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BIG BACTERIA

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■ **Abstract** A small number of prokaryotic species have a unique physiology or ecology related to their development of unusually large size. The biomass of bacteria varies over more than 10 orders of magnitude, from the 0.2 μm wide nanobacteria to the largest cells of the colorless sulfur bacteria, *Thiomargarita namibiensis*, with a diameter of 750 μm. All bacteria, including those that swim around in the environment, obtain their food molecules by molecular diffusion. Only the fastest and largest swimmers known, *Thiovulum majus*, are able to significantly increase their food supply by motility and by actively creating an advective flow through the entire population. Diffusion limitation generally restricts the maximal size of prokaryotic cells and provides a selective advantage for μm-sized cells at the normally low substrate concentrations in the environment. The largest heterotrophic bacteria, the 80 $\times$ 600 μm large *Epulopiscium* sp. from the gut of tropical fish, are presumably living in a very nutrient-rich medium. Many large bacteria contain numerous inclusions in the cells that reduce the volume of active cytoplasm. The most striking examples of competitive advantage from large cell size are found among the colorless sulfur bacteria that oxidize hydrogen sulfide to sulfate with oxygen or nitrate. The several-cm-long filamentous species can penetrate up through the ca 500-μm-thick diffusive boundary layer and may thereby reach into water containing their electron acceptor, oxygen or nitrate. By their ability to store vast quantities of both nitrate and elemental sulfur in the cells, these bacteria have become independent of the coexistence of their substrates. In fact, a close relative, *T. namibiensis*, can probably respire in the sulfidic mud for several months before again filling up their large vacuoles with nitrate.

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THE SCALE OF LIVING ORGANISMS

Within the past decade, several uncultured bacteria were consecutively announced as the largest known prokaryotes: *Epulopiscium fishelsoni* (3), *Beggiatoa* sp. (48), and *T. namibiensis* (83). Over the years, big bacteria have been described as "megabacteria" or "gigantobacteria" or given names such as "*Titanospirillum*" (20, 30). The current holder of the biovolume record, a chain-forming, spherical sulfur bacterium, *T. namibiensis*, was discovered only recently in the sea floor off the coast of Namibia (83). The cells may reach 750 μm diameter, clearly visible to the naked eye. They form chains of cells that, due to their light refracting sulfur globules, shine white on the background of black mud and thus appear as a string of pearls (*Thiomargarita* = sulfur pearl).

Also the rod-shaped heterotrophic bacterium, *Epulopiscium fishelsoni*, found in fish guts may reach a giant size of 80 μm diameter and 600 μm length (3, 10). The largest reported Archaea are probably the extremely thermophilic *Staphylothermus marinus*, which in culture may occasionally have cell diameters up to 15 μm (19). The smallest prokaryotes are found among both the Archaea and the Eubacteria. The disk-shaped cells of the archaea, *Thermodiscus*, have diameters down to 0.2 μm and a disk-thickness of 0.1–0.2 μm (87). Under the collective designation of nanobacteria or ultramicrobacteria, a range of cell forms with diameters down to 0.2–0.3 μm have been found in both natural samples and cultures (92). Altogether, the biovolumes of prokaryotic cells may cover a range of more than 10 orders of magnitude, from <0.01 μm^3 for the smallest prokaryotic cells to 200,000,000 μm^3 for the largest.

When all living organisms are considered, from bacteria to whales, the size scale is so vast that it is difficult to comprehend. Whereas the smallest prokaryotic cells are at the resolution of the light microscope, 0.2 μm, the blue whales at the other end of the spectrum may grow to 30 m in length, 8 orders of magnitude larger than the nanobacteria. The maximum span in biomass between bacteria and whales is the third power of their difference in size (or somewhat less, because whales are not spherical), i.e., $1{:}10^{22–23}$. This is similar to the volume ratio between humans and the earth.

It is therefore not surprising that the world, as it appears in the microscale of bacteria, is also vastly different from the world that we humans can perceive and from which we have learned to appreciate the physical laws of nature. These are the classical laws of Newton relating mass, force, and time with mass movement and flow, and with properties such as acceleration, inertia, and gravitation. As we go down in scale and into the aqueous microenvironment of bacteria, these

properties lose their significance. Instead, viscosity becomes the strongest force affecting motion, and molecular diffusion the fastest transport. Viscosity affects how bacteria swim through their aqueous environment, and it affects their chemotactic mechanisms for oriented movement in chemical gradients. Molecular diffusion determines the flux of solutes to and from the bacterial cells and thereby sets limits to their size. When cells are small they can more efficiently take up substrate from the surrounding medium and compete for substrates at low concentration. What, then, drives a small number of prokaryotic species to grow to extreme size? What is so special about their biology or their environment that large cell size provides competitive advantage, and do they have common traits that may explain the evolution of gigantism? To answer these questions, we first discuss some fundamental properties of the microscale environment and bacterial behavior and then provide examples of bacterial adaptations related to large cell size.

DIFFUSION AND THE SIZE LIMIT OF PROKARYOTES

Molecules move through water in random directions by molecular diffusion. The time, *t*, required for a mean diffusion distance, *L*, is described by a simple but important equation that holds the secret of diffusion:

$$t = \pi L^2/4D, \tag{1}$$

or rearranged:

$$L = (4Dt/\pi)^{1/2}, \tag{2}$$

where *D* is the diffusion coefficient for a given temperature and specific type of molecule. These equations express that the distance molecules are likely to travel by diffusion increases with the square root of time, not with time itself as in the locomotion of objects and fluids that we generally know from our macroworld. This is a point where our intuition fails: The velocity of movement depends on the time over which we observe the movement:

$$\text{Velocity} = L/t = (4D/t\pi)^{1/2}.$$

As an example, oxygen molecules, which typically diffuse 1 mm in an hour, will take a day to diffuse 2 cm and 1000 years to reach 10 meters. Over the μm-scale of normal bacteria, however, they will take only 10^{-3} second. For 1-μm large bacteria, one could hardly envision a transport mechanism that would outrun diffusion within a millisecond. Accordingly, in contrast to the large eukaryotic cells, they have no plumbing system for internal transport, such as a cytoskeleton, actin filaments, or microtubules. In larger cells, hydrodynamic flows that are caused by stochastic thermal fluctuations, Brownian movements, may play a role in the transport of molecules. For diffusion inside bacterial cells, these Brownian accelerations do not seem to be important.

The scale and time of diffusion inside the bacterial cell are critical for its structural and functional organization and set constraints on the regulation of enzymatic

reactions (34). The rapid thermodynamic equilibration excludes intracellular solute gradients. This can be illustrated by the mixing time, t_{mix}, i.e., the time it takes before a molecule observed at some point can be found with equal probability anywhere else within the cell volume: $t_{mix} \cong L^2/D$, where L is now the linear size of the cell. For a bacterium of 1-μm size and a typical diffusion coefficient for small molecules of 10^{-5} cm^2 s^{-1}, the mixing time is about 1 ms. For larger molecules it is about 10 ms. The turnover rate for many intracellular enzymatic reactions is a few hundreds per second and, thus, substrate and product molecules can move through the entire volume of the cell many times within the duration of a single round of catalysis. This rapid diffusional communication may allow the catalytic function of different enzyme molecules in small bacteria to operate in a coupled or even synchronous mode as a dynamic network (86).

The rapid mixing by random Brownian motion in small bacterial cells has the additional consequence that the traffic time (i.e., the theoretical time it takes for any two molecules within a cell to meet each other) is extremely short (58). As an example, the traffic time in a 1-μm size bacterium is: $t_{traffic} = L^3/DR = 1$ s, where L is the diameter of the cell (10^{-4} cm), D is the sum of the diffusion constants of the substrate and enzyme molecules (ca 10^{-6} cm^2 s^{-1}), and R is the sum of their radii (ca 10^{-6} cm). Statistically, in every second, any substrate molecule will have met any enzyme molecule in the bacterial cell. Due to this rapid diffusional mixing, spatial chemical separation and pattern formation are practically impossible within the cytoplasm of normal bacteria in the absence of membrane-bound compartments. The situation is very different in the largest prokaryotic cells such as *Epulopiscium*, with a size of up to 80×600 μm, or *Thiomargarita*, in which the thin layer of peripheral cytoplasm is essentially a two-dimensional film with a thickness of 1–2 μm and an area of up to 2×10^8 μm^2. In a cell of 100-μm diameter, the mixing time is on the order of seconds to minutes, and the traffic time, which grows proportional to the cell volume (L^3), is tens of hours. This may allow chemical and functional microenvironments to exist within the cytoplasm and thus enable kinetic regimes completely different than those in small cells. The consequences of such nonbounded compartmentalization for the physiology and genetic control of large prokaryotic cells are not understood and provide an interesting problem for future research.

The transport of substrate molecules to bacterial cells from their aqueous environment takes place by molecular diffusion. Even under turbulent conditions in the ocean, the viscosity of water dampens fluctuations smaller than a dimension known as the Kolmogorov scale or viscous length, L_v, which is in the order of 1–6 mm, smallest in the most vigorous turbulence (54). Below this dimension, the shear is linear and isotropic, and contrary to intuition, turbulence is not important for the substrate flux to the bacterial cell. Thus, microorganisms of <100-μm size are always surrounded by a diffusion sphere that is not affected by the surrounding turbulence (42).

Because substrate molecules in nature generally occur at very low, often submicromolar concentrations, the substrate uptake and growth of bacteria may be

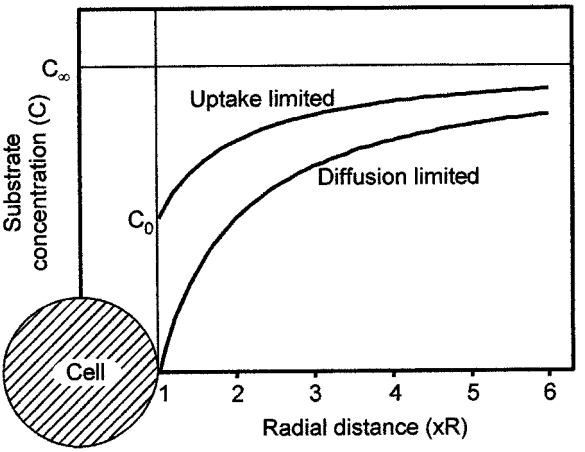


Figure 1 Radial gradients of substrate taken up by a spherical bacterium. (*A*) The cell is uptake limited in its capacity to transport substrate across the cell wall ($C_0 > 0$). (*B*) The cell is diffusion limited as its potential uptake rate exceeds the maximal diffusion supply ($C_0 = 0$). With distance from the cell, the concentrations approach that of the bulk solution (C_∞).

limited by diffusion rather than by physiological constraints. The substrate concentration, C_r, around a spherical cell increases with distance to the cell according to (7):

$$C_r = R/r\,(C_0 - C_\infty) + C_\infty, \quad r > R, \tag{3}$$

where *r* is the radial distance from the center of the spherical bacterium of radius R, and C_∞ and C_0 are the substrate concentrations in the bulk water and at the cell surface (Figure 1). The total diffusion flux, J, to the cell surface is:

$$J = 4\pi DR(C_\infty - C_0). \tag{4}$$

The maximal substrate uptake is reached when the bacterial cell can maintain a substrate concentration near zero at its surface ($C_0 = 0$) and thereby establish the maximal diffusion gradient, $C(r) = R/r(-C_\infty) + C_\infty$, and the maximal flux, $J = 4\pi DRC_\infty$. The maximal specific rate of metabolism from the substrate molecules (i.e., the metabolic rate per biovolume of the diffusion-limited cell) that has a volume of $4/3\,\pi R^3$ is the (44, 46):

$$\text{Specific metabolic rate} = (4\pi DC_\infty R)/(4/3\pi R^3) = (3D/R^2)C_\infty. \tag{5}$$

The relation shows that the biomass-specific metabolic rate of the diffusion-limited cell varies inversely with the square of its size and that smaller size may, thus, efficiently relieve diffusion limitation. Whereas the diffusion supply of substrate increases linearly with the radius of a cell (Equation 4), the specific metabolic rate tends to be lower in larger cells and thus grows with the square of the radius,

i.e., with the surface area of the cell rather than with its biomass. The cell could, therefore, theoretically grow somewhat larger before diffusion limitation would be reached. Many phytoplankton cells have sizes of 5–30 μm and can at low ambient concentrations be diffusion limited in their uptake of nutrients and even of CO_2 (79). Due to rapid diffusional dissipation, heterotrophic bacteria, which release extracellular enzymes to feed on particulate organic matter in their surroundings, have a limited foraging distance of about 10 μm within which they obtain a positive return of released substrate molecules relative to the investment in enzymes (V.A. Vetter, PhD thesis). The advantage of minimal distance to the hydrolyzable particles may be an additional competitive factor favoring small cells at high numbers.

