thiovulum* veil (sheet) is shown for comparison (B).

motactic behavior and of the interrelations between the bacterial populations and their chemical environment.

A preliminary experiment of this type is reported here. To study the bacteria at high magnification, a simple microaquarium was built and fixed on a microscope stage (Fig. 5). The aquarium was filled with anoxic water from the *Beggiatoa* mats, and a *Beggiatoa* tuft was placed near the open side. A calomel reference electrode was connected to the aquarium via a salt bridge. The oxygen microelectrode was attached to a micromanipulator in a horizontal position so that the electrode tip and bacteria could be observed simultaneously at 100 to 400 times magnification.

Within 1 to 2 h, most *Beggiatoa* filaments had oriented themselves in a zone 1.5 mm from the air-water interface through which oxygen diffused into the aquarium (Fig. 5). The filaments were lying on the lower glass wall in a narrow band which was sharply bounded toward the oxic side. No *Beggiatoa* filaments occurred at oxygen concentrations above 10% air saturation. There was a less sharp boundary on the anoxic side where filaments lay scattered throughout the preparation. That observation indicated that the gliding *Beggiatoa* cells actively avoided higher oxygen concentrations. This is in accordance with their growth pattern on the sediments where a smooth *Beggiatoa* mat created microoxic or anoxic conditions for the whole population (cf. Fig. 2).

Similar observations were made with *Thiovulum* cells. A small piece of a veil was placed in the anoxic water of the microaquarium, and the bacteria soon formed a new veil parallel to the water surface at a distance of 1 to 2 mm. Initially, the cells swam around seemingly at random, but once they were established in the veil the amplitude of their movements perpendicular to the water surface did not exceed about 100 μm. The oxygen microelectrode showed that they were positioned just around the boundary where oxygen reached zero. The individual cells in the veil moved rapidly within an oxygen gradient from 0 to 1 to 3% air saturation.

DISCUSSION

The present results have shown that *Beggiatoa* and *Thiovulum* species live under very low O_2 and H_2S concentrations of a few micromoles per liter, irrespective of the high concentrations in the surrounding macroenvironment (above the 1-mm scale). The bacteria live as true microaerophiles when they grow in mats or veils. They are then surrounded by unstirred boundary layers which separate them and protect them from the surrounding high and fluctuating O_2 and H_2S concentrations of the macroenvironment. Dissolved substrates reach the bacteria through these boundary layers by molecular diffusion along steep gradients.

The presence of unstirred boundary layers in water adjacent to solid surfaces is a phenomenon well known from fluid dynamics (26). Their importance has not been fully appreciated in aquatic ecology or microbiology, mainly be-

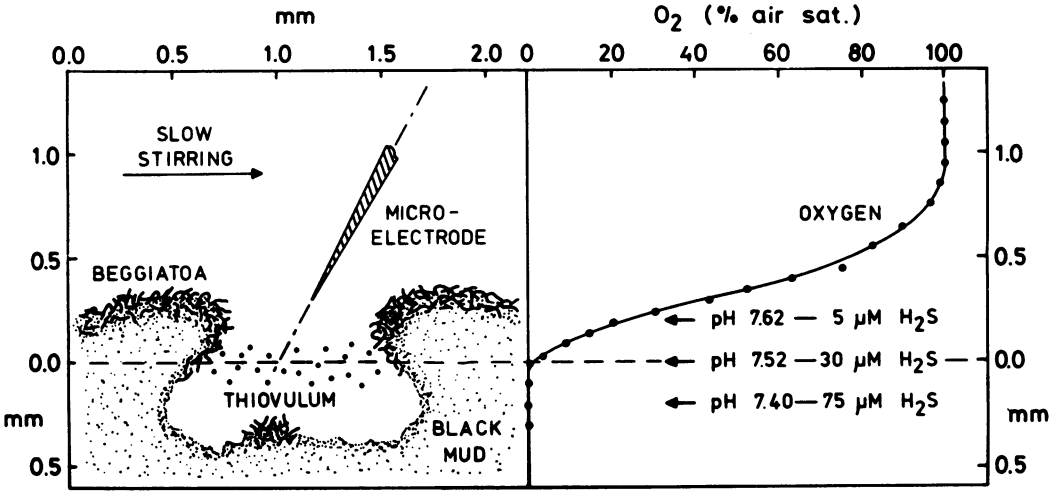

FIG. 4. *Thiovulum* cells swarming, without veil formation, at the oxygen-sulfide interface within a hollow in a *Beggiatoa* mat. Microelectrode measurements of O_2, H_2S, and pH were made along the broken line in the center.

