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Oxygen Responses and Mat Formation by *Beggiatoa* spp.

MOGENS M. MØLLER, LARS P. NIELSEN, AND BO B. JØRGENSEN*

Institute of Ecology and Genetics, University of Aarhus, DK-8000 Aarhus C, Denmark

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The behavioral response of single *Beggiatoa* sp. filaments moving on a gas-permeable membrane was studied by the combined use of microscopy and oxygen microelectrodes during controlled oscillations of oxygen tension. The bacteria reacted to increasing oxygen by reversing the direction of movement. The same step-up phobic response to oxygen was observed when a filament tip or loop glided into a stable microgradient of increasing oxygen. The response was sensitive to a change in oxygen tension of <5% of air saturation min^{-1}. The response time was 20 to 50 s. Frequently, only part of the filament responded, which led to the formation of sharp bends, loops, and coils. This partial response facilitated the positioning of the long filaments within the narrow O_2-H_2S interface. The structure of whole *Beggiatoa* mats on sediment surfaces varied from loose to dense in relation to shallow or steep oxygen gradients in the 0.3- to 2-mm-thick, unstirred boundary layer. In an illuminated sediment *Beggiatoa* spp. lived together with photosynthetic organisms and migrated vertically in accordance with light/dark variations. The combined effect of phobic responses to light and oxygen can explain this migration.

The filamentous, colorless sulfur bacteria *Beggiatoa* spp. often grow abundantly at the surface of sulfide-rich sediments, decomposing plant residues, and cyanobacterial mats (14, 19, 22, 26). Most reports on their occurrence in freshwater or marine habitats describe their conspicuous mass aggregations as mats or tufts covering solid surfaces. Such mats of *Beggiatoa* spp. or the closely related *Thioploca* spp. may cover large areas of the sea-bed in coastal up-welling regions or in eutrophic fjords (10, 13, 29).

Beggiatoa mat formation occurs where diffusing sulfide from below and oxygen from above meet. The filaments oxidize sulfide, with oxygen competing efficiently with the spontaneous chemical oxidation, thereby establishing steep, opposed gradients of oxygen and sulfide with a coexistence zone of only about 50-μm thickness (15). Under these gradient conditions the bacteria may use the chemical energy of H_2S oxidation for CO_2 assimilation. Such a chemoautotrophic growth has been suggested and questioned repeatedly ever since it was originally put forward by Winogradsky (30). Chemoautotrophy was finally confirmed, however, for a marine strain of *Beggiatoa* sp. based on $^{14}CO_2$ fixation measurements and on the presence of the CO_2-fixing enzyme ribulose-1,5-bisphosphate carboxylase (23).

The dynamic transition zone between oxygen and sulfide is potentially very unstable and moves as the environmental conditions change. The ability of the gliding *Beggiatoa* filaments to follow these movements has been observed in photosynthetically active sediments, where the oxygen and sulfide zones showed a diurnal vertical migration (12, 14, 22). The *Beggiatoa* spp. formed a white coating on the surface during the night when the sediment was anoxic and rich in sulfide. During the day, the sulfur bacteria had migrated down and were hidden below the oxic surface.

One of the cellular mechanisms which may be the basis for such behavior is a negative response to light. A photophobic response in *Beggiatoa* spp. was recently demonstrated by Nelson and Castenholz (22) for filaments from a pure culture. It was suggested by the authors to be an important response for the bacteria, which could otherwise be exposed to inhibitory levels of light, of photosynthetically produced oxygen, and perhaps of intracellular peroxide.

The *Beggiatoa* populations which live below the photic region in sediments must have additional behavioral responses to orient themselves optimally in relation to their chemical environment. Responses to oxygen and sulfide would seem to be obvious possibilities to enable such an orientation.

It was the aim of the present study to search for responses of *Beggiatoa* spp. to oxygen and to analyze the behavioral patterns which lead to the complex and dynamic mat structures.

MATERIALS AND METHODS

Sources of *Beggiatoa* spp. A laboratory sulfuretum was established to provide an ample supply of *Beggiatoa* spp. for the present study. Organic-rich sediment with a high concentration of sulfide in the pore water and with dense populations of *Beggiatoa* spp. at the surface was collected from Kalø Lagoon in northern Aarhus Bay, Denmark. This locality is described in further detail by Hansen et al. (12) and Troelsen and Jørgensen (28). The sediment consisted mainly of fine organic detritus and had a very homogeneous texture. Fifty liters of the sediment was incubated in the laboratory in an aquarium. The sediment was covered by constantly aerated seawater (salinity, 23‰; temperature, 17 to 21°C) and exposed to a diurnal light-dark cycle under fluorescent light. Within the following day, *Beggiatoa* filaments migrated up and formed a dense white mat, which was visible on the sediment surface during dark periods. Filamentous cyanobacteria developed dark green mats, which covered the sediment surface during light periods. With only one exception, all experiments were performed with samples from this source. The filaments were 100 μm to >10 mm long and the cells ranged in width from 1 to 40 μm, with the majority being 18 to 30 μm wide. Filaments without internal

* Corresponding author.

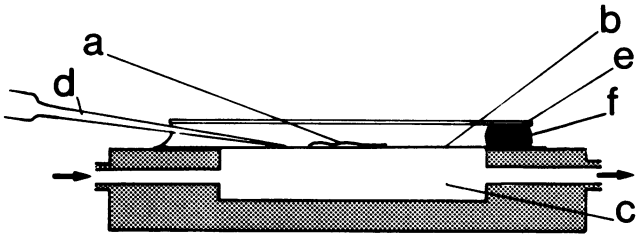

FIG. 1. Gas exchange microaquarium. *Beggiatoa* filaments (a) moved on a gas-permeable Teflon membrane (b) which separated the microaquarium from a gas compartment (c) through which the appropriate gas constantly flowed. An oxygen microelectrode (d) was introduced into the microaquarium and positioned with the sensing tip close to the selected filament. The aquarium was about 1 mm deep and sealed on top by a cover slip (e) supported by a frame of Vaseline (f). Only one side of the aquarium, where the electrode could be inserted, was exposed to air. The cover slip and Vaseline were renewed for each new preparation.

elemental sulfur were very rare and were never used in the experiments.