According to Equation 5, a diffusion-limited bacterium could potentially increase its specific rate of metabolism fourfold if the cell diameter were only half as large. Thus, at low substrate concentration, microorganisms may avoid substrate limitation by forming smaller cells, which is generally also observed both in natural environments and in laboratory cultures. The average bacterial size in soils and lake water is around 0.1 μm^3, whereas in the nutrient-poor ocean it is even smaller. In fact, spherical cells have the worst possible shape for efficient substrate uptake. A mechanism to improve the surface-to-volume ratio must be long and thin like the spirillae, develop appendages like the prosthecate bacteria, or even be flat like the square bacteria (44, 72, 95).

The giant bacteria *Epulopiscium fishelsoni* appear to break these rules of diffusion limitation and size. The organisms live in the intestinal tract of fish and may divide once a day in synchrony with the diel feeding cycle of the host. Koch (45) made theoretical calculations of whether these large cells could cover their rate of growth through substrate diffusion. By assuming an extremely high substrate concentration of 0.1% glucose, Koch found that the cells could reach 410 μm diameter before diffusion limitation, i.e., an even larger size than observed. The actual substrate concentration and the physiology of *Epulopiscium* are not known, however, and the large size remains unexplained.

The transport of substrates to the microbial cells may take place not only by passive molecular diffusion but also by advection caused either by the movement of the fluid relative to the microorganism or by their active swimming. The ratio of transport by convection to that by diffusion is expressed by the Péclet number:

$$\mathrm{Pe} = \mathrm{uL/D}, \tag{6}$$

where u is the relative velocity between the organism and the fluid, and L is the characteristic length of the organism (or rather the thickness of its diffusive boundary layer, which for small organisms is of similar magnitude) (42). When the Péclet number is large, $\gg 1$, fluid flow or swimming strongly enhance substrate availability relative to diffusion alone.

The Péclet number for normal motile bacteria is $\ll 1$, which means that the bacteria cannot gain more substrate by swimming around as compared to passively waiting for the substrate to reach them by diffusion (43, 76). With diffusion

coefficients typically around 10^{-5} cm^2 s^{-1} for dissolved organic substrates, organisms must be larger than about 10 μm before they gain more substrate by swimming. As an example, phytoplankton cells must approach a size of 40 μm before they may increase the uptake of nutrients such as nitrate or phosphorus by sinking or swimming (42). These conclusions are independent of the substrate concentration for diffusion-limited cells and indicate that small bacteria do not swim to catch more substrate but to reach environments of higher substrate concentration and a higher energy level of the cell (90). Due to the rapid diffusion at small scale, ordinary bacteria of 1-μm size are surrounded by a substrate-depleted microenvironment that they cannot escape because their "diffusion-halo" follows them as fast as they can swim.

There is a notable exception to this generalization, namely the large unicellular sulfur bacteria, *T. majus*. These cells have a radius, R, of about 8 μm and achieve extremely high swimming speeds of up to 600 μm s^{-1} (15, 25). The bacteria are covered by many flagella, which may help to overcome the high viscous drag due to the large cell. It is not understood, however, how they generate their high swimming speed and whether they have particularly high rotational velocity of the flagella. The individual cells of *Thiovulum* were found to have a mean respiration rate of 2–3 $\times$ 10^{-16} mol O_2 s^{-1} and to be close to diffusion limitation when swarming at the oxic-anoxic interface (41). By swimming, they may theoretically increase their substrate uptake by a factor, G, of (43): $G = 1 + (2\mu R/\pi D)^{1/2} = 1 + (2 \times 600 \times 8/3.14 \times 10^3)^{1/2} = 4$. Although a fourfold-higher substrate availability would appear to be an important selective factor for the evolution of high swimming speed, the adaptation of these amazing bacteria is much more complex, as discussed below.

CHEMOTAXIS

Motile microorganisms have diverse receptors in the cell membrane that detect the ambient concentrations of solutes and cause a response in the direction or speed of movement. As an example, the enteric bacteria, *Escherichia coli*, perform an oriented movement in chemical gradients by a "run-and-tumble" mechanism of chemotaxis, in which cells typically swim at a speed of 15–30 μm s^{-1} and tumble less frequently when moving up an attractant gradient than when moving down the gradient (7). Without an orientation, the bacteria would disperse by random walk with an effective "diffusion coefficient" of ca 0.2 10^{-5} cm^2 s^{-1}, proportional to the square of the swimming speed (55). The chemotactic response adds an oriented drift to the random movement that, however, is only a small percentage of the actual swimming speed.

Many eukaryotic unicellular organisms such as the large ciliates have sufficient size and speed to move in a straight oriented path through the water. Small bacteria with normal swimming speeds are unable to perform directional swimming. They are knocked out of direction by Brownian motions, which cause random accelerations of their rotational movement. The magnitude of this rotational diffusion is

inversely related to cell volume and swimming velocity. Natural populations of aquatic bacteria are commonly only 0.2–0.6 μm long, significantly smaller than the enteric bacteria. They must therefore swim faster than 100 μm s^{-1} in order to overcome the effect of Brownian movement and perform chemotactic movement (59). Swimming speeds of 100–400 μm s^{-1} have been recorded in natural populations of marine bacteria (60). As the power requirement increases with the square of the speed, the metabolic demand of high-speed swimming may consume most of the energy generation of the cell and is therefore dependent on the substrate availability and cellular energy level (60, 90). Through rapid shifts in swimming speed and tumbling frequency, even the smallest bacteria have fast and efficient mechanisms to find nutrient patches and concentrate in zones of optimal nutrient availability (5, 8, 61).

The large *T. majus* is a notable exception to the general mechanisms of bacterial chemotaxis: Their extraordinarily large cell size and high swimming speed allow them to perform directional swimming relative to a chemical gradient. This was shown by video recordings of *Thiovulum* cells swimming in a microscope slide chamber within steep oxygen microgradients (15, 25). Similar to the protozoa, *Thiovulum* rotate and swim in a helical path with 3–10 rotations s^{-1} at a radius of 5–40 μm, a pitch of 40–250 μm, and a tangential velocity of 150–600 μm s^{-1} (Figure 2*A*). The organisms are microaerophilic and distribute around the oxic-anoxic interface with the highest cell densities at 4% air saturation of oxygen (Figure 2*B*). The translational swimming velocity varies with the oxygen concentration. When swimming toward higher oxygen concentration, they increase swimming speed and follow a U-shaped path that effectively brings them back to the preferred oxygen zone within 0.5–2 s (Figure 2*C*). When swimming toward the anoxic zone, the speed is reduced and again the cells tend to follow a U-path. An acceleration or deceleration of the rotational velocity will theoretically lead to a change in swimming direction (12), and this may be the mechanism behind the observed U-turns. As a result of their variation in swimming velocity and the ability to rapidly return to their optimal zone, cell populations of *Thiovulum* are very efficient in aggregating at oxic-anoxic interfaces where they oxidize hydrogen sulfide (see below). The extraordinary efficiency of their chemotactic behavior was demonstrated by the observation that *Thiovulum* in anoxic water were able to chase a swimming phyto-flagellate, *Euglena* sp. (41). Apparently, the bacteria could track the comet's tail of oxygen that the photosynthesizing flagellate left behind.

THE SEDIMENT-WATER INTERFACE

Solid-water interfaces such as the surface of sediments, rocks, macroalgae, or detritus in aquatic environments are sites of intense microbial activity. Energy-rich substrates such as small organic molecules or inorganic species such as H_2S, NH_4^+ or Fe^{2+} exist together with electron acceptors such as O_2 or NO_3^- and may support the energy metabolism of a wide range of heterotrophic or chemoautotrophic

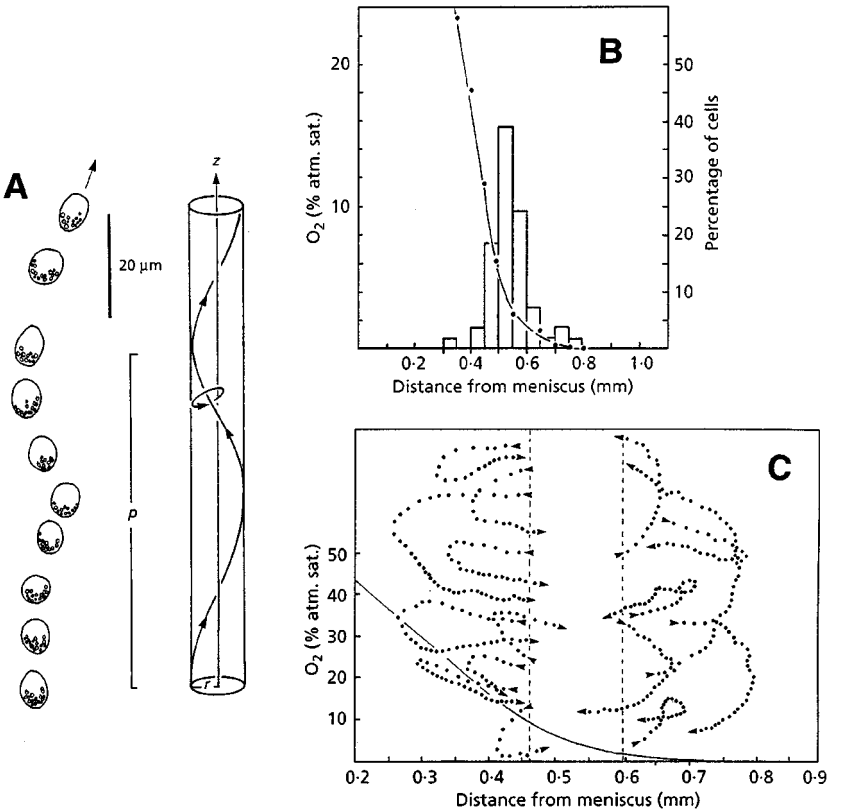


Figure 2 Motility and chemotactic behavior of the colorless sulfur bacteria, *T. majus*. (*A*) A swimming cell recorded by video under the microscope at 40 msec time intervals. The helical swimming path is shown with pitch (*p*), radius (*r*), and tangential and rotational velocity components. (*B*) Distribution of free-swimming *Thiovulum* cells at the oxic-anoxic interface in a microslide preparation. The bacteria aggregated at around 4% air saturation of oxygen. (*C*) Swimming tracks of individual *Thiovulum* cells that entered a zone of superoptimal or suboptimal oxygen concentration and returned by a U-shaped path. Recorded by video in a microslide preparation at 40 msec time intervals. (Reproduced from T. Fenchel, 1994).

bacteria. Due to rapid assimilation by microorganisms, the energy substrates and oxidants are generally counter-diffusing along steep gradients and coexist only within a narrow zone. The analysis of the specific bacterial populations and their metabolic activity within these steep diffusion gradients requires high-resolution techniques such as the combination of fluorescent in situ hybridization and chemical microsensors (77, 82).

Typically, a resolution of 50–100 μm is required to adequately describe the relevant chemical gradients of bacterial microenvironments, e.g., at a sediment

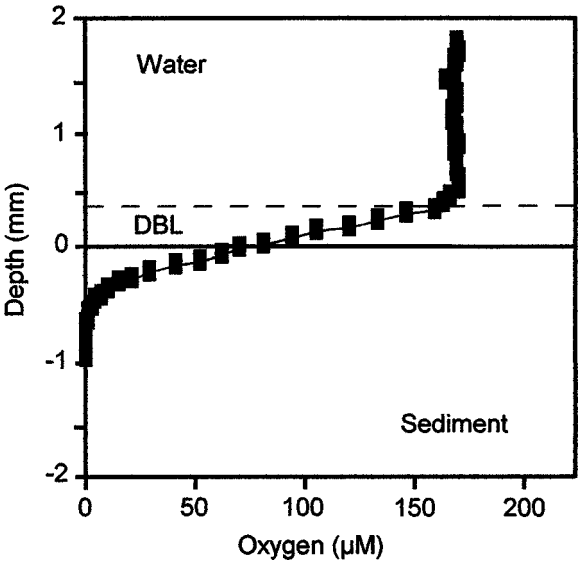


Figure 3 Oxygen microprofile at the sediment-water interface showing the vertical gradient in the diffusive boundary layer (DBL). The profile was measured directly on the seabed at 16 m water depth in the Baltic Sea. During the summer, the oxygen concentration in the overlying water was only ca 70% of air saturation and the oxygen uptake of the sediment was very high, 40 mmol m^{-2} d^{-1}. (Unpublished data of J.K. Gundersen and B.B. Jørgensen).

surface or in a biofilm (78). In the ca. 0.5 mm thick water film adjacent to the non-porous sediment surface, turbulent mixing is strongly impeded by water viscosity so that molecular diffusion over this small scale dominates over eddy diffusion (31). As a consequence, the steep oxygen gradient caused by intensive microbial respiration in the surface sediment is extended up into the free water phase and reveals the existence of a diffusive boundary layer (DBL) (Figure 3).

