Aerobic Sulfate Reduction in Microbial Mats

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Measurements of bacterial sulfate reduction and dissolved oxygen (O_2) in hypersaline bacterial mats from Baja California, Mexico, revealed that sulfate reduction occurred consistently within the well-oxygenated photosynthetic zone of the mats. This evidence that dissimilatory sulfate reduction can occur in the presence of O_2 challenges the conventional view that sulfate reduction is a strictly anaerobic process. At constant temperature, the rates of sulfate reduction in oxygenated mats during daytime were similar to rates in anoxic mats at night: thus, during a 24-hour cycle, variations in light and O_2 have little effect on rates of sulfate reduction in these mats.

IN MARINE SEDIMENTS ALONG CONTINENTAL margins, sulfate reduction is an important metabolic process, responsible for about 50% of the total carbon oxidized (*1*). In microbial mats, sulfate reduction may be even more important in the mineralization of organic matter (*2*). Sulfate reduction has generally been thought to occur only after more energetically favorable electron acceptors such as O_2, nitrate, and metal oxides have become depleted (*3*). We show here that, in hypersaline marine microbial mats, sulfate reduction can occur at rapid rates in the presence of high O_2 tensions. A better understanding of the physiological capabilities of sulfate-reducing bacteria will allow a more complete view of the range of environments in which sulfate reduction occurs, and of the importance of this process in the mineralization of organic matter.

We studied sulfate reduction in the evaporating ponds of the Exportadora de Sal, located in Guerrero Negro, Baja California Sur, Mexico. Well-laminated mats, dominated by the cyanobacterium *Microcoleus chthonoplastes* (Fig. 1), are developed in these ponds in a salinity range of 65 to 120 per mil (*4*). During the day, light penetrates to a depth of 1 to 2 mm in the upper photosynthetic portion of the mats (*5, 6*) and sustains rapid rates of photosynthetic O_2 production and high concentrations of dissolved O_2 (*7*). Dissolved sulfide accumulates below the zone containing O_2 (*7*). At night, when there is no photosynthetic O_2 production, sulfide moves upward and may even penetrate the mat-water interface.

Rapid rates of sulfate reduction, approaching the fastest rates ever reported in a sediment, were measured in the upper aerobic zone of the mat (Fig. 2) at several times of the year. Sulfate reduction was completely inhibited by the addition of molybdate, demonstrating that the reduction of sulfate was mediated by bacteria (*8*). Sulfate reduction in the aerobic zone probably did not occur within reducing microniches. Sulfate-reducing bacteria are about 1 to 2 μm in size (*9*), and a reduced microenvironment requires numerous cells occupying a volume with dimensions of several tens to hundreds of micrometers (*10*). Oxygen microsensors with tip diameters of 5 to 20 μm have been routinely used to study these mats, and such microenvironments have never been observed. Furthermore, we believe that the sulfate reduction rates we measured reflect a dissimilatory rather than an assimilatory process. This is an important distinction because assimilatory sulfate reduction, a metabolic process that provides S principally to S-containing amino acids, is common among aerobic organisms (*11*). Assimilatory processes cannot make a large contribution to the measured rates because our analytical procedure does not measure S in amino acids (*12*). Also, the isotopic composition of reduced inorganic S (present as elemental S, an intermediate product of sulfide oxidation by O_2) in the surface aerobic portion of the mat is −27 per mil (relative to Canyon Diablo troilite); this value indicates that this S is derived principally from the dissimilatory reduction process (*13*).

In the conventional view, sulfate-reducing bacteria are thought to be obligate anaerobes whose ability to reduce sulfate is completely inhibited by even traces of O_2 (*9*). The inhibition mechanisms are not well understood, but O_2 is thought to inactivate or oxidize several of the enzymes and proteins used in the reduction process. For example, (Fe)hydrogenase in its reduced form is irreversibly oxidized by O_2 (*14*). Also, cytochrome c_3 is readily oxidized in the presence of O_2 (*9*) and is thus presumably made unavailable as an electron donor for the enzymes involved in the sulfate reduction process. Sulfate-reducing bacteria can survive exposure to O_2 and even use O_2 as a terminal electron acceptor for carbon metabolism (*9, 15*). Despite this O_2 tolerance, however, dissimilatory sulfate reduction has not yet been reported to occur in the presence of free O_2 in pure cultures. Rapid rates of sulfate reduction in the presence of high O_2 tensions in mats, as we observed, may indicate that there is a novel biochemical pathway for sulfate reduction or that there are complex microbial interactions, the details of which are not yet known.