Temporal oxygen variations. The response of single *Beggiatoa* filaments to controlled, temporal changes in oxygen tension were studied in a gas exchange microaquarium (Fig. 1). The microaquarium was built from transparent Plexiglas and could be mounted on a microscope stage. Oxygen diffused freely through the gas-permeable membrane. The oxygen tension experienced by the *Beggiatoa* filaments could therefore be regulated by passing different mixtures of nitrogen and oxygen through the gas compartment.

Oxygen was measured by a microelectrode with a sensing tip of about 5 μm in diameter. The construction and application of these oxygen microelectrodes have been described by Baumgärtl and Lübbers (1) and by Revsbech (25). The electrode was attached to a micromanipulator, and the current was read on a picoammeter connected to a strip-chart recorder. A calomel reference electrode was connected to the microaquarium via a salt bridge. The electrode tip was always positioned less than 100 μm from the *Beggiatoa* filaments. The diffusion time over such a distance is only a few seconds, and delays between measured oxygen tensions and tensions experienced by the filaments were therefore insignificant.

Beggiatoa tufts for experimentation in the microaquarium were first transferred with a pipette from the laboratory sulfuretum to a small petri dish. Under the dissection microscope a few intact filaments were then carefully transferred with a pipette and deposited on the Teflon membrane of the microaquarium, which was finally filled up with oxic or anoxic seawater.

Spatial oxygen gradients. Studies of behavioral responses of *Beggiatoa* spp. were performed with more simple microaquaria made from a microscope slide and a cover slip held 2 mm apart by a frame of Vaseline. The aquarium was filled with seawater, and a small amount of sulfide-rich mud together with a tuft of *Beggiatoa* spp. were placed in it. As the water gradually became anoxic, the tuft of filaments dispersed. When one side of the aquarium was left open, a stable oxygen gradient developed within a 1- to 2-mm-wide zone at the air-water interface, as measured with the oxygen microelectrode. The responses of filaments that entered this zone were observed under the microscope at ×100 magnification.

Studies on whole populations. To study *Beggiatoa* populations also under more natural conditions, intact sediment cores with *Beggiatoa* mats from the sulfuretum were carefully collected in Plexiglas tubes and immersed in a circulating seawater system. In this system the mat surface could be exposed to different velocities of water flow with controlled oxygen tension. An oxygen microelectrode was mounted in a nearly vertical position on a micromanipulator. The electrode tip and the *Beggiatoa* mat could then be observed simultaneously under the dissection microscope and measurements of oxygen concentration could be related precisely to the mat structure. Vertical distances were measured also by means of the micromanipulator, using the electrode tip as a marker.

The mats were kept in darkness in most cases and only studied for short periods under dim light to minimize light responses and photosynthetic activities in the sediment. When it was specifically decided to include the effects of light, the mats were illuminated by a slide projector with a 250-W halogen lamp and a heat filter. Light intensities were measured by a quantum meter (Lambda Instruments) with an underwater quantum sensor for visible light of 400 to 700 nm. The light intensity at the sediment surface was 2,000 μEinsteins m^{-2} s^{-1}, which is about 50% above full sunlight in shallow water.

A special technique was applied to observe the positioning of *Beggiatoa* filaments which had migrated into the sediment. A needle was attached to the micromanipulator in place of the fragile electrode. The needle tip was lowered stepwise into the sediment, and for each step the overlying sediment was briefly pushed aside with another needle for observation. The whole procedure was carried out quickly before the filaments responded to changes in light and oxygen gradients.

The light penetration into the sediment was also determined. A glass petri dish with seawater was placed over the quantum sensor and shielded from light on the sides by black tape (17). A small amount of surface sediment was suspended evenly in the petri dish. The sediment precipitated within a few minutes and achieved the same loose appearance as the original sediment surface. The thickness of the precipitated layer was measured by a needle attached to the micromanipulator, and the light intensity was read. This procedure was repeated several times with successively increasing sediment thicknesses.

RESULTS

Responses to temporal oxygen variations. To determine whether oxygen could directly induce behavioral responses of *Beggiatoa* spp., single filaments were exposed to repeated oscillations of oxygen tension between 30 and 300% of air saturation in the gas exchange microaquarium (Fig. 1). The *Beggiatoa* spp. in this experiment were randomly oriented on the membrane of the microaquarium and were exposed to similar oxygen tensions over the entire length of the filament.

The bacteria used were not from the laboratory sulfuretum but were collected from sediments of the brackish Ringkøbing Fjord in western Jutland, Denmark. They were 3 μm wide. A number of filaments were placed in the microaquarium and allowed to glide over the Teflon membrane. Only those filaments which were straight were then used to assay for oxygen response. These filaments were usually the shorter specimens of <0.6 to 0.8 mm. During the following experimental variations in oxygen tension, any reversal in the direction of movement of the leading part or the whole filament was scored. As these filaments often

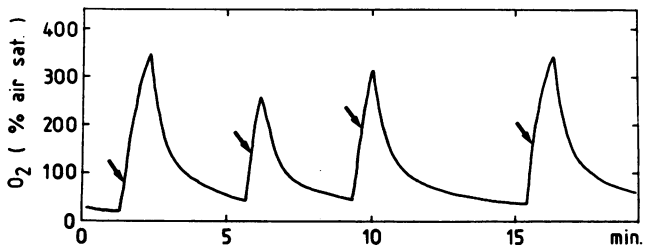

FIG. 2. Responses of a *Beggiatoa* filament to temporal variations in the oxygen tension of the gas exchange microaquarium. The arrows indicate when the filament reversed.

remained straight after a reversal, it was possible to assay such filaments through repeated cycles of oxygen variation.

One example of the results is shown in Fig. 2. The filament reversed its direction of movement every time the oxygen tension increased. The reversal came 20 to 30 s after the start of increase. When the oxygen decreased, there was no reversal. A total of 56 similar oxygen increases were performed with 30 filaments. In 87% of the cases, the filaments reversed within 1 min after onset of oxygen increase. As a control, 18 of these filaments were also observed during a total of 240 min under decreasing or constant oxygen concentration at near zero air saturation. Under these conditions the frequency of reversals was only 5 to 10% min^{-1}.