Diffusive boundary layers coat all solid surfaces in the aquatic environment and play an important role in the exchange of dissolved ions, gases, and organic molecules (38). The one-dimensional diffusive flux, J, through the DBL can be calculated from:

$$J = D(C_\infty - C_0)/\delta_{eff.}, \quad (7)$$

where C_∞ and C_0 are the oxygen concentrations in the bulk water and at the sediment surface, respectively, and $\delta_{eff.}$ is the effective thickness of the diffusive boundary layer (39). For a sediment with very high oxygen respiration capacity, the diffusive boundary layer is a true barrier that limits the diffusion flux and thereby regulates the respiration rate of the entire microbial community (41). With a typical diffusive boundary layer thickness of 500 μm ($=0.05$ cm) and

an O_2 concentration in the overflowing water of 250 μM ($= 0.25\ 10^{-6}$ mol cm^{-3}), the theoretical maximum of oxygen flux to the surface is reached when the O_2 concentration at the surface is zero:

$$J = 2\ 10^{-5}\ 0.25\ 10^{-6}/0.05 = 1 \times 10^{-10}\ \text{mol cm}^{-2}\ \text{s}^{-1} = 85\ \text{mmol m}^{-2}\ \text{d}^{-1}. \quad (8)$$

An active biofilm, such as a *Beggiatoa* mat on a sulfidic sediment surface, may indeed approach this limit. The respiration rate of both the individual organism and the entire community is then restricted by boundary layer diffusion. Several of the largest sulfide oxidizing bacteria have developed elegant means to overcome this transport barrier.

The mean diffusion time of oxygen or other small molecules through the DBL is several minutes. The DBL thus provides not only a chemical barrier but also a time delay relative to the changing chemistry in the water phase. Some of the filamentous bacteria are able to stretch up from the substrate and into the water above. Many small sessile organisms, such as the colonial stalked ciliates, *Vorticella* and *Zoothamnium*, have developed special structures to rise above the surface on which they are attached and thereby to penetrate up through the DBL and into the water flow where oxygen and food particles are more abundant. *Zoothamnium niveum* growing on highly sulfidic mangrove peat in the Carribbean was found to be overgrown by chemoautotrophic sulfide oxidizing bacteria (69). At 5–30 s intervals, the colonies contract their stalks measuring up to 15 mm in height when expanded and less than 1 mm when contracted. The extremely rapid contraction at Reynolds number R = 2500 presumably exchanges the water adhering to the surface of the symbiotic cells, whereas the slow expansion at R = 30–100 may allow sulfidic water from the boundary layer to be carried up into the oxic water and thus provide the bacteria with both sulfide and oxygen. The small bacteria thus use the ciliate as a vehicle to move rapidly back and forth between their substrates and to gain access to the free-flowing water without being flushed away.

BIG BACTERIA

The size range and biovolumes of prokaryotic organisms are shown by some selected examples in Figure 4 and Table 1. For the large cells, the maximum sizes are quoted, whereas for the smallest cells, minima are given to stress the full size range. In some cases, the large cell sizes quoted are occasional extremes relative to the mean size of the whole population. In some pure cultures, large cells may develop in particularly rich media. For example, the thermophilic archaea, *S. marinus*, are normally 0.5–1 μm wide but may form giant cells up to 15 μm in diameter at high yeast extract concentration (19). For *T. namibiensis*, the mean cell diameter is 150–200 μm and only occasionally cells of up to 750 μm are found (Figure 4*A*). Furthermore, the cells are mostly comprised of a large liquid vacuole and only 2% of the biovolume (i.e., up to 4,000,000 μm^3) consists of active cytoplasm. Similarly, *Epulopiscium* spp. are commonly 10–20 μm wide and 70–200 μm long, thus yielding a biovolume of 5,000–50,000 μm^3. The filamentous sulfur

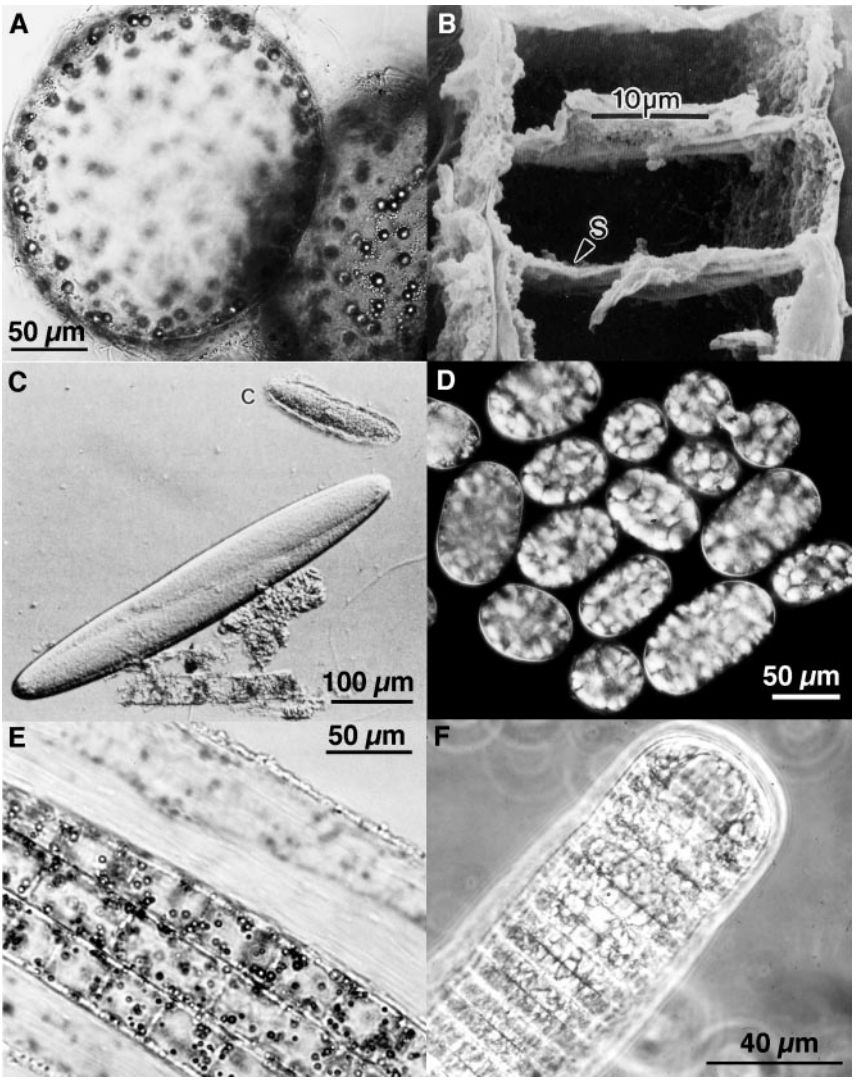


Figure 4 Micrographs of large bacteria. (*A*) The colorless sulfur bacterium *Thiomargarita namibiensis*. At the periphery of the cell several sulfur inclusions are visible, whereas the inner part of the cell appears hollow. (*B*) Scanning electron micrograph of a broken *Beggiatoa* filament revealing the hollow nature of the cell. (Reproduced from J.M. Larkin and M.C. Henk, 1996). (*C*) A gut symbiont of surgeonfishes, *Epulopiscium fishelsoni*, next to a much smaller ciliate. (Reproduced from K.D. Clements and S. Bullivant, 1991). (*D*) The sulfur bacterium *Achromatium oxaliferum* containing many large inclusions of calcite. (Reproduced from H.D. Babenzien). (*E*) Light micrograph of the sulfur bacterium *Thioploca araucae*, showing three trichomes with sulfur inclusions within a common sheath. (*F*) Micrograph of a large, marine *Oscillatoria* (cyanobacterium) isolated from intertidal mats. (Reproduced from C. Castenholz).

TABLE 1 Size scale of prokaryotic cells, from the largest to the smallest

Organism	Characteristics	Size[a] (μm)	Biovolume (μm)	Reference
Thiomargarita namibiensis	Spherical sulfur bact.	750	200,000,000	83
Epulopiscium fishelsoni	Heterotrophic gut bact.	80×600	3,000,000	10
Beggiatoa spp.	Filamentous sulfur bact.	160×50[b]	1,000,000	66
Achromatium oxaliferum	Ellipsoid sulfur bact.	35×95	80,000	33
Thioploca araucae	Filamentous sulfur bact.	43×30[b]	40,000	85
Lyngbya majuscula	Filamentous cyanobact.	80×8[b]	40,000	13
Prochloron sp.	Phototrophic bact.	30	14,000	11a
Macromonas mobilis	Rod-shaped sulfur bact.	14×30	3,500	86a
Thiovulum majus	Spherical sulfur bact.	18	3,000	97
Staphylothermus marinus	Archaea	15	1800	19
Titanospirillum velox	Rod-shaped sulfur bact.	5×30	600	30
Magnetobacterium bavaricum	Magnetotactic bact.	2×10	30	85a
Escherichia coli	Heterotrophic bact.	1×2	2	68a
Mycoplasma pneumoniae	Pathogenic bact.	0.2	0.005	34a
Thermodiscus sp.	Archaea	0.2×0.08	0.003	87

[a]Where only one number is given this is the diameter of spherical cells.

[b]Multicellular filaments of one to several cm length; the height of a single disk-shaped cell is indicated.

bacteria and cyanobacteria cover a wide range of diameters. Thus, freshwater and marine strains of *Beggiatoa* comprise filament widths from <1 μm and up to 200 μm (Figure 4*B*), whereas cyanobacteria may reach 80 μm diameter (Figure 4*F*) (13, 48, 88).

It is striking that many of the particularly large prokaryotes are either cyanobacteria or sulfide oxidizers, the latter being easily recognizable from their sulfur inclusions. There are many morphological similarities between filamentous cyanobacteria and sulfur bacteria, which has in the past led to the assumption that large sulfur bacteria might be apochlorotic cyanobacteria (52). According to 16S rDNA sequences, however, there is no close phylogenetic relationship between the two

groups (1, 47, 74, 91). The observed similarities in morphology and behavior between cyanobacteria and sulfide oxidizers (e.g., between *Oscillatoria* and *Beggiatoa* or between sheath-building bundles of *Microcoleus* and *Thioploca*) evolved rather independently as adaptations to a life as gliding benthic bacteria in steep environmental gradients.

For the large Epulopiscidae, for cyanobacteria and for several other megabacteria, the selective advantage of large size is not clear. The giant cells of the uncultured genus, *Epulopiscium*, have a unique intracellular structure and reproductive biology (Figure 4*C*). These symbiotic bacteria have been found in the guts of herbivorous surgeonfish from the Red Sea and the Great Barrier Reef of Australia. They were originally placed in the kingdom Protista (20), but 16S rRNA sequence analysis has now placed them among the bacteria, notably the anaerobic, gram-positive, spore-forming clostridia (3, 10). The cells contain large amounts of DNA that forms a mesh of numerous nucleoids along the periphery. They have an unusual cortex, which in electron micrographs appears to consist of vesicles, capsules, and tubules, structures better known from the protists. Based on the ultrastructural analysis, the vesicles have been suggested to have an excretory function in the removal of waste products. Thus, it may be speculated that the constraints of diffusion limitation owing to the large cell size may be overcome by an intracellular system of transport organelles (80).