cause measuring techniques have had insufficient resolving power to demonstrate that they existed. The unstirred boundary layers are created by the viscous forces (internal friction) in the water, which cause a thin water film to stick to the solid surface. The viscous forces in this film dominate the inertial forces which drive the turbulent movement of the surrounding water. The thickness of the unstirred boundary layer depends, among other things, on the roughness of the solid surface and on the degree of turbulence of the stirred water.

We will discuss how the gradient bacteria, *Beggiatoa* and *Thiovulum* species, have adapted to a life governed by diffusion through such unstirred boundary layers.

Substrate diffusion and consumption. The microgradients of oxygen and pH above the *Beggiatoa* mat shown in Fig. 1 indicated a sharp transition between a stagnant boundary layer adjacent to the mat and the stirred bulk water above. The thickness of the boundary layer was found to vary with the rate of stirring. The linearity of the oxygen gradient at 0 to 0.5 mm indicated that there was a constant diffusion coefficient and no oxygen consumption within the layer. The sulfide gradient was also linear at 0.1 to 1.5 mm below the oxygen zone, which showed that it was governed by diffusion with little influence from production or consumption of H_2S in this layer. Thus, the whole consumption of oxygen must have taken place in the uppermost 100 μm of the *Beggiatoa* mat, whereas sulfide was consumed in the zone from 50 to 100 μm depth where it met and coexisted with oxygen in a dynamic steady state.

The transport of oxygen and sulfide by molecular diffusion is a rapid process in dimensions below 1 mm. The diffusion time of an oxygen molecule through the unstirred layer can be calculated from the Einstein-Smoluchowski relation:

$$t = L^2/2D = 1 \text{ min}$$

where t is the diffusion time, L is the diffusion path (500 μm), and D is the diffusion coefficient of O_2 at 20°C (2.06×10^{-5} cm^2 s^{-1} [4]).

Since the microgradients show a pure diffusion transport of both O_2 and H_2S, it is possible to calculate the flux and the consumption rate for both substrates. The flux is calculated from Fick's first law:

$$F = -\phi \cdot D \cdot dC/dx$$

where F is the flux, ϕ is the porosity of the system (ϕ is 1.0 in the water phase and is estimated to be 0.9 in the *Beggiatoa* mat), D is the diffusion coefficient at 20°C (2.06×10^{-5} cm^2 s^{-1} for O_2 [4], 1.52×10^{-5} cm^2 s^{-1} for H_2S-HS^- as calculated from radiotracer measurements by Jørgensen et al. [13] from a cyanobacterial mat, 1.56×10^{-5} cm^2 s^{-1} for H_2S gas alone [4]), and dC/dx is the vertical gradient of O_2 or H_2S concentration.

As the thickness of the consumption layer is known as well as the substrate concentrations, in this layer, it is possible to calculate the consumption rate per volume as well as the turnover time (residence time) of the substrate pool. As the substrate concentration has a gradient within the layer, the calculated turnover time represents a mean value for the total pool. The mean turnover time is simply the total pool size