Beggiatoa filaments, 6 μm wide, from the laboratory sulfuretum were also tested for oxygen response in a series of experiments with the gas exchange microaquarium. The filaments were transferred in deoxygenated water to avoid exposure to high oxygen. Straight filaments gliding over the Teflon membrane were then observed. Initially the oxygen tension was constant and below 2% of air saturation. Under these conditions they reversed at random time intervals with a frequency of 5 to 10% min^{-1}, like the filaments under anoxia in the former experiments. In each experiment one or two of the straight filaments were selected randomly. If they had not shown any reversal within a standard period of 6 min, the oxygen tension was gradually increased and reversals were scored. The oxygen tension was raised linearly with an increase rate between 2 and 13% of air saturation min^{-1}. A total of 26 filaments were tested.

An example of the results is shown in Fig. 3. This filament reversed 2.7 min after the oxygen increase was initiated. The oxygen tension was then 6% of air saturation. None of the 26 filaments showed a response within the first 25 s after the oxygen increase was initiated. A total of 85% of the filaments then reversed over the next 3.5 min.

Of five filaments tested at oxygen increase rates of <5% of air saturation min^{-1}, all showed a response within 1 min, i.e., before the oxygen tension had reached 5% of air saturation. This means that the observed response to oxygen is sensitive even to increase rates and O_2 tensions below these values.

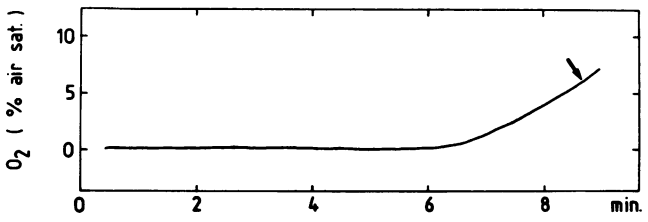

FIG. 3. Oxygen tension as experienced by a *Beggiatoa* filament in the gas exchange microaquarium. After 6 min the oxygen tension was slowly increased, and the filament showed a reversal 2.7 min later (arrow).

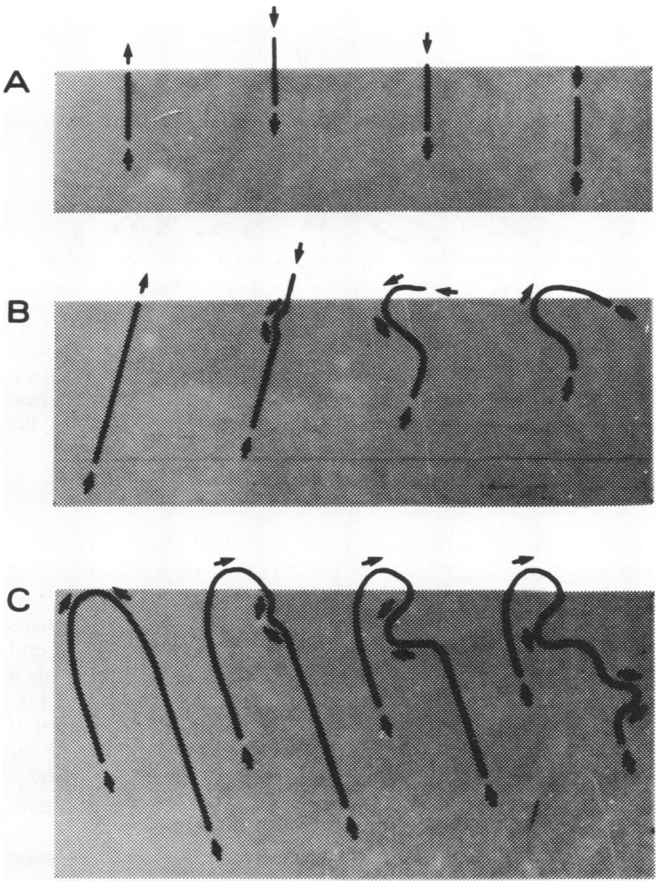

FIG. 4. Time sequence, I to IV (see text), of the behavior of three *Beggiatoa* filaments as they entered an oxic zone by gliding on a glass slide. The anoxic zone is shaded and arrows show the direction of movement of the filament. Each sequence lasted about 2 min. (A) The entire filament reversed after entrance into the oxic zone. (B) Only the leading end reversed (II) and a bow was created (III). At the end, the filament resumed the original unidirectional movement, but now with the leading end pointing in a new direction (IV). (C) A U-shaped filament moved with the central part leading (I). A short region of this leading part reversed and a bow was created (II and III). Later an adjacent filament part also reversed and the filament continued to glide away, with the latter bow in front (IV). Commonly, such bows on the filaments were slightly raised above the surface of the glass slide.

Similar experiments were carried out with 10 sigma units of catalase $liter^{-1}$ added to the seawater in the microaquarium to show whether a protection against possible harmful effects of peroxide would change the response pattern. There were no detectable effects of the catalase.

Responses at anoxic-oxic interfaces. The responses of single *Beggiatoa* filaments as they entered an oxic zone were observed in the simple microaquarium on glass slides. Figure 4 shows three examples of responses.

A simple reversal of the entire filament was mainly restricted to short, straight filaments (Fig. 4A). Occasionally we observed that the straight filament vibrated without

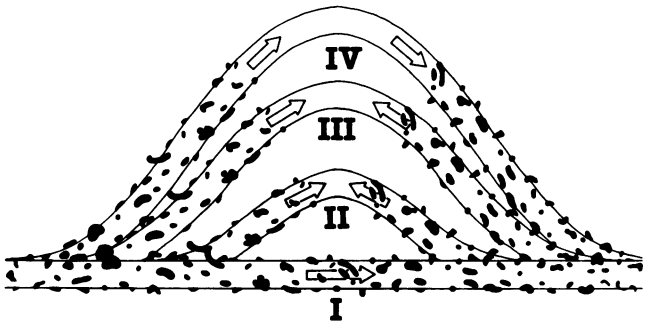

FIG. 5. Time sequence of 1-min duration showing the formation of a bow on a part of a *Beggiatoa* filament which moved on a glass slide. The slime sheath, which adhered to the surface, was stained by black India ink particles. The direction of movement of the filament relative to the sheath is indicated by arrows.

reversal, possibly because a part of it had tried to reverse, but was still being pushed or pulled in the opposite direction by the rest of the filament.

Figure 4B and C represents other, more typical responses and illustrates three general stages: (i) only the leading end reversed initially and caused a lateral movement of a subterminal filament region; (ii) subsequently, adjacent filament parts reversed as well, thereby creating a new region of lateral movement; (iii) the reversed parts commonly reversed back again. The consequence of these responses was that the filaments tended to curl up and the entire organism obtained a new direction of movement.