Epulopiscium reproduce in a viviparous mode by the formation inside the parent cell of one or two vegetative daughter cells (63, 80). Ultimately, the parent cell ruptures destructively and extrudes the active daughter cells. This unusual reproduction may hypothetically have developed from the spore formation in clostridia. A phylogenetically related bacterium, *Metabacterium polyspora*, which is an intestinal symbiont of rodents and also grows to unusual size, forms two or more refractile endospores per cell and may point toward the evolution of the *Epulopiscium* reproduction (2). It is not clear for any of these organisms whether the large cell size is related to their special mode of reproduction, or whether its selective advantage may be the protection from protozoan predation (cf. Figure 4*C*) or something completely different. For the time being, they remain the most enigmatic group of gigantobacteria.

Many of the large bacteria harbor massive cell inclusions of known or unknown function that reduce the volume of metabolically active cytoplasm and, possibly, the diffusion limitation. The colorless sulfur bacteria, *Achromatium oxaliferum*, which occur widespread in limnic and brackish sediments, may be the largest free-living single-celled prokaryotes. They have highly variable sizes with biovolumes of $<$1,000 to 80,000 μm^3 and are unique among the prokaryotes in that they store calcium carbonate in the cell (4, 33). Dense spherical inclusions of calcite and elemental sulfur make the bacteria easily recognizable in microscopic preparations (Figure 4*D*). The calcite takes up a large portion of the total cell volume and provides the organisms with such a high density that they may be purified by gentle swirling of a sediment suspension in a petri dish. Their physiology and in particular the role of the calcium carbonate inclusions are not yet clear. Among

the proposed functions are the neutralization of acidity developed by sulfur oxidation, the increase of the internal CO_2 partial pressure to facilitate autotrophic growth, or the regulation of cell buoyancy. The bacteria oxidize sulfide to sulfate via internally stored sulfur globules and appear to play a role for sulfide oxidation in those lake sediments where they occur in high biomass (29). Phylogenetically, the strains of *Achromatium* constitute a separate cluster of 16S rRNA sequences among the sulfide oxidizing γ-Proteobacteria, which also include *Chromatium* spp. and *Beggiatoa* spp., for example.

Multicellular bacteria integrate the individual cells into a structure or an organism of much larger size. The large filamentous sulfur bacteria may consist of a row of hundreds to a thousand disk-shaped cells and reach a length of up to 7 cm (35). Some many-celled magnetotactic bacteria have fixed structures with intercellular connections between 10–30 cells and flagella oriented toward the outside. They are characterized as multicellular organisms (81). Other prokaryotes grow in obligate or facultative syntrophic consortia consisting of tens to hundreds of cells. Examples are the structured and uniform aggregates recently found in deep-sea sediments associated with gas hydrates, which apparently perform anaerobic methane oxidation and consist of a central colony of about one hundred archaea overgrown by a few hundred sulfate-reducing bacteria (9). The external substrates of these aggregates, sulfate and methane, are present in high and nonlimiting concentration, whereas the diffusion-limited substrate transfer takes place internally between the archaea and the sulfate-reducing bacteria. Structured consortia collectively named *Pelochromatium* or *Chlorochromatium* are specialized in phototrophic sulfide oxidation and consist of a dozen phototrophic epibionts attached to a motile, colorless central bacterium (22, 71).

ECOLOGICAL NICHES OF BIG BACTERIA

The large sulfide oxidizers comprise a unique group among the big bacteria as they have a number of obvious advantages from their large size that are related to their particular physiology and ecology. Vast amounts of H_2S are produced in aquatic environments, particularly in the seafloor, yet this excellent energy source is often available only within narrow redox-boundaries. Evolution has led to amazing strategies for the exploitation of this energy source, in competition with less specialized microorganisms or with the autocatalytic H_2S oxidation by O_2. Examples of adaptations among the large sulfide oxidizers to the physical and chemical environment at the sediment-water interface are discussed in the following section, starting with the most common life-forms and ending with the most exotic ones.

Holding on in Flowing Water

Many filamentous bacteria that live from dissolved organic matter or from sulfide in flowing water grow with one end of the filament attached to a solid surface. The

sulfur bacteria *Thiothrix* spp. are specialized to such environments where oxygen and sulfide are mixed in a turbulent flow (52). The filaments have diameters of only a few μm but grow to many mm in length. Dense "fouling" of *Thiothrix* can be found on stones, plants, or free-floating aggregates in a broad range of habitats such as sulfidic springs, eutrophic streams, or activated sludge sewage systems. The organisms require a solid substrate for attachment and a well-mixed water flow carrying oxygen and sulfide. In contrast to filamentous sulfur bacteria gliding on surfaces, e.g. *Beggiatoa*, which may be swept away by turbulent water flow (28), the sessile filaments can withstand even strong currents. At the same time, diffusion limitation around the cells is minimal because the water flow erodes the boundary layers and facilitates the substrate flux to the swaying tufts.

The disadvantage of this passive mode of life is that the filaments depend on a continuous flux of substrates from the flowing water and cannot move if the flow or the O_2 and H_2S concentrations change. The attached organisms have several mechanisms to overcome this problem. *Thiothrix* can release swarm cells (gonidia) from the unattached end of the filament. The swarm cells are distributed by the current and will later settle on other solid surfaces where they are capable of a gliding movement. One pole of the swarm cells has fimbriae that can attach either to a surface or to other swarm cells (50, 96). Once attached, the cells grow to form new multicellular filaments and rosettes. Although most of the gonidia may not survive, these swarm cells enable a *Thiothrix* population to survive also in changing environments and to effectively spread to new areas.

The attached *Thiothrix* need to be metabolically flexible and are able to adapt their activity in response to changes in the availability of oxygen or sulfide. A study of large *Thiothrix* filaments from a sulfurous cave in Italy showed that during an intermittent absence of external sulfide, *Thiothrix* still consumed oxygen for the oxidation of internal sulfur, but at only 4% of the sulfide-supported rate (F.P. Van den Ende, PhD thesis). Through the storage of internal sulfur, thick mats of *Thiothrix* may bridge periods of less active venting. In addition to adapting their metabolic rate, *Thiothrix* may also change their type of metabolism and grow autotrophically, heterotrophically, or mixotrophically on different reduced sulfur species (51, 56, 68, 96). In a periodically aerated activated sludge system where *Thiothrix* grows in the suspended flocks, the filaments span anoxic periods by using internally stored sulfur and possibly nitrate as alternative electron acceptors (67).

A particularly elegant solution for sessile filamentous bacteria to overcome their lack of motility is to attach to a living animal that inhabits a suitable environment. Filaments resembling *Thiothrix* have been found to grow on marine oligochetes (14), ostracods (L.P. Nielsen, M.M. Møller, MSc thesis), and on mayfly larvae (49), all of which were living in sulfide-rich environments. Whether these filaments are only commensals or whether they also protect their host against H_2S has yet to be clarified. A most conspicuous association of animals with attached filaments is found around hydrothermal vents of the Mid-Atlantic Ridge. Dense swarms of the eyeless shrimp, *Rimicaris exoculata*, aggregate around the vents, where the hot sulfidic vent water mixes with cold oxygenated seawater (93). The mouthpart and

the inner carapace of these shrimps is covered with filamentous sulfur-containing bacteria that, however, are phylogenetically only distantly related to *Thiothrix* (73). The diet of the shrimps seems to be a mixture of free-living sulfur oxidizing bacteria and its own epibionts (27, 75). Thus, at least this association seems to be of mutual benefit for both animals and attached filamentous bacteria.

Life in One-Dimensional Opposed Diffusion Gradients

Motile filaments such as *Beggiatoa* spp. can actively seek out microenvironments where they find an optimal nutrient supply. Where opposed gradients of oxygen and sulfide are established on the surface of a sediment or on decomposing plant material, *Beggiatoa* filaments grow as dense mats and give the surface a shining white appearence. Such communities can be found in coastal marine environments where sulfide is produced by sulfate reduction within the sediment, while oxygen diffuses down from the overlying water. Studies on natural mats of *Beggiatoa* (41) as well as on laboratory cultures of autotrophic marine strains (65) have demonstrated the ability of *Beggiatoa* to accumulate and grow at O_2-H_2S interfaces. The bacteria live from the oxidation of H_2S with O_2 and need a high supply of both, yet they tolerate only low concentrations, particularly of oxygen as they appear unable to break down peroxide. The solution to this dilemma is their efficient separation and uptake of both substrates that meet and overlap only within the thin *Beggiatoa* mat. As a result of their efficiency, the *Beggiatoa* become strongly diffusion limited, and the respiration rate of the entire community is regulated by the steep gradient and molecular diffusion of oxygen through the diffusive boundary layer (41, 65). Although the *Beggiatoa* inhabit only a <1 mm thick zone at the sediment-water interface, they may be responsible for up to 70% of the total oxygen consumption of the sediment (16) and may consume all the sulfide produced (16, 41).

The *Beggiatoa* are several mm to 1 cm long, yet the zone of coexisting oxygen and sulfide is mostly less than 100 μm thick. How do these large filaments manage to accumulate and coil up as a thin film exactly at the O_2-H_2S interface? Møller et al (62) showed that *Beggiatoa* have a phobic response to oxygen concentrations above 5% air saturation. They react with a delay of 20 to 30 seconds by reversing their direction of gliding. Thus, when short filaments glide into an area of higher oxygen concentration, they simply glide back out of this area (Figure 5*A*). For long filaments, however, only the leading part becomes exposed to oxygen before the organism responds. In this case, the reversal of gliding direction is restricted to the exposed front end, while the rest of the filament continues forward. As a result, the middle part is forced out to one side, which just happens to be at the oxic-anoxic interface (Figure 5*B*). Repetition of such partial reversals at other parts of the filaments leads to the formation of further loops (Figure 5*C*). As this happens again and again, the net effect is that the whole long filament ends up very close to the desired O_2-H_2S interface.

Beggiatoa filaments also have a phobic response to light (64). The organisms often live at the sediment surface in shallow water among dense populations of

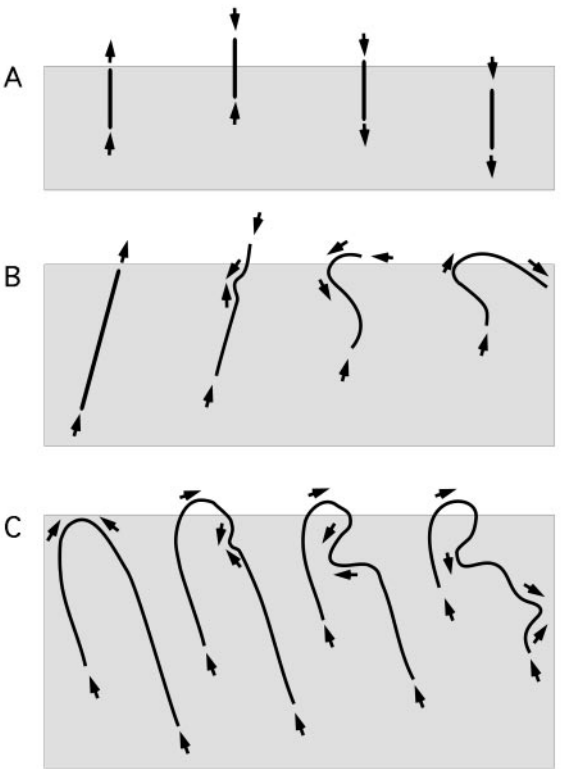


Figure 5 Possible chemotactic response of single *Beggiatoa* filaments gliding into the oxic zone. (*A*) The entire filament reverses the gliding direction. (*B*) The leading part reverses and a bow is created. The end of the filament resumes its original movement but with the leading end pointing into a new direction. (*C*) A short region of a U-shaped filament reverses and a bow is formed. (Reproduced from M.M. Møller, L.P. Nielsen, and B.B. Jørgensen, 1985).

benthic diatoms or cyanobacteria. The intermittent exposure to light may be indirectly harmful for these microaerophilic filaments as it induces an intensive photosynthesis and results in high oxygen concentrations. During the night, the interface between oxygen and sulfide is often found at or even above the sediment surface due to intensive respiration. *Beggiatoa* follow the oxygen to the surface and may be positioned above the phototrophic organisms at the end of the night. With the first morning light, they need to move down below the algae and may have to pass through a zone of starting oxygen production. In this situation, the phobic response to light may override the phobic response toward oxygen and thus enable the filaments to escape down through a rising oxygen peak and deeper