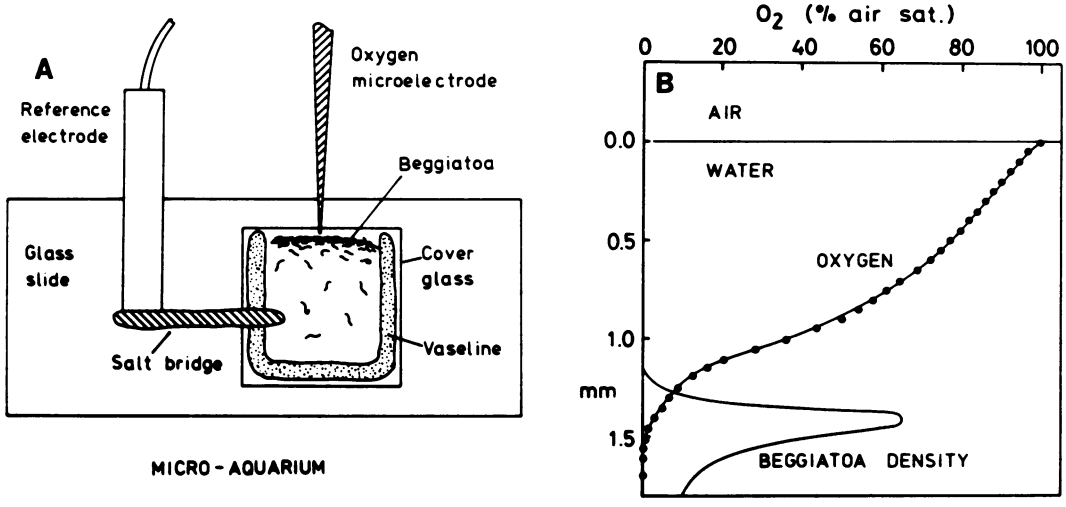

FIG. 5. Illustration of *Beggiatoa* cells in a 1-mm-deep microaquarium with anoxic seawater. Oxygen diffused 1.5 mm into the seawater from the ambient air and reached zero concentration within the densest *Beggiatoa* zone (A). The filaments formed a band on the bottom glass slide. This created both vertical and horizontal gradients within the aquarium, and the oxygen gradient, therefore, appeared nonlinear (B).

within the consumption layer divided by the flux into the layer, under the reasonable assumption that the diffusion gradients are in steady state. Diffusion steady states in these dimensions are established within a few minutes. The results are shown in Table 1 together with similar calculations for a *Thiovulum* veil based on data from Fig. 2 (sheet). The thickness of the O_2 consumption zone in the *Thiovulum* veil was determined from microscopic observations of the cells concurrent with measurements of O_2 microgradients. A thickness of 50 μm was in accordance with the lower curved part of the O_2 gradient in Fig. 3, which indicated consumption.

The fluxes of oxygen and sulfide into the thin *Beggiatoa* mat were 0.99×10^{-10} and 0.44×10^{-10} mol cm^{-2} s^{-1}, respectively. The part of the oxygen consumption which was due to sulfide oxidation will depend on the oxidation product. An oxidation only to elemental sulfur would consume 0.22×10^{-10} mol of O_2 cm^{-2} s^{-1}, whereas a complete oxidation to sulfate would consume 0.88×10^{-10} mol of O_2 cm^{-2} s^{-1}. The actual value must lie somewhere in between, depending on how fast the *Beggiatoa* cells accumulated elemental sulfur. The remaining oxygen consumption could be used to oxidize other substrates such as dissolved organic molecules.

Oxygen penetrated only 100 μm into the *Beggiatoa* mat before it was all consumed. In this 0 to 100 μm layer, the average O_2 concentration was 6 μmol $liter^{-1}$. Due to the small pool size, the turnover time was very short, only 0.6 s. This shows how extremely dynamic the chemical microenvironment of the gradient bacteria can be. Similar calculations for the 50-μm-thick layer of O_2-H_2S coexistence showed a turnover time for the H_2S pool of 0.6 s. Oxygen had a turnover time of only 0.1 s in the *Thiovulum* veil.

The calculated fluxes of oxygen and sulfide are in good agreement with directly measured rates of oxygen uptake and of sulfide production from sulfate reduction measured in organic-rich coastal sediments, similar to those on which the *Beggiatoa* and *Thiovulum* species were growing (12).