It can be calculated from the steepness of the oxygen gradient (50 to 100% of air saturation mm^{-1}) and the gliding speed of the *Beggiatoa* spp. (about 3 $\mu m\ s^{-1}$) that these experienced an increase in oxygen tension of 10 to 20% of air saturation min^{-1} when moving perpendicularly across the anoxic-oxic interface.

Mechanisms of lateral movement. The lateral movements of the filaments due to partial reversals were difficult to explain, and the phenomenon was therefore investigated in detail. The secretion of polysaccharide slime seems to be an integral part of the gliding movement in *Beggiatoa* spp. The slime surrounds the entire filament as a thin sheath, which adheres to solid surfaces on which the bacteria glide. A reversal of the direction of movement over the whole filament must therefore be associated with a reversed longitudinal displacement of the filament relative to the slime sheath. The mechanism of this displacement is still not resolved (19). The gliding movement was always accompanied by a left-handed helical rotation of the filament, unless surrounding objects or bends on the filament prevented this. In the case of partial reversals, physical constraints in terms of flexibility of the bending filament, torsion due to opposed directions of rotational movement, and adhesion strength to solid substrates must all be important for the resulting bows and bends and overall changes in the direction of movement.

To study the slime displacement patterns that created the observed movements, the sheaths were made visible by staining with India ink. India ink consists of black graphite particles that adhere readily to slime sheaths. It has been used also in previous studies of filamentous gliding bacteria (e.g., see reference 11). A suspension of India ink was added to the simple microaquarium and the described filament movements were again observed under the microscope. The behavioral effects of light stimulation on *Beggiatoa* spp. were found to be similar to those of oxygen (cf. reference 22). Light responses were experimentally preferable here, because discrete filament regions could be specifically stimulated. A 500-μm wide, intense light spot formed with the microscope lamp was therefore conveniently used for well-controlled stimulation in these experiments.

The leading 100 to 300 μm of a moving filament was stimulated with a light spot for 20 s. About 35 s after initiation of stimulation, the direction of sheath displacement suddenly changed on an extended part of the filament including the stimulated tip. The sheath stretched, and within a few seconds it ruptured at a point up to 2 mm from the stimulated tip. From the point of rupture the sheath was then displaced in opposite directions along the filament. New ruptures could subsequently appear in the same way at points more and more distant from the leading tip, sometimes ending up with reversal of sheath displacement over the entire filament length after a couple of minutes. Usually, however, the original direction of sheath displacement was resumed or the initial, partial reversal was retained.

A rupture of the slime sheath would often force a lateral bow to arise on the filament, especially when the sheath adhered to a solid surface, e.g., to the glass slide (Fig. 5).

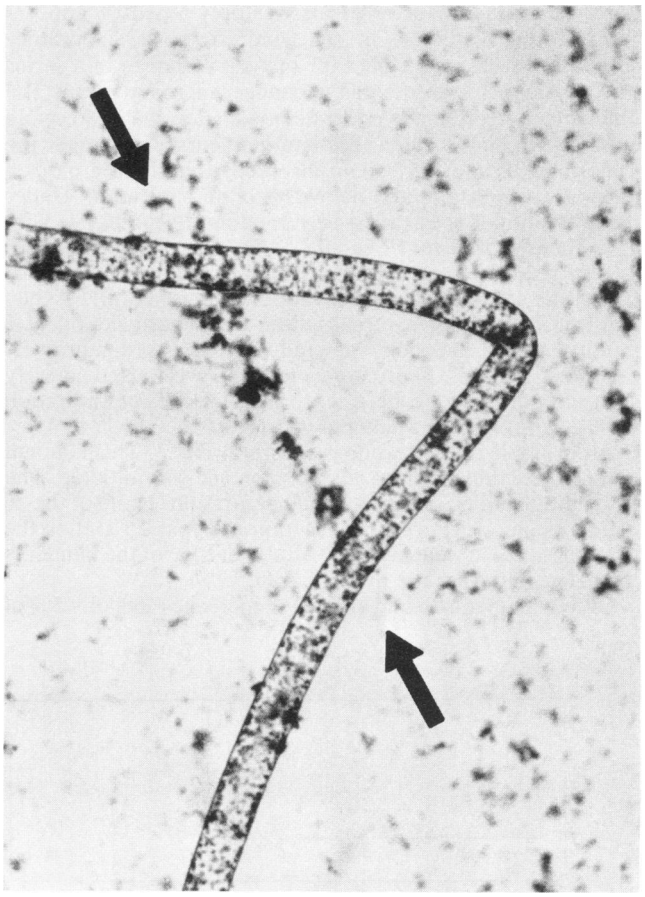

FIG. 6. Photomicrograph showing a 23-μm-wide *Beggiatoa* filament that had just been pressed out from the slime sheath and formed a sharp bend. India ink particles reveal the empty sheath that tied the bend together (arrows). The photomicrograph corresponds to the 60-s insert shown in Fig. 7B.