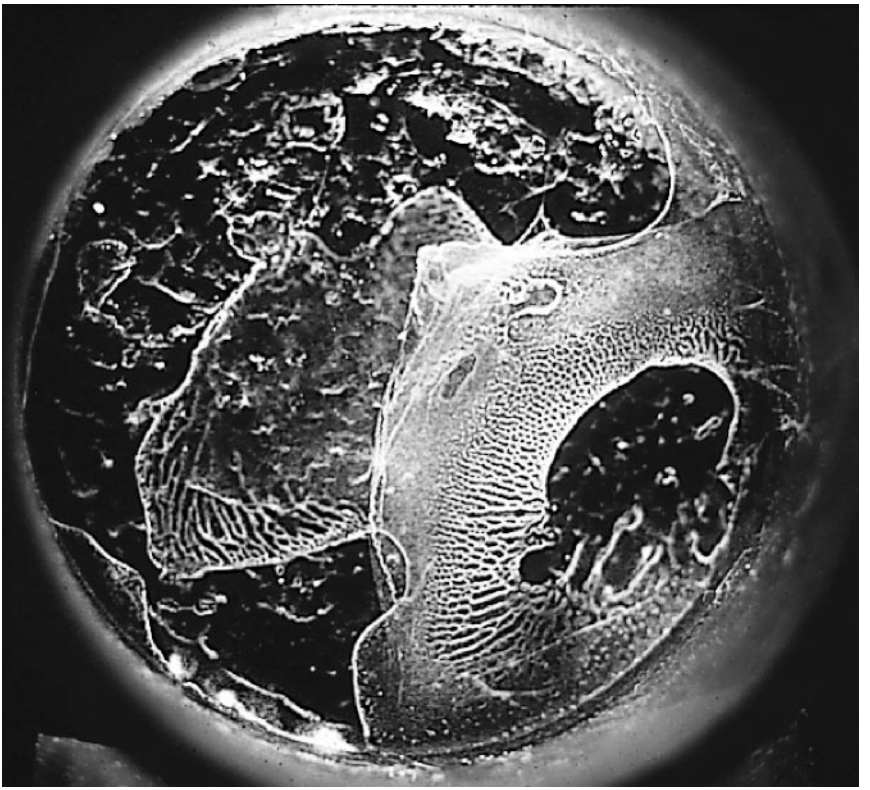


Figure 6 A veil of the colorless sulfur bacteria, *Thiovulum* sp., suspended over and partly covering a sulfidic mud surface. The 4-cm wide core of organic-rich sediment was taken from Limfjorden, Denmark, and kept under oxic seawater for several days. A reticulate pattern and large rounded apertures are seen in the net, which are related to the advective water flow established by the bacterial community. (Photo by B.B. Jørgensen).

into the sediment (26). During the daytime, the O_2-H_2S interface may also be very dynamic so that the filaments on a day with passing clouds may have to travel continuously up and down to track their desired microenvironment (26, 62).

Fast Swimmers and Organized Communities

Another elegant solution to a life as sulfide oxidizer in the benthic diffusive boundary layer is observed in the unicellular sulfur bacteria, *T. majus*. These highly motile, spherical cells form a unique veil that is draped over the sediment surface as an attached or partly floating thin blanket (Figure 6). The bacteria are chemoautotrophs and oxidize H_2S to elemental sulfur and sulfate with oxygen. *Thiovulum*

cells have a diameter of 9–18 μm (97) and are the fastest prokaryotic swimmers known. With their many flagellae, they can obtain swimming velocities of up to 615 μm s^{-1}, an order of magnitude faster than most other bacteria (15, 25). The microaerophilic *Thiovulum* cells swarm to a transition between oxygen and sulfide and there secrete a slime thread. The entire population of *Thiovulum* then spreads out with their slime threads sticking together and forms a fine two-dimensional mesh of 10^5–10^6 cells cm^{-2} (41, 97). The veil effectively separates the flowing oxic seawater above the sediment from a stagnant boundary layer of H_2S-enriched water. A sharp O_2-H_2S interface develops, similar to that of *Beggiatoa*, but with the important difference that the veil creates its own diffusive boundary layer suspended in the water column. By concerted swimming, the bacteria actively move the veil up or down and are thereby even able to control the fluxes of oxygen and sulfide so that they match the stoichiometry of their chemolithotrophic metabolism (41).

The slime threads of *Thiovulum* may also attach to solid surfaces and establish a more stable veil. The bacteria in the veil continue to swim and rotate, but because they are now tethered by their stretched slime threads, up to 100 μm long, they instead create a downward water flow with a velocity of about 200 μm s^{-1} across the veil (17). This flow brings oxic water, and possibly circulating sulfide, to the bacteria and thus increases their potential for oxic respiration beyond the passive diffusive flux across their diffusive boundary layer. The inflow is compensated by more focused outflows through numerous small openings in the veil. As a result, the veil develops fine patterns of alternating dense bacterial films and open spaces, with the appearance of a fine lace (Figure 6).

The role of convective transport across the veil relative to molecular diffusion can be estimated from the Péclet number (Equation 6): $Pe = u\,L/D$, where the flow velocity, u, is 150 μm s^{-1}; the length scale, L, is the thickness of the diffusive boundary layer, typically 500 μm; and the diffusion coefficient, D, of oxygen is 2×10^{-5} cm^2 s^{-1} or 2×10^3 μm^2 s^{-1}. Thus, $Pe = (150 \times 500)/(2 \times 10^3) = ca\,40$, which indicates that convective flow is much more important than molecular diffusion for the transport of oxygen to the attached bacteria (17). This is a unique example of bacteria that develop spatially organized structures in order to generate a convective water transport by their flagellar activity and thereby enhance the availability of their substrate. The mechanism is based on the concerted action of a large population and cannot be achieved by the individual organism. Both the oriented high-speed swimming and the flagella-induced water flow are properties that require a minimum size of the organisms.

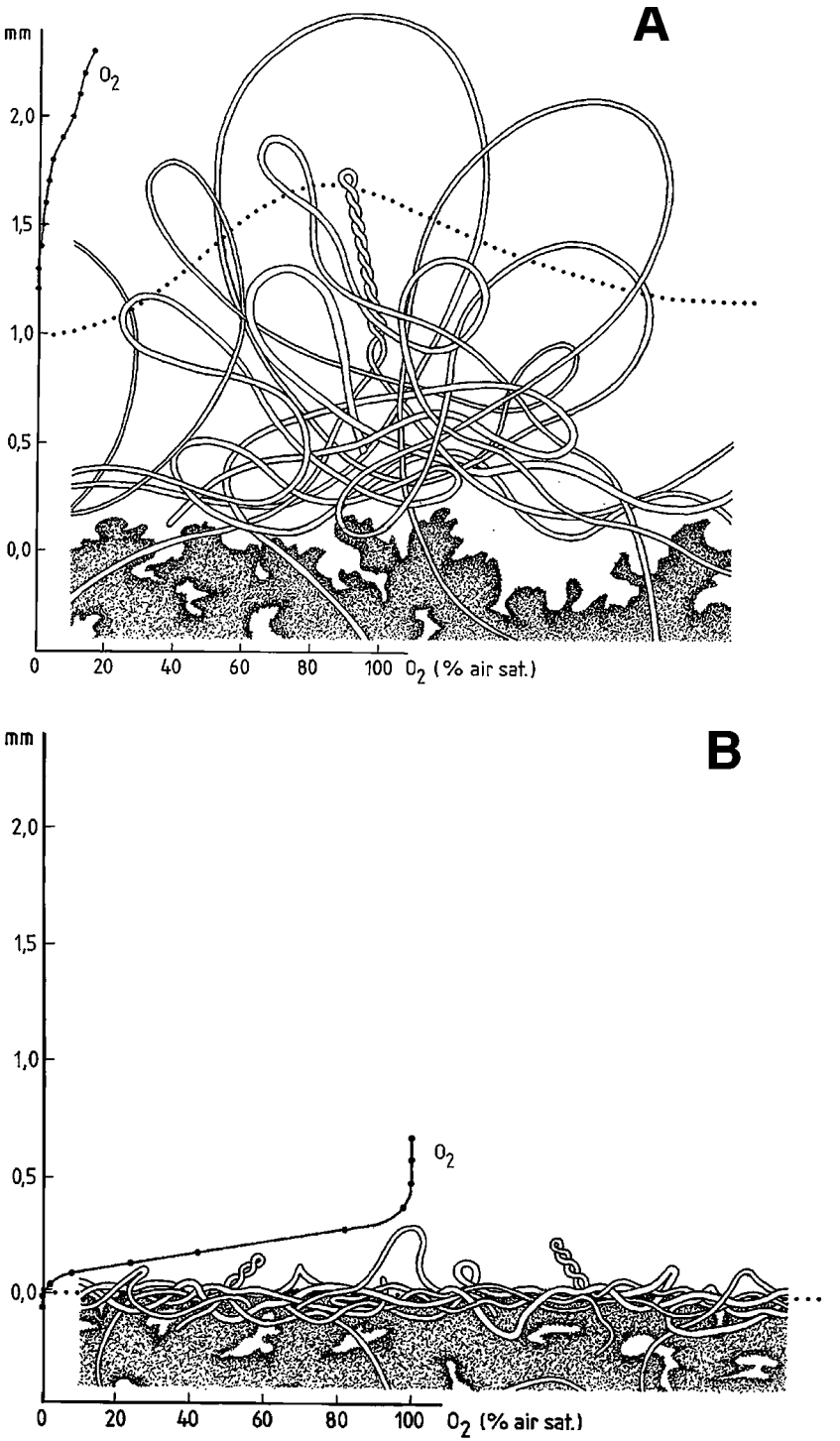
Breaking Through the Diffusive Boundary Layer

The respiration of *Beggiatoa* communities is limited by the oxygen flux through the diffusive boundary layer. If the flow of the overlying water ceases, the oxygen concentration and its gradient tend to drop and the oxygen-sulfide interface

moves up slightly above the sediment surface. The chemotactic mechanism of partial reversals in *Beggiatoa* is thereby suppressed, and long filament ends and loops instead stretch out of the sediment and through the diffusive boundary layer. Thereby, the filaments reach into more oxic water and improve their access to oxygen relative to the rest of the benthic community (Figure 7*A*). Because of their large size and their accumulation at the sediment-water interface, the filaments form a loose mat that can rise a few mm above the sediment. In contrast to attached filaments such as *Thiothrix*, the entire *Beggiatoa* community can rapidly retract from the flowing water if the oxygen concentration should again become high (62). This response is simple, elegant, and efficient. It again relates to the mechanism of partial reversals in individual filaments. If the upper part of a loop gets into contact with high oxygen, only this exposed part of the filament reverses its direction of movement (Figure 8). When filaments glide, they rotate and push the thin slime sheath backward relative to the direction of movement. A reversal of movement is accompanied by a reversal of the rotation and the direction of slime extrusion. Opposed directions of rotation and pushing of the slime sheath build up a torsion that ultimately causes the filament to bend double and break out through the slime sheath at the point where this is pulled in opposite directions. As a consequence, the loop flips down onto the sediment surface or the whole free filament loop coils up (Figure 8). These mechanisms lead to a sudden collapse of the entire loose mat structure and thus to a smooth mat, typical for flowing oxic water (Figure 7*B*). This happens much faster than if the filaments had to escape from the surface by normal gliding and chemotaxis.

A close relative of *Beggiatoa*, *Thioploca* spp. live as bundles of 15–40 μm thick filaments in a common sheath (Figure 4*E*) that may penetrate vertically more than 10 cm down into the sediment (40, 53, 84, 85). The *Thioploca* filaments, up to 7 cm long, are more rigid than *Beggiatoa*, probably as a result of longer cells with less flexible junctions. They do not form interwoven mats, but instead each filament stretches up from the sediment surface, which makes the whole community superficially resemble a lawn of white grass. The *Thioploca* reach up through the diffusive boundary layer and into the flowing water from which they take up nitrate for their respiration (see below). The efficiency of this behavior is demonstrated by the observation that the total areal nitrate uptake of the sediment increases tenfold when the *Thioploca* extend out of their sheaths compared to when they are retracted (35; L.P. Nielsen, personal communication). The *Thioploca* appear to be obligate anaerobes or perhaps microaerophiles, and they normally escape higher oxygen concentrations by gliding down and retreating into their sheaths (35).