We explored the effects of O_2, temperature, and light on rates of sulfate reduction in the surface photosynthetic part of the mat. In two constant-temperature experiments at 20° and 30°C, sulfate reduction rates were measured at noon and midnight.

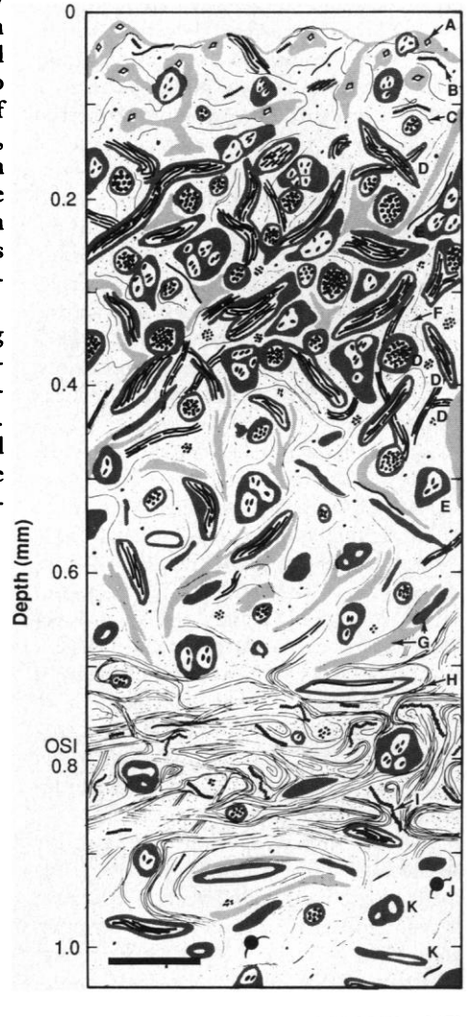

Fig. 1. Schematic diagram depicting the daytime depth distribution of bacterial populations in a cyanobacterial mat from the Exportadora de Sal. Although the O_2-sulfide interface (OSI) is shown at 0.8 mm, it may occur between 0.7 and 4 mm depending on the time of year and the salinity of the pond; A, diatoms; B, *Spirulina* sp. (cyanobacteria); C, *Oscillatoria* spp. (cyanobacteria); D, *Microcoleus chthonoplastes* (cyanobacteria); E, nonphotosynthetic bacteria; F, unicellular cyanobacteria; G, fragments of bacterial mucilage; H, *Chloroflexus* spp. (green bacteria, capable of anoxygenic photosynthesis if light penetrates to the OSI); I, *Beggiatoa* spp. (nonphotosynthetic sulfide-oxidizing bacteria); J, unidentified grazer; K, abandoned cyanobacterial sheaths. Adapted from (*28*).

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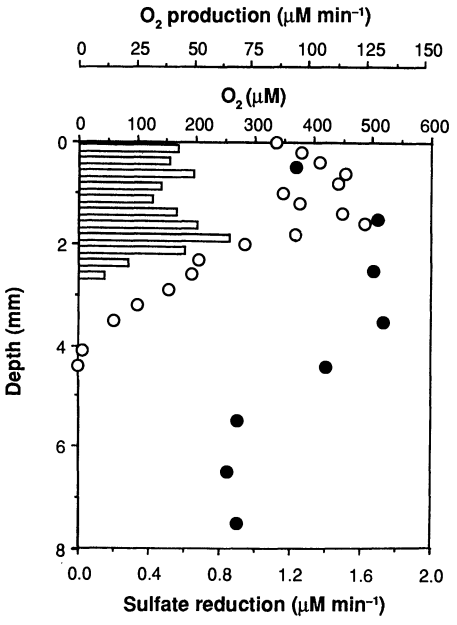
At noon, light intensity was near maximum, as were rates of oxygenic photosynthesis and also the depth of O_2 penetration. At midnight, the mats had experienced 6 hours of complete darkness. Although profiles of O_2 were not measured at night during this particular experiment, the sulfide-O_2 interface at other times of the year always migrated upward to and sometimes penetrated the mat surface at night (*16*). Maximum daytime rates of sulfate reduction were observed within the aerobic zone of the mat (Figs. 2 and 3). Also, both day and night rates of sulfate reduction at 20°C were one-half to one-fourth the rates at 30°C. A similar dependence of sulfate reduction rate on temperature has been observed in anoxic marine sediments (*17*) and in the permanently anoxic zone of other microbial mats (*2*).