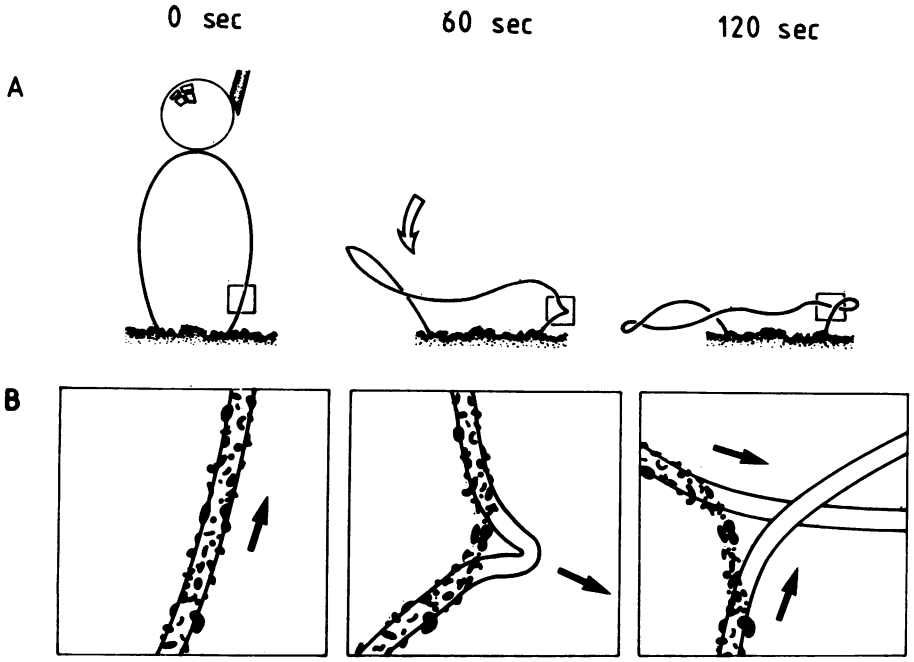

FIG. 7. Time sequence showing the collapse of a filament loop after it had been touched by an air bubble (A). Initially, the loop stretched freely 3 mm up from the sediment. (B) Enlargement of the boxed regions of (A) to show the interaction between filament and slime sheath when a sharp bend was formed. The slime sheath was stained by India ink particles. Arrows indicate the direction of filament movement relative to slime sheath.

Initially, the whole filament moved in one direction (I). Then the right part reversed and the sheath ruptured. A part of it detached from the slide as a bow was formed (II). The bow enlarged (III) until finally the reversed part reversed back again and the filament continued to move unidirectionally, but now through the fixed bow (IV).

Sometimes the rupture of the sheath was incomplete and the two filament parts instead pushed the convergence area out through the side of the sheath in a sharp bend. In the photomicrograph in Fig. 6 such a bend is held together by the stained sheath. As the *Beggiatoa* filaments continued to glide, the sharp bend either was extended into a loop or moved down along the filament, evidently without causing any damage to the cells.

The formation of sharp bends could only occur if the filament part was free to make the lateral movements, for instance, when filaments in a mat formed loops stretching freely out from a sediment surface. An example of a sharp bend formation on a loop is shown in Fig. 7. The water above the mat was stagnant and anoxic, and filaments formed numerous loops stretching up into the water. The tip of a loop was now touched by a small air bubble held at the point of a syringe needle (Fig. 7). After less than 20 s the bubble was removed. After a short lag time the filament suddenly collapsed and simultaneously sharp bends appeared on the filament (Fig. 7B). Twenty-nine filaments with diameters from 20 to 30 μm were tested in this way and 13 of these collapsed. The response time, from onset of stimulation until collapse, was 52 ± 14 s (standard deviation). The actual collapse was very rapid; a few seconds after it began the whole loop was lying flat along the sediment surface.

Responses of whole populations. We tried to evaluate the observed single-filament oxygen responses in relation to the behavior of a whole *Beggiatoa* population growing as a mat on a sediment surface.

Sediment surfaces are, like any surface submerged in water, always covered by a film of unstirred water called the diffusive boundary layer. The exchange of molecules between water and sediment takes place through this layer only by means of molecular diffusion (cf. reference 16). The flux of oxygen to the sediment is therefore influenced by the thickness of the boundary layer, i.e., by the surface structure and the amount of stirring in the bulk water. To study the *Beggiatoa* community under different oxygen conditions, it was therefore sufficient to change the flow velocity of the bulk water. A *Beggiatoa* mat in darkness was exposed to aerated seawater with three degrees of flow velocity: stagnant, moderate, and strong flow. Oxygen measurements were made in combination with microscopic observations of the bacterial population. The results are shown in Fig. 8A to C.

Under stagnant water, slow convective movements were the only stirring in the water. The boundary layer was several millimeters thick and the oxygen flux was therefore very limited (Fig. 8A). The mat was loose and fluffy with numerous filament loops and coils stretching several millimeters above the surface. The whole mat was above the sediment, only supported at a few points, and the structure was characterized by diffuse filament tufts on a layer of horizontally oriented filaments. All available oxygen was consumed by the uppermost loops of *Beggiatoa* filaments that extended into the oxic region. The isopleths of oxygen could oscillate, but oxygen was never detected below 1 mm above the sediment.

Under moderate water flow the boundary layer was only about 0.7 mm thick, and the mat was correspondingly much more compressed (Fig. 8B). The filaments formed only small loops and had many irregular bends. From the studies with the microaquaria, such multiple bends are indicative of frequent, partial reversals of the gliding bacteria. The tufts