This ability of *Beggiatoa* and *Thioploca* to reach up through the ca 0.5 mm thick diffusive boundary layer and thereby to increase the access to their electron acceptor in the overflowing water is clearly related to their large size. Only an organism of many mm to cm length and with sufficient rigidity would be able to withstand the viscous drag of the flowing water. At the same time, the diffusive



boundary layer may function as a protective barrier against exposure to harmfully high oxygen concentrations.

Surviving Anoxia with a Storage Tank of Nitrate

Like all other large sulfur bacteria, *Beggiatoa* can bridge an intermittent absence of sulfide by oxidizing internal sulfur inclusions, as long as oxygen is available. In eutrophic coastal environments typically inhabited by *Beggiatoa* there may, however, be summer periods where the entire bottom water becomes anoxic and where aerobic respiration is thus excluded for days or even months. There is now evidence that during such periods the larger forms of *Beggiatoa* may use nitrate as an alternative electron acceptor for the oxidation of sulfide or internal sulfur, thus bridging times of complete anoxia (Figure 9*B*). A seasonal study on the distribution of *Beggiatoa* in a eutrophic Danish fjord, Limfjorden, showed that 5–23 μm wide *Beggiatoa* filaments frequently occurred down to 4 cm depth in the sediment, i.e., much deeper than the few mm penetration of O_2 (37). These *Beggiatoa* accumulate nitrate internally in liquid vacuoles at up to 100–200 mM concentration (M. Mußmann, H.N. Schulz, B. Strotmann, T. Kjær, L.P. Nielsen, R. Rosselló-Mora, R. Amann, B.B. Jørgensen, manuscript in preparation). Measurements of nitrate profiles through a freshwater mat of *Beggiatoa* using a microelectrode have also indicated an uptake of nitrate by these organisms (89).

Hydrothermal vents, hydrocarbon seeps, methane hydrates, and other systems with high sulfide concentrations are frequently associated with mats of unusually large *Beggiatoa* having 40–200 μm diameter (6, 9, 36, 48, 66) (Figure 4*B*). These filaments were shown to accumulate nitrate in a central vacuole at up to 160 mM concentration (57). The high availability of sulfide from the outflow of pore fluid in these areas may explain the large biomass of mats that can be more than 1 cm thick (36). Yet, it seems unlikely that the mat communities could obtain sufficient electron acceptor from the overlying water only through diffusion. As a possible explanation, it was found that the pulsating hydrothermal emissions from vents in the Gulf of California induced a small-scale convective pore water flow by which oxygenated and nitrate-rich cold seawater penetrated into the *Beggiatoa* mats (32). Through their internal accumulation of nitrate, the large *Beggiatoa* are apparently able to buffer the intermittent pulses by having sufficient storage capacity. Their large size, furthermore, allows the formation of a coarse and strong

Figure 7 Structure of a *Beggiatoa* mat on the sediment surface at low and high flow velocities. (*A*) Under stagnant water conditions, oxygen is consumed 1 mm above the sediment. The *Beggiatoa* filaments form large loops to reach into oxygenated water. (*B*) At high flow velocity in the bottom water, oxygen penetrates down to 0.2 mm above the sediment surface. The *Beggiatoa* filaments coil up as a dense mat directly at the sediment surface. (Reproduced from M.M. Møller, L.P. Nielsen & B.B. Jørgensen, 1985).

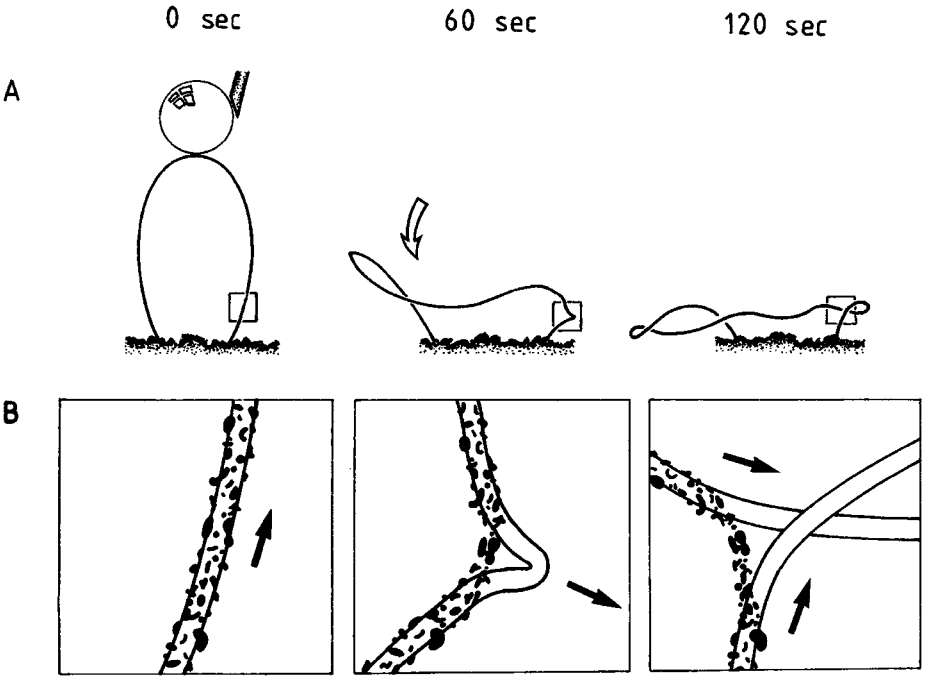


Figure 8 Time sequence showing the response of a filament loop touched by an air bubble. (*A*) After being touched by the air bubble the loop bends at one side and sinks down with parts of the filament coiling up. (*B*) Enlargement of the boxed region to show the interaction between filament and slime sheath when a bend is formed. (Reproduced from M.M. Møller, L.P. Nielsen & B.B. Jørgensen, 1985).

mesh that facilitates the penetration of fluid without sweeping away the entire mat community.

Monopolizing Substrates by Commuting

The marine *Thioploca* (Figure 4*E*) also possess a central vacuole in which they store nitrate at up to 500 mM concentration (21, 40). These bacteria are particularly abundant in shelf sediments along the Chilean and Peruvian coast and may even be the dominant benthic organism in terms of biomass (24). The marine *Thioploca* seem not to use oxygen as an electron acceptor, but only nitrate that they reduce to ammonium (Figure 9*A*; 70). They retreat into their sheaths if bottom water oxygen concentrations are above 10% air saturation, even when nitrate is available (35). Prolonged periods of oxygenated bottom water during winter or during El Niño events reduce the population of *Thioploca* dramatically and, because of their low growth rates, they recover only slowly after such an event (70, 85).

Similar to *Beggiatoa*, each *Thioploca* filament secrets slime while gliding, but instead of leaving a thin slime sheath behind, the sheath material of *Thioploca*

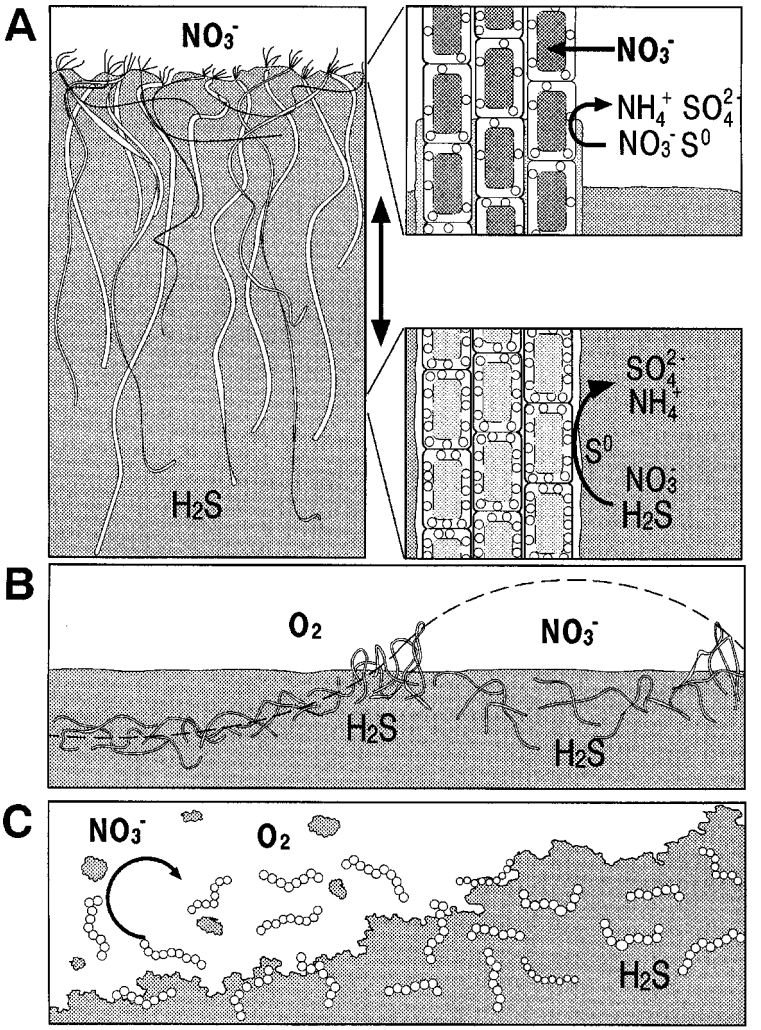


Figure 9 Physiology of nitrate-storing sulfur bacteria. (*A*) *Thioploca* filaments use their vertical sheaths to commute between sediment surface where they take up nitrate and several cm depths where they reduce H_2S and store it as sulfur. (*B*) *Beggiatoa* filaments follow the oxygen-sulfide interface (dashed line) up into the overlying water. During times of anoxia, they may survive by using internally stored nitrate as electron acceptor. (*C*) *Thiomargarita* can only take up nitrate if the loose sediment gets resuspended. During these times they can easily get into contact with oxygenated water, which they tolerate. When the sediment settles down again, sulfide concentrations become very high. *Thiomargarita* bridge these periods by surviving on internally stored nitrate.

accumulates as the trichomes are gliding up and down in the sediment. Thus, simply by accumulating slime the trichomes preserve successful routes through the sediment, which serve as highways and guide the filaments in their chemotactic movement. Unused *Thioploca* sheaths disintegrate rapidly (85). Sediments that are densely populated by *Thioploca* often contain very low or undetectable concentrations of free hydrogen sulfide in spite of high sulfate reduction rates (18). The sulfide is very efficiently oxidized back to sulfate by the bacteria using the nitrate the organisms picked up from the overlying seawater. By commuting up and down in the sediment, the long filaments may continuously perform their chemolithotrophic metabolism. When they stretch up into the overlying water, they fill their nitrate reservoirs. When they are down in the sediment, they oxidize sulfide and store elemental sulfur in the cells as an intermediate energy reserve. This mode of life is so efficient, the *Thioploca* community can keep the sulfide spatially separated from a potential free oxidant such as nitrate or oxygen. Thereby, they monopolize this process and compete effectively with all the "normal" sulfide oxidizers that lack the double storage capacity and instead depend on the coexistence of their substrates (Figure 9*A*).

Although the vertical sheaths are useful in sediments where *Thioploca* can maintain low sulfide concentration, they also force the migration path of the filaments and thus, restrict their chemotactic flexibility. Under high sulfide concentrations, *Thioploca* have been observed to abandon their sheaths and live as freely gliding filaments on the sediment surface (84, 85), thus adapting the life mode of *Beggiatoa*. Another potential disadvantage of living in bundles enclosed by a sheath is that the trichomes compete for sulfide and are limited by the diffusion flux of H_2S from the surrounding sediment. Depending on the local sulfate reduction rates, there seems to be an optimal or maximal diameter of sheaths with a tendency for wider sheaths in sediments of higher sulfate reduction rates (85). The availability of sulfide is probably enhanced due its nearby production by filamentous sulfate reducing-bacteria *Desulfonema* spp., which may grow densely on the outer surface of the sheaths (23).