For mats held at 30°C nighttime and daytime rates of sulfate reduction were similar, whereas nighttime rates were somewhat lower than daytime rates for mats at 20°C. Cohen (*18*) suggested that sulfate reduction in mats can be stimulated by light, although he did not indicate whether constant temperature was maintained in his experiments. Our results do not allow us to separate the effects of light and O_2 on sulfate reduction. Distinction of the effects is particularly difficult in mats because the two are integrally linked; the oxygenated photic zone in a well-lit mat can become completely bathed in sulfide within minutes after the onset of darkness (*19*). Our results do, however, suggest that, overall, only minimal differences in sulfate reduction rates occur in the upper photosynthetic zone of mats whether the mats are bathed in light and well oxygenated or held in darkness and fully anoxic.

Sulfate reduction has been found in association with free O_2 in other environments. For example, using a technique different than the one we utilized, Cohen (*18*) detected maximum rates of sulfate reduction at the O_2-sulfide interface in microbial mats. Also, alkalinity-CO_2 relations imply that sulfate reduction might be occurring at low free-O_2 concentrations just above the O_2-sulfide interface in the Cariaco Trench (*20*). Rates of sulfate reduction as high as 125 μM per hour have been measured in the surface 2 mm of carbonate sediment from Mangrove Bay, Bermuda (*21*), where dissolved O_2 in parallel cores extended to depths between 2 and 5 mm (*22*). The sediment surface in Mangrove Bay is well illuminated and supports various sea grasses.

Whether sulfate reduction occurs in the aerobic zone of more typical, nonphotosynthetic clastic marine sediments is uncertain because measurements of O_2 penetration and sulfate reduction have not been performed with the needed spatial resolution.

Fig. 2. Depth distribution of sulfate reduction (●), dissolved O_2 (○), and O_2 production rate by photosynthesis (bars), measured in February 1989. We measured O_2 profiles using O_2 microsensors (*23*), O_2 production rates with the light-dark shift technique (*29*), and sulfate reduction rates with radiolabeled $^{35}SO_4^{-2}$ (*30–32*). The concentrations of dissolved O_2 are from single determinations, although several similar profiles were also measured in the mats. Most O_2 production rates are from single determinations, with replicate measurements at some depths showing a variation of about 10%. Sulfate reduction rate measurements are from single determinations; however, a replicate core sectioned in 5-mm intervals gave the same average reduction rates as the 1-mm intervals shown in the figure. Concentrations of dissolved O_2 and rates of O_2 production and sulfate reduction were measured within a 10-cm^2 area of mat.