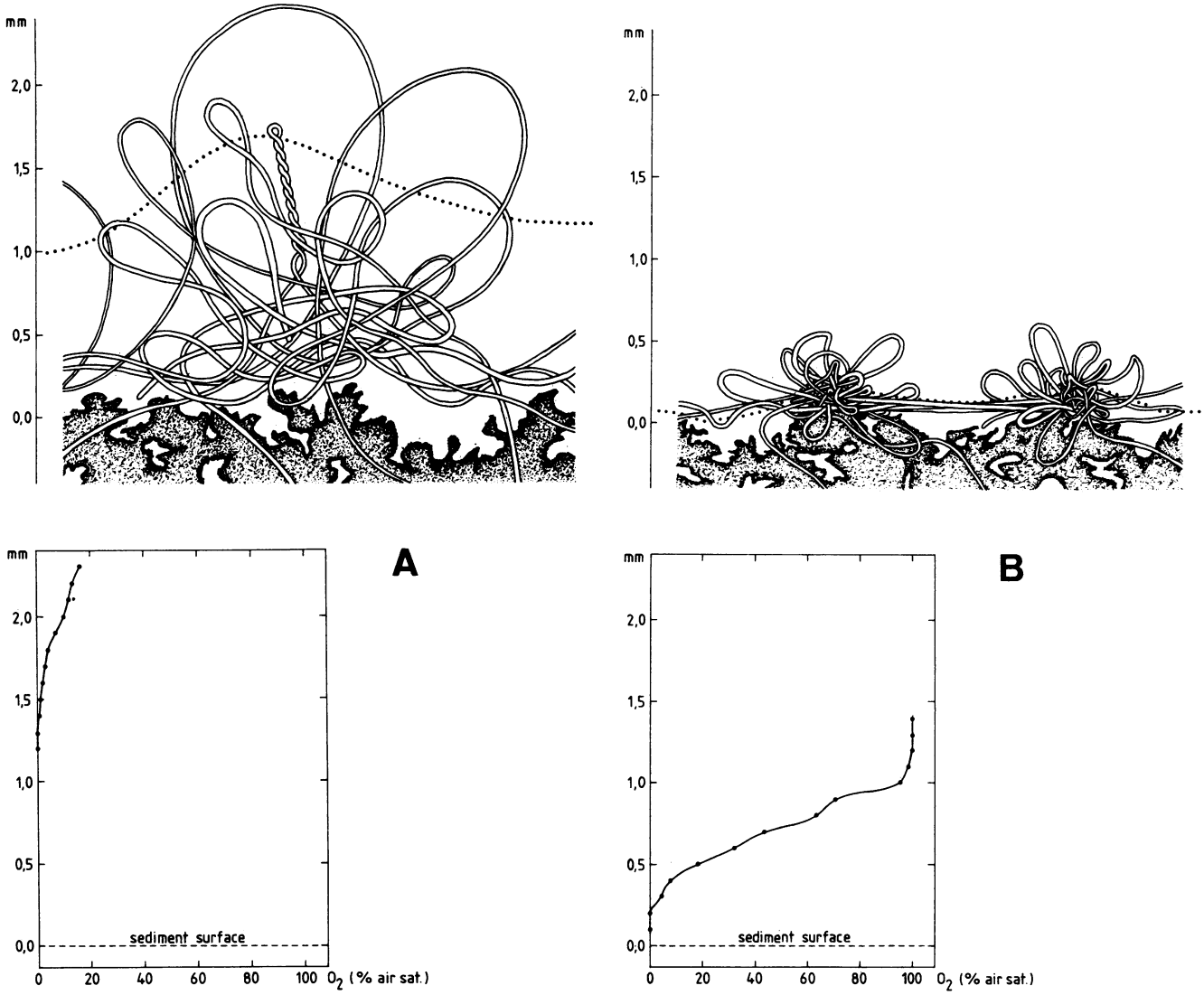

FIG. 8. Structure of a *Beggiatoa* mat on a sediment surface under different experimental conditions. The dotted lines indicate the oxic-anoxic interface. The oxygen gradients and their position relative to the sediment surface are also shown. (A) Darkness, stagnant water; (B) darkness, moderate flow velocity; (C) darkness, high flow velocity; (D) light, moderate flow velocity. The shaded filaments are *Oscillatoria* sp. The light profile is shown below, as well as the O_2 profile and the upper boundary of *Beggiatoa* spp. before and after addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Note differences in O_2 scale.

had very compact centers, and they were interconnected by dense bundles of straight filaments. The tufts were not stable, and at any one time some were developing and some were disintegrating. The tufts were even pulled around by the straight filament bundles.

Under high flow velocity of water the mat had a very smooth surface, from which only scattered small loops protruded (Fig. 8C). The filaments formed a dense, smooth layer at the very surface of the sediment and oxygen penetrated 30 to 40 μm down into the mat before it was consumed. The unstirred boundary layer was compressed to one-fourth of its extension under medium flow.

Tight coiling of a filament (Fig. 8A and C) was commonly observed. A coil occurred when a rotating filament reversed partially. The reversed part would also reverse the direction of rotation relative to the other part, whereby a torsion would build up between the two parts. As they simultaneously moved against each other the torsion could eventually be released in the formation of the observed left-handed coil. The formation of coils has previously been described in filamentous cyanobacteria (9).

When a *Beggiatoa* mat from stagnant water was suddenly exposed to higher oxygen tension by an increase in the water flow, the fluffy mat soon collapsed as many widely extended loops suddenly bent down in a manner similar to that shown in Fig. 7. Multiple bends and bows appeared on the filaments, and the tufts contracted, forming dense clumps with only short loops protruding. These clumps gradually flattened out and the mat finally reached an appearance such as that seen in Fig. 8C.

The observed differences in structure in Fig. 8A to C were the results of a change in oxygen conditions and were not due to changes in water flow. This was demonstrated by exposing the mat to a constant, medium flow velocity of