Waiting for the Electron Acceptor

In contrast to *Beggiatoa* and *Thioploca*, the largest of all known sulfur bacteria *T. namibiensis* is not motile (Figures 4*A* and 9*C*) (83). Thus, like *Beggiatoa*, the cells cannot actively follow the overlapping zone of sulfide with oxygen or nitrate (Figure 9*B*); neither can they shuttle between different horizons of the sediment like *Thioploca* (Figure 9*A*). The sediments inhabited by *Thiomargarita* are a semifluid diatom ooze with very high sulfide concentrations of >10 mM (V. Brüchert, unpublished data). The highest density of *Thiomargarita* is usually found near the sediment surface (83), but living cells containing nitrate can be found down to >10 cm (D. Riechmann, unpublished data). Apparently, *Thiomargarita* cells can only come in contact with nitrate when the loose sediment becomes suspended into the water column (Figure 9*C*). This may happen as a result of storms, by wave pumping, or through methane eruptions, which are known to

occur regularly in this area and are the cause of massive fish kills (11). Extensive methane eruptions are known from the sediments off Walvis Bay and have, in extreme cases, caused a part of the sea floor to rise to the water surface and float around as an island for several hours (94). Also, a less violent ebullition of methane may carry *Thiomargarita* cells high enough up into the water column to get into contact with oxygen. The enormous cell size of *Thiomargarita* is probably an adaptation to bridge long periods without access to an electron acceptor. As *Thiomargarita* consists of 98% vacuole, they have much greater storage capacity for nitrate than *Thioploca* and *Beggiatoa*. If one assumes a rate of nitrate reduction per volume of cytoplasm similar to that of *Thioploca* (70), then an average-sized *Thiomargarita* should be able to respire for at least 40–50 days without taking up new nitrate (83). The observed survival periods of *Thiomargarita* are in fact much longer. Mud samples from Namibia, which were kept in a cold room without addition of nitrate, contained healthy cells after more than two years.

In contrast to *Beggiatoa* and *Thioploca*, *Thiomargarita* are not harmed by high oxygen concentrations but can survive exposure to 100% air-saturated water and may also use oxygen as an electron acceptor for the oxidation of sulfide (H.N. Schulz & D. DeBeer, unpublished data). By measuring radial oxygen microprofiles around single cells of *Thiomargarita*, it was shown that the cells take up oxygen and the uptake of oxygen is greatly enhanced in the presence of sulfide and vice versa. The tolerance toward oxygen might be another adaptation to resuspension up into the water column. It seems likely that, once the highly sulfidic sediments get resuspended, *Thiomargarita* may not only take up nitrate and survive the exposure to oxygen but can also gain energy by using oxygen for oxidizing internal sulfur or sulfide mixed up into the water column. Similar to the sessile *Thiothrix*, *Thiomargarita* thus compensate for the lag of motility by a high physiological flexibility.

SUMMARY

The size of prokaryotic organisms is generally restricted by the limitations of molecular diffusion of substrates from their nutrient-poor environment. Higher substrate concentrations may allow the development of larger cells, yet only a small number of bacterial species show true gigantism. Because none of these has so far been grown in pure culture, we have only limited understanding of their basic properties such as genetic regulation of growth and metabolism, intracellular differentiation and diffusion, or energetics and kinetics of enzymatic activity. The largest heterotrophs, *Epulopiscium* spp., have unique reproduction and cell structures, for which the function and possible relation to cell size remain unknown.

The sulfur bacteria have been studied most extensively, and for these the advantage of large size seems evident. Large filaments may reach out through the diffusive boundary layer, which otherwise limits the food supply of the entire community. Large cells with a nitrate-filled vacuole and a large sulfur reservoir can

respire for long periods in the absence of external resupply. Large cells that can also swim fast may overcome Brownian motions and thereby strongly increase the efficiency of their chemotactic motility.

On the background of these competitive adaptations found in sulfur bacteria, why are there not more physiological types of bacteria that have developed large size to break the thermodynamic limitations of microscale? Hydrogen sulfide appears to be unique in that its oxidation provides a high energy yield and a solid intermediate, elemental sulfur, which can be stored as a readily available energy reserve in the cell. This is not the case for nitrogen or for metal ions, whereas organic carbon is an excellent storage product in the form of poly-glucose or poly-β-hydroxy butyrate. Other energy-rich substrates such as methane cannot be stored as they are membrane-permeable gases. This is also the case for the oxidant, O_2. In contrast, nitrate can be accumulated as an electron acceptor up to 0.5 molar concentration and yields nearly as much energy by redox reactions as does oxygen.

The giants among the prokaryotes were discovered only within the last decade or two, although in hindsight they should have been the easiest organisms to observe. In fact, their large size confused their identification as bacteria. Keeping an open mind toward the potential extremes of prokaryotes, it is likely that we can await many more surprises from the enigmatic world of big bacteria.

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LITERATURE CITED

1. Ahmad A, Barry JP, Nelson DC. 1999. Phylogenetic affinity of a wide, vacuolate, nitrate-accumulating *Beggiatoa* sp. from Monterey Canyon, California, with *Thioploca* spp. *Appl. Environ. Microbiol.* 65:270–77
2. Angert ER, Brooks AE, Pace NR. 1996. Phylogenetic analysis of *Metabacterium polyspora*: clues to the evolutionary origin of daughter cell production in *Epulopiscium* spp., the largest bacteria. *J. Bacteriol.* 178:1451–56
3. Angert ER, Clements KD, Pace NR. 1993. The largest bacterium. *Nature* 362:239–41
4. Babenzien HD. 1991. *Achromatium oxaliferum* and its ecological niche. *Zentralbl. Mikrobiol.* 146:41–49
5. Barbara GM, Mitchell JG. 1996. Formation of 30- to 40-micrometer-thick laminations by high-speed marine bacteria in microbial mats. *Appl. Environ. Microbiol.* 62:3985–90
6. Barry JP, Greene HG, Orange DL, Baxter CH, Robison BH, et al. 1996. Biologic and geologic characteristics of cold seeps

in Monterey Bay, California. *Deep-Sea Res. Part I-Oceanogr. Res. Pap.* 43:1739–62

7. Berg HC. 1983. *Random Walks in Biology*, p. 142. Princeton, NJ: Princeton Univ. Press
8. Blackburn N, Fenchel T. 1999. Influence of bacteria, diffusion and sheer on microscale nutrient patches, and implications for bacterial chemotaxis. *Mar. Ecol. Prog. Ser.* 189:1–7
9. Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, et al. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407:623–26
10. Clements KD, Bullivant S. 1991. An unusual symbiont from the gut of surgeonfishes may be the largest known prokaryote. *J. Bacteriol.* 173:5359–62
11. Copenhagen WJ. 1953. The periodic mortality of fish in the Walvis region; a phenomenon within the Benguela Current. *Invest. Rep. Div. Fish. S. Afr.* 14:8–35

11a. Cox G. 1986. Comparison of *Prochloron* from different hosts. I. Structural and ultrastructural characteristics. *New Phytol.* 104:429–45

12. Crenshaw HC, Edelsteinkeshet L. 1993. Orientation by helical motion. II. Changing direction of the axis of motion. *Bull. Math. Biol.* 55:213–30
13. Demoulin V, Janssen MP. 1981. Relationship between diameter of the filament and cell shape in blue-green algae. *Br. Phycol. J.* 16:55–58
14. Dubilier N. 1986. Association of filamentous epibacteria with *Tubificoides benedii* (Oligochaeta, Annelida). *Mar. Biol.* 92:285–88
15. Fenchel T. 1994. Motility and chemosensory behavior of the sulfur bacterium *Thiovulum majus*. *Microbiology* 140:3109–16
16. Fenchel T, Bernard C. 1995. Mats of colourless sulphur bacteria. I. Major microbial processes. *Mar. Ecol. Prog. Ser.* 128:161–70
17. Fenchel T, Glud RN. 1998. Veil architecture in a sulphide-oxidizing bacterium enhances countercurrent flux. *Nature* 394:367–69
18. Ferdelman TG, Lee C, Pantoja S, Harder J, Bebout BM, et al. 1997. Sulfate reduction and methanogenesis in a Thioploca-dominated sediment off the coast of Chile. *Geochim. Cosmochim. Acta* 61:3065–79
19. Fiala G, Stetter KO, Jannasch HW, Langworthy TA, Madon J. 1986. *Staphylothermus marinus* sp. nov. represents a novel genus of extremely thermophilic submarine heterotrophic archaebacteria growing up to 98°C. *Syst. Appl. Microbiol.* 8:106–13
20. Fishelson L, Montgomery WL, Myrberg AA. 1985. A unique symbiosis in the gut of tropical herbivorous surgeonfish (Acanthurida: Teleostei) from the Red Sea. *Science* 229:49–51
21. Fossing H, Gallardo VA, Jørgensen BB, Huettel M, Nielsen LP, et al. 1995. Concentration and transport of nitrate by the mat-forming sulphur bacterium *Thioploca*. *Nature* 374:713–15
22. Frostl JM, Overmann J. 1998. Physiology and tactic response of the phototrophic consortium *Chlorochromatium aggregatum*. *Arch. Microbiol.* 169:129–35
23. Fukui M, Teske A, Assmus B, Muyzer G, Widdel F. 1999. Physiology, phylogenetic relationships, and ecology of filamentous sulfate-reducing bacteria (genus *Desulfonema*). *Arch. Microbiol.* 172:193–203
24. Gallardo VA. 1977. Large benthic microbial communities in sulphide biota under Peru-Chile subsurface countercurrent. *Nature* 268:331–32
25. Garcia-Pichel F. 1989. Rapid bacterial swimming measured in swarming cells of *Thiovulum majus*. *J. Bacteriol.* 171:3560–63
26. Garcia-Pichel F, Mechling M, Castenholz RW. 1994. Diel migrations of microorganisms within a benthic, hypersaline mat community. *Appl. Environ. Microbiol.* 60:1500–11
27. Gebruk AV, Pimenov NV, Savvichev

AS. 1993. Feeding specialization of bresiliid shrimps in the tag site hydrothermal community. *Mar. Ecol. Prog. Ser.* 98:247–53

28. Grant J, Bathmann UV. 1987. Swept away: Resuspension of bacterial mats regulates benthic-pelagic exchange of sulfur. *Science* 236:1472–74
29. Gray ND, Pickup RW, Jones JG, Head IM. 1997. Ecophysiological evidence that *Achromatium oxaliferum* is responsible for the oxidation of reduced sulfur species to sulfate in a freshwater sediment. *Appl. Environ. Microbiol.* 63:1905–10
30. Guerrero R, Haselton A, Sole M, Wier A, Margulis L. 1999. *Titanospirillum velox*: a huge, speedy, sulfur-storing spirillum from Ebro Delta microbial mats. *Proc. Natl. Acad. Sci. USA* 96:11584–88
31. Gundersen JK, Jørgensen BB. 1990. Microstructure of diffusive boundary layers and the oxygen-uptake of the sea floor. *Nature* 345:604–7
32. Gundersen JK, Jørgensen BB, Larsen E, Jannasch HW. 1992. Mats of giant sulphur bacteria on deep sea sediments due to fluctuating hydrothermal flow. *Nature* 360:454–56
33. Head IM, Gray ND, Babenzien HD, Glöckner FO. 2000. Uncultured giant sulfur bacteria of the genus *Achromatium*. *FEMS Microbiol. Ecol.* 33:171–80
34. Hess B, Mikhailov A. 1995. Microscopic self-organization in living cells. A study of time matching. *J. Theor. Biol.* 176:181–84

34a. Himmelreich R, Hilbert H, Plagens H, Pirkl E, Li BC, Herrmann R. 1996. Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*. *Nucleic Acids Res.* 24:4420–49