***Beggiatoa* mats.** It was the early work of Winogradsky (27) at the end of the last century on enrichment cultures of *Beggiatoa* cells which led to the concept of chemoautotrophic metabolism in these organisms. Since then, the autotrophic growth of *Beggiatoa* cells, as well as their ability to use H_2S as an energy source, has been questioned repeatedly (5, 20). Recent studies on pure cultures of *Beggiatoa* cells have shown that most strains grow heterotrophically (18, 20, 24), and in the one *B. alba* strain investigated, the typical autotrophic CO_2-fixing system is absent (23). A report on chemolithotrophic heterotrophy in chemostat-grown *B. alba* (10) was later questioned by Kuenen and Beudeker (15a). Such a metabolism would, however, agree well with a habitat at the oxic-anoxic interface in sediments and water.

Although the chemolithotrophy on H_2S remains questionable, the present study gives strong indication that *Beggiatoa* cells growing in mats have the ability to oxidize all sulfide ascending from the sediment very fast and efficiently.

TABLE 1. Diffusion flux and consumption of O_2 and H_2S in a *Beggiatoa* mat and a *Thiovulum* veil

Genus	Substrate	Flux (mol cm^{-2} s^{-1})	Uptake zone (μm)	Uptake rate (μmol $liter^{-1}$ s^{-1})	Avg concn (μmol $liter^{-1}$)	Turnover time (s)
Beggiatoa	O_2	0.99×10^{-10}	100	9.9	6	0.6
	H_2S	0.44×10^{-10}	50	8.8	5	0.6
Thiovulum	O_2	1.3×10^{-10}	50	26	3	0.1

Bacteria which oxidize H_2S with oxygen must compete with the spontaneous autooxidation which takes place concurrently. The chemical oxidation of H_2S in seawater and freshwater has been studied in the laboratory as well as in the chemocline of stratified water bodies. The half-life of sulfide is generally in the range of 1 to 3 h at air saturation of oxygen and 20°C (1, 6, 8). Almgren and Hagström (1) found a half-life of 38 to 81 min for sulfide at 1 to 5 μmol $liter^{-1}$, i.e., at the same concentrations as in the O_2-H_2S interface of the *Beggiatoa* mat. The residence time of H_2S in the mat was only 0.6 s. Thus, the sulfide oxidation in the mat was 10,000- to 100,000-fold faster than in water. Although chemical catalysts are able to speed up the chemical oxidation 10- to 100-fold (7), the spontaneous reaction would still be less than 1% of that in the *Beggiatoa* mat. It is therefore concluded that the process was biologically catalyzed. As *Beggiatoa* constituted the completely dominating bacterial biomass at the O_2-H_2S interface, it is suggested that this organism was chiefly responsible for the rapid oxidation of H_2S.

It was only 10% of the 500-μm-thick *Beggiatoa* mat which was within the 50-μm-thick O_2-H_2S interface at any time. Since the filaments glide around continuously at the speed of 100 to 200 μm min^{-1} (9), they may at intervals have passed through the interface zone and rapidly accreted sulfur droplets. These were then slowly oxidized to sulfate above the sulfide zone or perhaps reduced back to H_2S below the oxygen zone (19).

***Thiovulum* veils.** The formation of *Thiovulum* veils serves the same purpose as the formation of *Beggiatoa* mats, namely to position the bacteria exactly at the interface between the oxic and the sulfide zones and to stabilize the interface. The veils are sufficiently rigid to withstand slow movements of the water, and they enable an expansion of the anoxic zone into the water. In this way, *Thiovulum* veils can outcompete the sediment-bound *Beggiatoa* filaments, which have sometimes been observed to glide up on the top side of *Thiovulum* veils.

The establishment of a new veil was observed under the microscope. Initially, swarming *Thiovulum* cells aggregated exactly at the oxic-anoxic interface. As the cells reached the interface, their rapid swimming changed into a vibrating motion as they were seemingly caught in the developing veil. The amplitude of their movements along the oxygen gradient was then only about 100 μm. An agglutination of slime threads secreted from the aggregating cells was suggested by Wirsen and Jannasch (28) as the mechanism of veil formation.

The chemotactic response of *Thiovulum* cells to traces of O_2 must be very sensitive, as was illustrated by the following observation. In a microscope preparation of anoxic porewater from a sulfuretum, a phyto-flagellate (*Euglena* sp.) was observed to swim around vividly, pursued by a flock of 5 to 10 *Thiovulum* cells. The bacteria were evidently able to trace the oxygen which, like a comet's tail, was trailing after the flagellate. When the microscope light was switched off for a few seconds, the *Thiovulum* cells immediately lost track of the flagellate and swam around at random, but in the light again they soon renewed the pursuit.