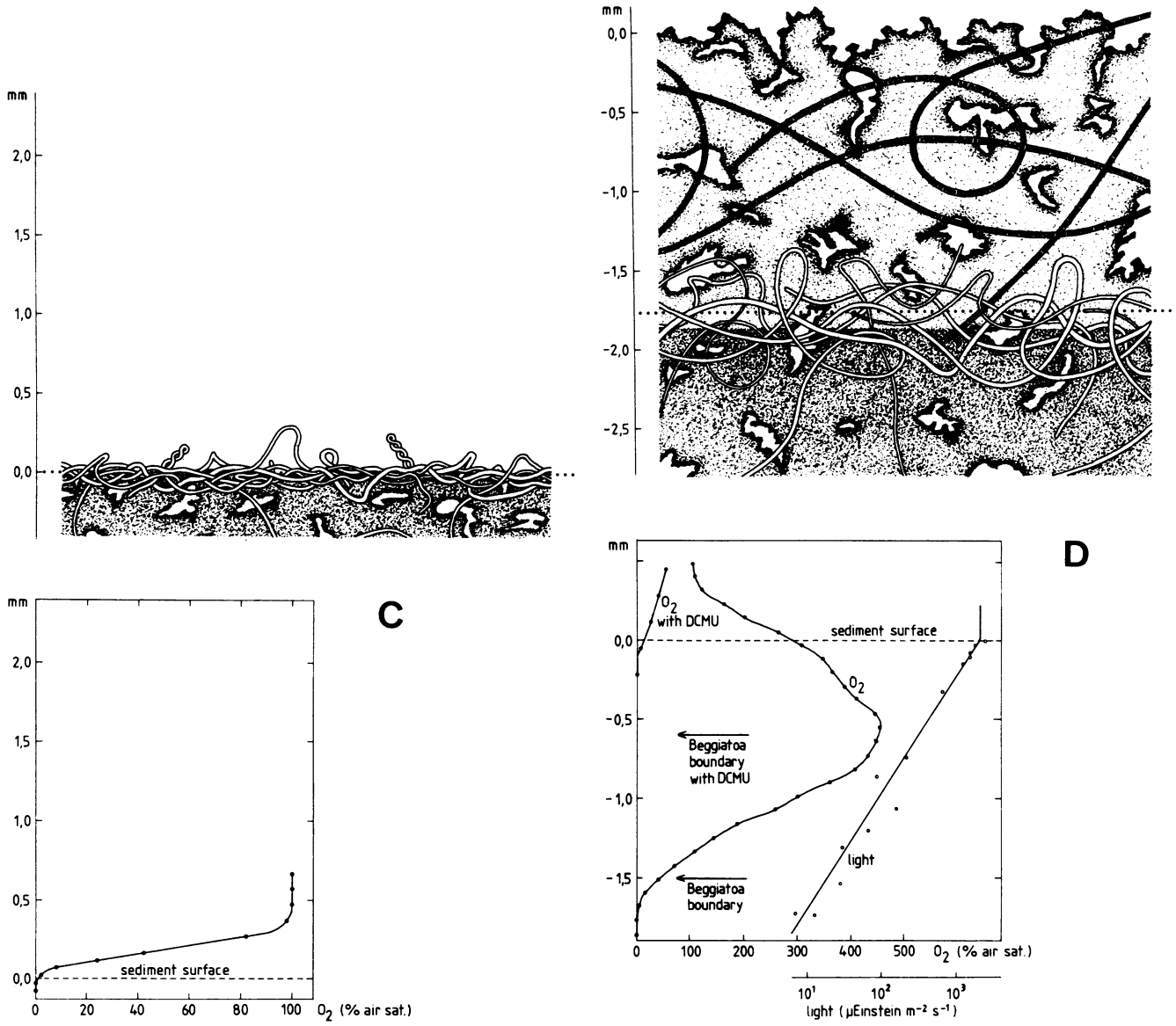

FIG. 8—*Continued*

water while varying the oxygen concentrations. Under a flow of oxygen-free water the mat developed the same loose structure as in stagnant water (Fig. 8A), whereas under oxygen-saturated water (500% of air saturation) all filaments migrated down into the sediment to a depth where oxygen approached zero (not shown).

When the sediment was exposed to light, *Oscillatoria* sp. and other phototrophic microorganisms created an oxygen maximum below the sediment surface (Fig. 8D). In that situation, *Beggiatoa* filaments migrated down and concentrated at the oxic-anoxic interface, 1.8 mm below the sediment surface. The restriction of the filaments toward the upper, oxic side was sharp and the uppermost filaments appeared irregular with small bows and bends, indicating frequent reversals. The restriction of *Beggiatoa* spp. towards deeper layers was more diffuse.

Phobic responses to either light or oxygen or both could in theory be responsible for the burial of *Beggiatoa* spp. and for the sharp upwards limitation of filaments in the photosynthetically active sediment (Fig. 8D). To check this, we added a specific inhibitor of oxygenic photosynthesis, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, to a final concentration of 10 μM in the water. The photosynthetic oxygen maximum thereby disappeared within a few minutes and the oxic-anoxic interface moved up to 0.1 mm below the sediment surface. The *Beggiatoa* layer also moved to a new stable position 0.9 mm above the former. This clearly indicated that the former position was in fact regulated by oxygen and not by light, which was still unchanged. The new position was 0.8 mm below the sediment surface and thus clearly below the oxic-anoxic interface. This indicated that the position was now regulated by light and not by oxygen. Thus, both

light and oxygen may regulate the upper limitation of *Beggiatoa* spp. depending on which of the two provides the most effective stimulation.

DISCUSSION

Induction of oxygen responses. The results on *Beggiatoa* behavior during temporal variations of O_2 showed a reversal in the direction of movement elicited by an increasing oxygen level, i.e., a step-up phobic response to oxygen (22). The observed response could, however, in theory also be a secondary response elicited by some other chemical gradient in the environment. Oxygen reacts chemically and biologically with many compounds, and therefore several chemical gradients are correlated with oxygen gradients in nature. The *Beggiatoa* filaments in the gas exchange microaquarium were experimentally exposed to seawater with oxygen tensions that oscillated within a factor of 10. The filaments usually responded each time the oxygen increased (Fig. 2). If some other external compound elicited this, it must have reacted quickly and reversibly with oxygen. Furthermore, it must have been present at a reasonable concentration and shown significant changes upon changes in oxygen tension, if it had to be sensed by *Beggiatoa* spp. We find it unlikely that such a compound exists in oxic seawater and therefore conclude that oxygen is the environmental factor to which *Beggiatoa* spp. directly responded.

The biochemical nature of the cellular response is not known as it is the first identified chemical response in *Beggiatoa* spp. Oxygen taxis in bacteria, though positive, has been known for some 100 years (2, 7). Much later studies of oxygen taxis in *Escherichia coli* and *Salmonella typhimurium* (27) and in *Euglena gracilis* (20) led to the suggestion that perturbations of the electron transport chain initiated oxygen responses in these organisms. More specifically, Miller and Diehn (20) pointed towards the terminal electron acceptor cytochrome oxidase as the primary receptor. Although *Beggiatoa* spp. possess a negative oxygen taxis, it could possibly have a similar oxygen receptor mechanism as it contains a full complement of cytochromes (4). Perturbations of the electron transport pigments were also suggested to underlie the light response of *Beggiatoa* spp. (22).

We could not find the lower limits of sensitivity in the oxygen response. *Beggiatoa* spp. responded well to oxygen tensions even below 5% of air saturation and to low O_2 increase rates of 2 to 5% of air saturation min^{-1}. This sensitivity seems quite sufficient for filaments, which migrate under natural conditions in the *Beggiatoa* mat, to allow them to establish a sharp zonation at the oxic-anoxic interface by simple responses to oxygen. The gliding speed was measured in a large number of specimens and was, on average, 3 $\mu m\ s^{-1}$ at 20°C. Under medium water flow, the oxygen fell from air saturation to zero within a boundary layer of 0.7-mm thickness. The oxygen gradient above the mat was thus 140% of air saturation mm^{-1}. *Beggiatoa* filaments which migrated vertically in this gradient thus experienced a temporal change in O_2 concentration of 25% of air saturation min^{-1}. In our experiments, *Beggiatoa* spp. responded to O_2 change rates 10-fold lower than this. They should still be able to respond to the oxygen gradient when moving at an angle of 6° from the horizontal, or when there was a very low water flow above the mat.

The response times recorded varied from 20 to 50 s or more. At the longer response times, however, the *Beggiatoa* filaments had frequently shown a slight vibration of the filament before reversal. Staining with India ink revealed that the vibrations were accompanied by a reversal of the slime sheath displacement on a part of the filament but without reversal of the direction of movement. We interpret that the reversed region was being dragged or pushed across the underlying substrate against its direction of slime movement. As only reversals relative to the substrate were scored as a response, the longer time intervals are representative of the behavior of the whole organism rather than representative of reactions at the cellular level.