35. Huettel M, Forster S, Klöser S, Fossing H. 1996. Vertical migration in the sediment-dwelling sulfur bacteria *Thioploca* spp. in overcoming diffusion limitations. *Appl. Environ. Microbiol.* 62:1863–72
36. Jannasch HW, Nelson DC, Wirsen CO. 1989. Massive natural occurrence of unusually large bacteria (*Beggiatoa* sp.) at a hydrothermal deep–sea vent site. *Nature* 342:834–36
37. Jørgensen BB. 1977. Distribution of colorless sulfur bacteria (*Beggiatoa* spp.) in a coastal marine sediment. *Mar. Biol.* 41:19–28
38. Jørgensen BB. 2000. Microbial life in the diffusive boundary layer. In *The Benthic Boundary Layer: Transport Processes and Biogeochemistry*, ed. BP Boudreau, BB Jørgensen, pp. 348–73. Oxford: Oxford Univ. Press
39. Jørgensen BB, DesMarais DJ. 1990. The diffusive boundary layer of sediments: oxygen microgradients over a microbial mat. *Limnol. Oceanogr.* 35:1343–55
40. Jørgensen BB, Gallardo VA. 1999. *Thioploca* spp: filamentous sulfur bacteria with nitrate vacuoles. *FEMS Microbiol. Ecol.* 28:301–13
41. Jørgensen BB, Revsbech NP. 1983. Colorless sulfur bacteria, *Beggiatoa* spp. and *Thiovulum* spp. in O_2 and H_2S microgradients. *Appl. Environ. Microbiol.* 45:1261–70
42. Karp-Boss L, Boss E, Jumars PA. 1996. Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. In *Oceanography and Marine Biology: An Annual Review*, ed. AD Ansell, RN Gibson, M Barnes, pp. 71–107. London: UCL Press
43. Koch AL. 1971. The adaptive responses of *Escherichia coli* to a feast and famine existence. In *Advances in Microbial Physiology*, ed. AH rose, JF Wilkinson, 6:147–217. London/New York: Academic
44. Koch AL. 1985. The macroeconomics of bacterial growth. In *Bacteria In Their Natural Environments*, ed. MM Fletcher, GD Floodgate, pp. 1–42. London: Academic
45. Koch AL. 1996. What size should a bacterium be? A question of scale. *Annu. Rev. Microbiol.* 50:317–48
46. Koch AL, Wang CH. 1982. How close to the theoretical diffusion limit do

bacterial uptake systems function? *Arch. Microbiol.* 131:36–42
47. Lane DJ, Harrison AP, Stahl D, Pace B, Giovannoni SJ, et al. 1992. Evolutionary relationships among sulfur-oxidizing and iron-oxidizing eubacteria. *J. Bacteriol.* 174:269–78
48. Larkin JM, Henk MC. 1996. Filamentous sulfide-oxidizing bacteria at hydrocarbon seeps of the Gulf of Mexico. *Microsc. Res. Tech.* 33:23–31
49. Larkin JM, Henk MC, Burton SD. 1990. Occurrence of a *Thiothrix* sp. attached to mayfly larvae and presence of parasitic bacteria in the *Thiothrix* sp. *Appl. Environ. Microbiol.* 56:357–61
50. Larkin JM, Nelson R. 1987. Mechanism of attachment of swarm cells of *Thiothrix nivea*. *J. Bacteriol.* 169:5877–79
51. Larkin JM, Shinabarger DL. 1983. Characterization of *Thiothrix nivea*. *Int. J. Syst. Bacteriol.* 33:841–46
52. Larkin JM, Strohl WR. 1983. *Beggiatoa, Thiothrix*, and *Thioploca*. *Annu. Rev. Microbiol.* 37:341–67
53. Lauterborn R. 1907. Eine neue Gattung der Schwefelbakterien (*Thioploca schmidlei* nov. gen. nov. spec.). *Ber. Dtsch. Bot. Ges.* 52:238–42
54. Lazier JRN, Mann KH. 1989. Turbulence and the diffusive layers around small organisms. *Deep-Sea Res. Part I-Oceanogr. Res. Pap.* 36:1721–33
55. Lovely PS, Dahlquist FW. 1975. Statistical measures of bacterial motility and chemotaxis. *J. Theor. Biol.* 50:477–96
56. McGlannan MF, Makemson JC. 1990. HCO_3-fixation by naturally occurring tufts and pure cultures of *Thiothrix nivea*. *Appl. Environ. Microbiol.* 56:730–38
57. McHatton SC, Barry JP, Jannasch HW, Nelson DC. 1996. High nitrate concentrations in vacuolate, autotrophic marine *Beggiatoa* spp. *Appl. Environ. Microbiol.* 62:954–58
58. Mikhailov A, Hess B. 1995. Fluctuations in living cells and intracellular traffic. *J. Theor. Biol.* 176:185–92
59. Mitchell JG. 1991. The influence of cell size on marine bacterial motility and energetics. *Microb. Ecol.* 22:227–38
60. Mitchell JG, Pearson L, Bonazinga A, Dillon S, Khouri H, et al. 1995. Long lag times and high velocities in the motility of natural assemblages of marine bacteria. *Appl. Environ. Microbiol.* 61:877–82
61. Mitchell JG, Pearson L, Dillon S, Kantalis K. 1995. Natural assemblages of marine bacteria exhibiting high speed motility and large accelerations. *Appl. Environ. Microbiol.* 61:4436–40
62. Møller MM, Nielsen LP, Jørgensen BB. 1985. Oxygen responses and mat formation by *Beggiatoa* spp. *Appl. Environ. Microbiol.* 50:373–82
63. Montgomery WL, Pollak PE. 1988. *Epulopiscium fishelsoni* n. g., n. sp. , a protist of uncertain taxonomic affinities from the gut of an herbivorous reef fish. *J. Protozool.* 35:565–69
64. Nelson DC, Castenholz RW. 1982. Light responses of *Beggiatoa*. *Arch. Microbiol.* 131:146–55
65. Nelson DC, Jørgensen BB, Revsbech NP. 1986. Growth-pattern and yield of a chemoautotrophic *Beggiatoa* sp. in oxygen-sulfide microgradients. *Appl. Environ. Microbiol.* 52:225–33
66. Nelson DC, Wirsen CO, Jannasch HW. 1989. Characterization of large, autotrophic *Beggiatoa* spp. abundant at hydrothermal vents of the Guaymas Basin. *Appl. Environ. Microbiol.* 55:2909–17
67. Nielsen PH, de Muro MA, Nielsen JL. 2000. Studies on the in situ physiology of *Thiothrix* spp. present in activated sludge. *Environ. Microbiol.* 2:389–98
68. Odintsova EV, Wood AP, Kelly DP. 1993. Chemolithoautotrophic growth of *Thiothrix ramosa*. *Arch. Microbiol.* 160:152–57

68a. Ørskov F. 1984. Genus I. *Escherichia* Castellani and Chalmers 1919, 941[AL]. In *Bergey's Manual of Systematic Bacteriology*, ed. NR Krieg, JG Holt, 1:430–33. Baltimore: Williams & Wilkins
69. Ott JA, Bright M, Schiemer F. 1998. The ecology of a novel symbiosis between a marine peritrich ciliate and chemoautotrophic bacteria. *Mar. Ecol. Pubbl. Stn. Zool. Napoli* 19:229–43
70. Otte S, Kuenen JG, Nielsen LP, Paerl HW, Zopfi J, et al. 1999. Nitrogen, carbon, and sulfur metabolism in natural *Thioploca* samples. *Appl. Environ. Microbiol.* 65:3148–57
71. Overmann J, Tuschak C, Frostl JM, Sass H. 1998. The ecological niche of the consortium "*Pelochromatium roseum*". *Arch. Microbiol.* 169:120–28
72. Poindexter JS. 1981. Oligotrophy—fast and famine existence. *Adv. Microb. Ecol.* 5:63–89
73. Polz MF, Cavanaugh CM. 1995. Dominance of one bacterial phylotype at a Mid-Atlantic Ridge hydrothermal vent site. *Proc. Natl. Acad. Sci. USA* 92:7232–36
74. Polz MF, Odintsova EV, Cavanaugh CM. 1996. Phylogenetic relationships of the filamentous sulfur bacterium *Thiothrix ramosa* based on 16S rRNA sequence analysis. *Int. J. Syst. Bacteriol.* 46:94–97
75. Polz MF, Robinson JJ, Cavanaugh CM, Van Dover CL. 1998. Trophic ecology of massive shrimp aggregations at a Mid-Atlantic Ridge hydrothermal vent site. *Limnol. Oceanogr.* 43:1631–38
76. Purcell EM. 1977. Life at low Reynolds number. *Am. J. Phys.* 45:3–11
77. Ramsing NB, Kühl M, Jørgensen BB. 1993. Distribution of sulfate-reducing bacteria, O_2, and H_2S in photosynthetic biofilms determined by oligonucleotide probes and microelectrodes. *Appl. Environ. Microbiol.* 59:3840–49
78. Revsbech NP, Jørgensen BB. 1986. Microelectrodes: their use in microbial ecology. *Adv. Microb. Ecol.* 9:293–352
79. Riebesell U, Wolfgladrow DA, Smetacek V. 1993. Carbon dioxide limitation of marine phytoplankton growth rates. *Nature* 361:249–51
80. Robinow C, Angert ER. 1998. Nucleoids and coated vesicles of "*Epulopiscium*" spp. *Arch. Microbiol.* 170:227–35
81. Rodgers FG, Blakemore RP, Blakemore NA, Frankel RB, Bazylinski DA, et al. 1990. Intercellular structure in a many-celled magnetotactic prokaryote. *Arch. Microbiol.* 154:18–22
82. Schramm A, Larsen LH, Revsbech NP, Ramsing NB, Amann R, et al. 1996. Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes. *Appl. Environ. Microbiol.* 62:4641–47
83. Schulz HN, Brinkhoff T, Ferdelman TG, Marine MH, Teske A, et al. 1999. Dense populations of a giant sulfur bacterium in Namibian shelf sediments. *Science* 284:493–95
84. Schulz HN, Jørgensen BB, Fossing HA, Ramsing NB. 1996. Community structure of filamentous, sheath-building sulfur bacteria, *Thioploca* spp., off the coast of Chile. *Appl. Environ. Microbiol.* 62:1855–62
85. Schulz HN, Strotmann B, Gallardo VA, Jørgensen BB. 2000. Population study of the filamentous sulfur bacteria *Thioploca* spp. off the Bay of Concepcion, Chile. *Mar. Ecol. Prog. Ser.* 200:117–26
85a. Spring S, Amann R, Ludwig W, Schleifer K-H, van Gemerden H, Petersen N. 1993. Dominating role of an unusual magnetotactic bacterium in the microaerobic zone of a freshwater sediment. *Appl. Environ. Microbiol.* 59:2397–403
86. Stange P, Zanette D, Mikhailov A, Hess B. 1999. Self-organizing molecular networks. *Biophys. Chem.* 79:233–47
86a. Starr MP, Schmidt JM. 1981. Prokaryote diversity. In *The Prokaryotes*, ed. MP

Starr, H Stolp, HG Trüper, A Balows, HG Schlegel, pp. 3–42. Berlin: Springer
87. Stetter KO. 1999. Smallest cell size within hyperthermophilic Archaea ("Archaebacteria"). In *Size Limits of Very Small Microorganisms:Proceedings of a Workshop*, ed. NRC Steering Group Astrobiol. Space Stud. Board, pp. 68–73. Washington, DC: Natl. Acad.
88. Strohl WR. 1989. Genus I. Beggiatoa Trevisan 1942, 56[AL]. In *Bergey's Manual of Systematic Bacteriology*, ed. JT Staley, MP Bryant, N Pfennig, JG Holt, 3:2091–7. Baltimore: Williams & Wilkins
89. Sweerts J-PRA, DeBeer D, Nielsen LP, Verdouw H, Vandenheuvel JC, et al. 1990. Denitrification by sulfur oxidizing *Beggiatoa* spp. mats on freshwater sediments. *Nature* 344:762–63
90. Taylor BL, Zhulin IB. 1998. In search of higher energy: metabolism-dependent behaviour in bacteria. *Mol. Microbiol.* 28:683–90
91. Teske A, Ramsing NB, Küver J, Fossing H. 1995. Phylogeny of *Thioploca* and related filamentous sulfide-oxidizing bacteria. *Syst. Appl. Microbiol.* 18:517–26
92. Vainshtein MB, Kudryashova EB. 2000. Nanobacteria. *Microbiology* 69:129–38
93. Vandover CL, Fry B, Grassle JF, Humphris S, Rona PA. 1988. Feeding biology of the shrimp *Rimicaris exoculata* at hydrothermal vents on the Mid-Atlantic Ridge. *Mar. Biol.* 98:209–16
94. Waldron FM. 1900. On the appearence and disapperarence of a mud island at Walfish Bay. *Trans. S. Afr. Philos. Soc.* 11:185–88
95. Walsby AE. 1980. A square bacterium. *Nature* 283:69–71
96. Williams TM, Unz RF. 1985. Filamentous sulfur bacteria of activated sludge—characterization of *Thiothrix*, *Beggiatoa*, and Eikelboom Type-021N strains. *Appl. Environ. Microbiol.* 49:887–98
97. Wirsen CO, Jannasch HW. 1978. Physiological and morphological observations on *Thiovulum* sp. *J. Bacteriol.* 136:765–74

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