In the following, we will analyze the veil as a mechanism by which *Thiovulum* cells can grow in the free-water phase with an optimal utilization of sulfide and oxygen as substrates. *Thiovulum* cells may possibly utilize other reduced compounds as well, but the ability to grow autotrophically or mixotrophically at the expense of sulfide as an energy source was at least strongly indicated by the studies of Wirsen and Jannasch (28).

Even the invisible, gelatinous *Thiovulum* veil of 100 μm thickness was able to create unstirred boundary layers (Fig. 3). A main function of the veil must be to serve as a solid interface between the oxic and the sulfide zones of the water. The veil prevents mixing of oxygen and sulfide by microturbulence or by convection.

The diffusion time for an O_2 molecule to go through the 100-μm-thick veil is only 1 s, and yet oxygen reached only halfway through the veil before it was all consumed (Fig. 3). The overlap between the oxygen and sulfide zones must therefore have been extremely narrow, only about 50 μm, and the residence time of the compounds at the interface was correspondingly short, 0.1 s for oxygen. The sulfide turnover time must be approximately the same, and the rapid sulfide oxidation must therefore be biologically catalyzed.

The creation of a stable, microoxic environ-

ment requires the concerted action of a large population of *Thiovulum* cells growing in a veil. It could not be achieved by individual cells swimming freely in the unstable environment. This may explain why *Thiovulum* cells often form minute spheres of veils in oxic water. As long as the oxygen consumption of the bacteria can balance the oxygen diffusion through the boundary layer they can maintain anoxic conditions inside the sphere. Continuous measurements made in the sphere in Fig. 3 showed that anoxic conditions were maintained for more than 1 h. With time, however, individual cells began to detach from the veil and swarm out into the oxic water.

Diffusion limitation of *Thiovulum* cells. Careful observations on microgradients of oxygen in *Thiovulum* veils showed that the cells on the inner side of the veil were in anoxic water. For these cells to reach oxygen, it was necessary to move. Cell movements within the veil seemed random, with an amplitude of only 50 to 100 μm. This, however, would be sufficient to bring cells into contact with both oxygen and sulfide within seconds. The bacteria would not outrun diffusion by this movement since the diffusion time of oxygen over the same distance is also 1 s. The oxygen molecules, however, had a residence time of only 0.1 s and, therefore, they did not diffuse that far. A rapid, vibrating movement, therefore, could quite possibly help to bring the bacteria into contact with both oxygen and sulfide.

Estimates of cell density in the veils were done under a microscope by observing veils forming in microaquaria as well as by observing intact veils under the dissection microscope. An ocular grid was used for the cell counts. The densities varied between 10^5 to 10^6 cells cm^{-2}, with 5×10^5 as an average value (cf. reference 11). The density corresponded approximately to one compact cell layer, which was evidently enough to completely consume the high influx of oxygen.

The low concentration and rapid turnover of substrate in the veil may lead to diffusion limitation of the metabolic rate in individual cells. To evaluate this possibility, it is necessary to know the rate of substrate uptake per cell. If we assume an average cell density of 5×10^5 per cm^2, an oxygen uptake of 1.3×10^{-10} mol of O_2 cm^{-2} s^{-1} as calculated in Table 1, and an H_2S flux of 0.44×10^{-10}, similar to that reaching the *Beggiatoa* mat in the same sediment cores as the *Thiovulum* veil was overgrowing, we obtain the following rates: O_2 uptake = 2.6×10^{-16} mol of O_2 $cell^{-1}$ s^{-1}; H_2S uptake = 0.9×10^{-16} mol of H_2S $cell^{-1}$ s^{-1}.