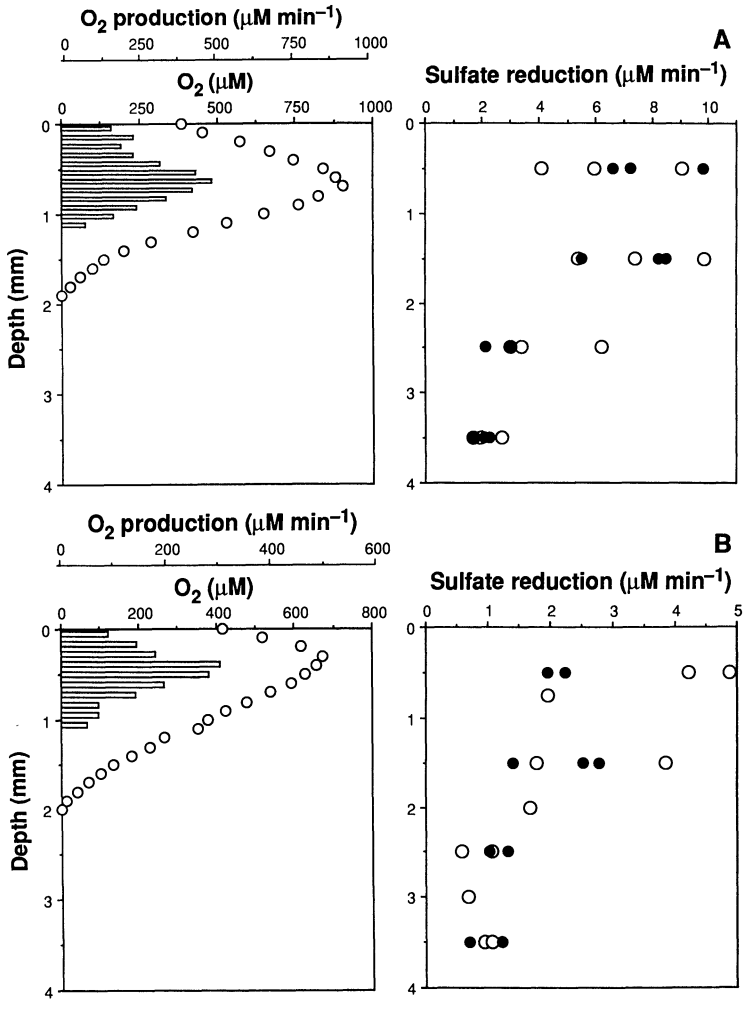


Fig. 3. Depth distributions of sulfate reduction rate [measured at noon (○) and midnight (●)], dissolved O_2, and O_2 production rate (measured at noon) at (**A**) 30°C and (**B**) 20°C in November 1989. Mats were incubated for 24 hours at the desired temperature in thermostatically regulated aquaria and were maintained at that temperature (within 1°C) during the time course of the experiment. At each temperature, sulfate reduction rates (measured in triplicate cores), rates of O_2 production, and O_2 depth distributions were measured within about a 50-cm^2 area of mat. Numerous O_2 profiles during the day, at various locations in the mat and at both temperatures, showed that the depth of O_2 penetration was fairly uniform between 1.6 and 2.0 mm.

Whereas O_2 may penetrate only 1 to 2 mm in a nearshore sediment (*23, 24*), sulfate reduction rates are rarely measured in less than 1 cm depth increments. High near-the-surface rates of sulfate reduction as reported in many nearshore sediments (*25, 26*), however, allow the possibility that sulfate reduction might occur in the aerobic zone. By contrast, in many continental margin sediments, particularly at low sedimentation rates and farther offshore, sulfate reduction is suppressed near the sediment surface and begins only below the zone of O_2 penetration (*24*). In this case, sulfate-reducing bacteria apparently cannot compete with other bacterial populations, such as iron-reducing bacteria (*27*), for organic substrate, although the details of these competitive interactions are not well known.

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31. Cores were injected vertically and incubated from 10 to 30 min under ambient conditions of light and temperature. Time course experiments showed that, with these short incubation times, no reduced ^{35}S was reoxidized back to sulfate, even in the aerobic zone, where sulfide was rapidly oxidized to elemental sulfur. Reduced ^{35}S was recovered with the use of a modification of the chromium reduction technique (*12, 32*), in which sulfide is liberated from dissolved sulfide, mineral sulfides, and elemental sulfur, but not organic sulfur compounds (*12*).
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Variations in Terrigenous Input into the Deep Equatorial Atlantic During the Past 24,000 Years

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Estimates of terrigenous fluxes at three different water depths at two sites in the equatorial Atlantic by normalization against excess ^{230}Th flux indicate that the flux of terrigenous material to the seafloor was significantly higher during the last glacial period than it is today. Fluxes started to decrease during deglaciation and reached minimal values in the middle of the Holocene. From 15,000 to 5,000 years ago, there was a substantial increase in flux with increasing water depth below 2,800 meters; this increase may reflect resuspension and lateral transport of slope and rise sediment, possibly because of intensification of deepwater circulation during that period.

THE INPUT OF TERRIGENOUS CLAYS into Atlantic deep-sea sediments has fluctuated with time. Clays usually have accumulated at higher rates during periods of low sea-level stand; this trend thus accounts in large part for the low amounts of $CaCO_3$ found in the glacial sections of Atlantic cores (*1–3*). Because of this apparent connection with climatic cycles, knowledge of changes in the mode of terrigenous input into deep-sea sediments is important for understanding the climate perturbations that occurred during the Quaternary.

We investigated variations in terrigenous flux in the equatorial Atlantic during the last 24,000 years by ^{230}Th profiling in six sediment cores taken at different water depths from the slopes of two rises (Sierra Leone Rise and Ceará Rise; Fig. 1). This method is based on observations from sediment trap studies suggesting that the annually averaged fluxes of excess ^{230}Th ($^{230}Th_{ex}$) to the seafloor are nearly constant and close to the expected rates of production from the radioactive decay of ^{234}U dissolved in the overlying water column. Thus, we contend that $^{230}Th_{ex}$ can be used as a reference against which the flux of other sedimentary components can be estimated (*3–6*). In contrast to methods where paleoflux estimates are obtained from average accumulation rates between dated sediment horizons, our method gives a flux estimate at each sampled point and thus allows better time resolution.

The $^{230}Th_{ex}$ mass balance (ψ) for sediments that accumulated in the Holocene and glacial sections of the six cores (Table 1) indicates the extent to which $^{230}Th_{ex}$ was brought to the site by post-depositional redistribution and lateral transport over the integrated periods (*4, 5,* 7). Values close to 1 indicate that the effect of these processes was small and that sediment accumulation at the site was dominated by the downward flux of material from the overlying water column. For these cases, ^{230}Th-normalized fluxes provide good estimates of pelagic settling fluxes. This situation was observed in most of the sections we analyzed. Exceptions were the glacial section of the deepest

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