The phototactic behavior of *Beggiatoa* spp. was found to have similar response times of 10 to 40 s (22). These are long response times compared with, e.g., *E. coli* and *S. typhimurium*, which react within less than 5 s to oxygen (27), and *Rhodospirillum rubrum*, which reacts within 1 s to light (5). The response time to light of filamentous cyanobacteria, which have many similarities to *Beggiatoa* spp., is several seconds (24).

Filaments kept at near zero oxygen tension showed spontaneous reversals with a frequency of 10% min^{-1} or less. This is similar to previous findings in *Beggiatoa* spp. of about 5% min^{-1} by Nelson and Castenholz (22) and in filamentous cyanobacteria of about 10% min^{-1} (6).

The relation of *Beggiatoa* spp. to oxygen has been thoroughly investigated but its ecological significance is unknown. The preference of *Beggiatoa* spp. for microoxic environments is well established (8, 18, 21), and pure-culture studies have shown that the growth of *Beggiatoa* spp. can be inhibited by oxygen concentrations near air saturation (3). Catalase released this inhibition to some extent, and it was theorized that this was due to the destruction of toxic peroxides formed under oxic conditions. However, avoidance of high oxygen concentrations may not be the primary ecological role for the negative oxygen response. Probably a more important role is to keep *Beggiatoa* spp. positioned close to the oxic-anoxic interface where sulfide oxidation frequently takes place. The accumulation of *Beggiatoa* spp. exactly at the interface was observed under different situations in both this and previous studies (14, 15). This precise positioning indicates a strong influence of the oxygen response.

The oxygen response of single filaments, which we have described, furthermore seems to provide a sufficient mechanism to explain the principal types of mat structure. At the oxic-anoxic interface the long filaments will frequently make partial reversals as a phobic response to oxygen increase. This induces the formation of many small loops and bows, and the filaments tend to curl up densely just at the interface. This corresponds well with the observed irregular configurations of the uppermost filaments in the mat (Fig. 8B, C, and D). Under stagnant conditions with shallow oxygen gradients, reversals are much less frequent due to anoxic conditions near the sediment surface. Large loops are therefore formed which give the mat a looser structure, since the loops tend to push the filaments apart and raise them above the sediment surface (Fig. 8A).

The peculiar formation of sharp bends was another characteristic response which was seen when a stagnant mat was exposed to oxygen. The functional formation of sharp bends has not been described among gliding bacteria before. To the free loops it represents an effective response to oxygen which is independent of whether the remaining filament parts are stimulated. This response allows large loops to rapidly collapse and create a smooth mat surface.

These simple mechanisms do not explain why the *Beggiatoa* spp. gather at the sediment surface and do not ran-

domly migrate deep down into the anoxic sediment. Additional tactic mechanisms of motility response must be involved to explain this, and one possibility could be a negative sulfide taxis.

We have not been able to uncover the mechanisms which lead to the formation of tufts rather than an even mat.

If *Beggiatoa* spp. live as chemoautotrophs from the oxidation of H_2S with O_2, the large size of the filaments seems to be a disadvantage, because the zone in which H_2S and O_2 coexist in the mat is only about 50 μm. It is not known to what extent *Beggiatoa* spp. can store reducing or oxidizing power in the cells outside this zone where neither H_2S nor O_2 is available, but elemental sulfur may possibly serve such a dual function. The free, intracellular pools of sulfide and oxygen must turn over instantaneously and cannot be transported as such. Two aspects of the behavioral response may, however, help the large filaments to exploit the O_2-H_2S zone efficiently. One is the long response time. If the filament reversed instantaneously and completely when it reached into the oxic zone, only a few cells at the tip would come in contact with oxygen. The response time of over 20 s allows a larger part of the filament to move up into the O_2-H_2S zone before it reverses the direction of movement and glides back. With a normal gliding speed, the *Beggiatoa* spp. will move 50 to 200 μm before reversal. The other aspect is that the response is usually limited to only the leading part of the filament. This may allow also the trailing part to continue its movement in the favorable direction. The result is a tendency of the filament to curl up within the O_2-H_2S zone.

The evolutionary reason for preserving the great length of the filament could very well be the advantage of this during stagnant situations with low oxygen supply to the sediment. Under these conditions where competition for oxygen must be strong in the sediment, *Beggiatoa* spp. reach several millimeters up into the water column and consume all of the available oxygen before it ever reaches the sediment.

In shallow environments, where *Beggiatoa* spp. grow on sediment surfaces exposed to daylight, phobic reactions to both light and oxygen act together. The result may be either enhanced or conflicting stimulations depending on whether light and oxygen have concurrent or opposed gradients. Nelson and Castenholz (22) calculated that 2% of full sunlight was enough to elicit the phototactic response. From our own light measurements we can calculate that this light intensity corresponded to a position of *Beggiatoa* spp. about 1.0 mm below the sediment surface in Fig. 8D. This agrees well with the actually observed position, 0.8 mm below the surface in the 3-(3,4-dichlorophenyl)-1,1-dimethylurea-poisoned sediment, and supports the assumption of light as the dominant factor in this special case. Oxygen normally forced the bacteria deeper into the sediment (Fig. 8D), which shows that the whole community responded to either factor depending on which provided the first or strongest stimulation.

The light response of *Beggiatoa* spp. can have an indispensable role in the diurnal migration of *Beggiatoa* spp. living in photosynthetic mats. Before sunrise, the photosynthetic organisms in the sediment are below *Beggiatoa* spp. Here they start oxygen production when the light intensity becomes sufficiently high in the morning (14). The *Beggiatoa* filaments could therefore easily be trapped on the surface above the developing oxygen maximum if they had to rely on the negative oxygen response alone. But if the impact of the light gradient is greater than the impact of the oxygen gradient, then the filaments may move down even through an oxygen maximum. Below this maximum, the oxygen gradient can guide the filaments further down to the oxic-anoxic interface within the dark sediment.

Further studies, especially on pure cultures of *Beggiatoa* spp., are needed before we understand the complex interactions of their behavioral responses of environmental qualities. In these dynamic responses lies a key to their success as gradient bacteria of the oxygen-sulfide interface.

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