The estimated rate of H_2S uptake can be compared with the autotrophic CO_2 fixation rates measured by Wirsen and Jannasch (28) in enrichments of *Thiovulum* cells. When growing under optimal conditions in veils, the bacteria assimilated up to 1.22 μg of CO_2 per 10^6 cells per h. With a complete oxidation of H_2S via elemental sulfur to sulfate and with an energy yield of 10% comparable to that of the thiobacilli (R. F. Beudeker, J. C. Gottshal, and J. G. Kuenen, Antonie van Leeuwenhoek J. Microbiol. Serol., in press), the rate of sulfide oxidation in these *Thiovulum* enrichments would have been 0.4×10^{-16} mol of H_2S $cell^{-1}$ s^{-1}. If the H_2S was oxidized to elemental sulfur only, the rate would be 1.6×10^{-16} mol of H_2S $cell^{-1}$ s^{-1}. Our estimate above of 0.9×10^{-16} is between these two rates.

The substrate concentration at which a nonmoving, spherical cell with a radius, R, and a substrate consumption rate, v, becomes diffusion limited is (14):

$$S = \frac{v}{4 \cdot \pi \cdot D \cdot R}$$

where S is the substrate concentration and D is the diffusion constant of the substrate. With the above calculated values for oxygen, $v = 2.6 \times 10^{-16}$ mol of O_2 $cell^{-1}$ s^{-1}, $D = 2.06 \times 10^{-5}$ cm^2 s^{-1}, and $R = 6 \times 10^{-4}$ cm, the limiting concentration for oxygen is 1.7 μmol of O_2 $liter^{-1}$. Thus, at 1.7 μmol of O_2 $liter^{-1}$, these *Thiovulum* cells should deplete the surrounding water sphere of oxygen to an extent where the concentration just reaches zero at the cell surface. At lower concentrations of oxygen in the surrounding water, the oxygen uptake of the bacteria will decrease because the availability of the oxygen is limited by the rate of molecular diffusion through the cells' own diffusion sphere. *Thiovulum* cells in the veil lived at oxygen concentrations between 0 and 5 to 10 μmol $liter^{-1}$, i.e., at or slightly above diffusion limitation. A similar calculation for the uptake of H_2S shows that the cells should become diffusion limited at 0.8 μmol of H_2S $liter^{-1}$. These calculations indicate that the *Thiovulum* cells not only create the steep microgradients through the stagnant boundary layer, but they assimilate oxygen and sulfide so efficiently that even the individual cells come close to diffusion limitation in their uptake rate. This must be the highest efficiency a bacterium of that size can reach in the competition with the chemical reaction between O_2 and H_2S.

The substrate limitation can be reduced a little because the cells swim. With a swimming rate, μ, of 50 μm s^{-1}, typical for bacteria, the substrate availability of the diffusion limited cell is increased by a factor, G, which is (14):

$$G = 1 + \sqrt{\frac{2 \cdot \mu \cdot R}{\pi \cdot D}} = 1.3$$

With the same values of the parameters as those used above, the increase in oxygen availability for the cell will be 30%. This is hardly enough to be the important explanation for cell movements in the veil. It can also be calculated that the cells do not gain more substrate by stirring the water within the veil. At a stirring rate of 50 $\mu m\ s^{-1}$ over the 100-μm-thick veil, the substrate transport by diffusion would still be faster than by stirring (cf. reference 21).

The large size of *Thiovulum*, 10 to 20 μm in diameter, is unusual for bacteria in general, but it is typical for several colorless sulfur bacteria which have specialized to grow between the opposed gradients of oxygen and sulfide. The need for these bacteria to establish and maintain a physical barrier between the oxygen and sulfide zones, which can withstand a certain degree of water turbulence, could possibly be a factor which selects for this large cell size. Steep diffusion gradients of the energy sources may then provide a sufficient substrate availability to allow the large cell size without significant diffusion limitation of individual cells. Competition from smaller bacteria with a more efficient substrate uptake may, e.g., in *Thiovulum* veils, force these to abandon the old slime web at intervals and establish a new one (27).

In conclusion, *Beggiatoa* and *Thiovulum* species represent optimal adaptations to life at the O_2-H_2S interface. Further studies of the physiology and chemotaxis of these bacteria should be made in pure cultures in combination with microelectrode measurements of the gradients to better understand the details of this adaptation.

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