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Dissolution of Barium from Barite in Sewage Sludges and Cultures of *Desulfovibrio desulfuricans*

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High concentrations of total barium, ranging from 0.42 to 1.58 mg · g^{-1} (dry weight) were found in sludges of two sewage treatment plants near Florence, Italy. Barium concentrations in the suspended matter decreased as redox potential values changed from negative to positive. An anoxic sewage sludge sample was aerated, and 30% of the total barium was removed in 24 h. To demonstrate that barium was solubilized from barite by sulfate-reducing bacteria, a strain of *Desulfovibrio desulfuricans* was used to study the solubilization of barium from barite under laboratory conditions. During cell growth with different concentrations of barite from 0.01 to 0.3 g · $liter^{-1}$ (the latter is the MIC) as the only source of sulfates in the cultures, the *D. desulfuricans* strain accumulated barium up to 0.58 μg · mg^{-1} (dry weight). Three times the quantity of barium was dissolved by bacteria than in the uninoculated medium (control). The unexpectedly low concentration of soluble barium (1.2 mg of Ba · $liter^{-1}$) with respect to the quantity expected (109 mg of Ba · $liter^{-1}$), calculated on the basis of the free H_2S evolved from the dissimilatory reduction of sulfate from barite, was probably due to the formation of other barium compounds, such as witherite ($BaCO_3$) and the transient species barium sulfide (BaS). The *D. desulfuricans* strain, growing on barite, formed visible aggregates. Confocal microscopy analysis showed that aggregates consisted of bacteria and barite. After 3 days of incubation, several autofluorescent crystals surrounded by a dissolution halo were observed. The crystals were identified as BaS by comparison with the commercial compound.

Barium is an alkaline earth element which occurs as a trace metal in igneous and sedimentary rocks. In nature it occurs principally in combined states as barite ($BaSO_4$) and witherite ($BaCO_3$). Ba is used industrially in a variety of forms. Barite is the most widely used of these salts, with a worldwide production in 1985 of 5.7 million tons (39).

The toxicity of barium is closely related to its solubility and for insoluble salts increases with decreasing pH (16, 32). Barium toxicity in animals and humans has been studied extensively. The ionic form (Ba^{2+}) is known to cause muscular paralysis due to physiological antagonism to potassium by blocking the K^+ channels of the Na-K pump in cell membranes (30).

Barium toxicity in eukaryotic and prokaryotic cells is related to the bioconcentration of the metal. Barium is known to act as an antagonist to Ca^{2+} and K^+ in several biochemical reactions. Growth studies have demonstrated that barium is generally toxic to bacteria, fungi, mosses, and algae. A low concentration of Ba^{2+} (10 to 100 μmol · $liter^{-1}$) inhibits the growth of *Nitrobacter agilis* (36).

Although not much is known about the biogeochemical cycle of barium, it seems to be related to the carbon cycle, since barium carbonate accumulates in a variety of marine biota (algae, mollusk shells, and corals) (18). Studies on Ba distribution have been of some historical importance because of its possible use as a stable analog to radium (6, 7). In marine environments with decaying biological debris, barium precipitates as the mineral barite ($BaSO_4$) (3, 9). Even though recent investigations have shown that Ba may serve as an indicator of palaeoceanographic and modern conditions (10, 11, 27), a better understanding of its biogeochemistry is required before confident interpretations of its distribution and reaction pathways are possible.

Barium has a low solubility from barite (2.47 mg · $liter^{-1}$ at 25°C), but it has been observed that laboratory cultures of sulfate-reducing bacteria use barite as a sulfate source for anaerobic respiration (4, 26, 31). No detailed physiological observations of the environmental consequences of this have yet been reported.

The aim of this study was to investigate the mobilization of barium in sewage sludge treatment plants in order to interpret the high concentrations of soluble barium found in treated effluent, groundwater, and the water supply of Florence, Italy (12, 22, 23). Since sulfate-reducing bacteria have been indicated as good candidates for barium solubilization from barite (4, 26, 31), this study used field and laboratory experiments to investigate the role of this group of bacteria in the solubilization of barium from sewage sludge and commercial barite.

MATERIALS AND METHODS

Sewage sludge sampling. From March to July 1993, 26 sewage sludge samples were collected, in individual 1-liter, acid-washed polyethylene bottles, from two sewage treatment plants near Florence, Italy: a mixed sewage plant at Caccini receiving hospital and urban wastes and a plant at Torre receiving only urban sewage. The samples were split into two subsamples: one was filtered immediately through Millipore membranes (pore size, 0.45 μm) under N_2, pressure, and the other was analyzed for the total barium content of the suspended matter (dry weight). During sampling, the temperature was recorded and the pH and redox potential were measured immediately in each sample by using a pH meter and redox meter unit (Hanna Instruments). The measurements were made under N_2 flow to avoid disturbance and contact with air.

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Count of sulfate-reducing bacteria. The most-probable-number technique was used to count sulfate-reducing bacteria in sewage sludge from the treatment plants. Samples were collected with a Whirl-pak (Nasco). In the laboratory the sample was distributed in 13-ml borosilicate vials with a 1-ml sterile syringe and diluted 1:10 with Postgate medium E (29) for each series of five replicates. After 7 days of incubation at 28°C, growth in the vials was evaluated by observing blackening of the cultures due to iron sulfide precipitation. The bacterial count was obtained by means of most-probable-number tables based on five replicates (20).

Bacterial cultures. *Desulfovibrio desulfuricans* LS was cultivated routinely in Postgate medium E. To study solubilization of barium from barite, the strain was grown on modified Postgate medium D without sulfates. The medium was finally adjusted to pH 7.6 with NaOH solution and supplemented with 4 ml of a resazurin solution (1 g · liter^{-1}) (colorless; redox potential, ≤−110 mV) after the boiling point was reached. The medium then was cooled under N_2 flux. Sulfates had previously been added to the vials as barite at 0, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 1.0 g · liter^{-1}. The barite crystals in the vials were purged with N_2 for 3 min before the anoxic medium was added. The strain was grown with 0.1 g of $BaSO_4$ · liter^{-1} three consecutive times to avoid interference of ferrous sulfate from Postgate medium E.

Microbial growth on barite. The *D. desulfuricans* strain was grown in liquid Postgate medium D in the presence of the electron acceptors $BaSO_4$ and $NaSO_4$ at concentrations of 0.01 to 1 g · liter^{-1}. After 3 days of incubation at 28°C, the bacterial cells were fixed (1:1, vol/vol) with 5% paraformaldehyde in buffer solution and stained with acridine orange (0.5 mM). They were counted in two replicate samples by epifluorescence microscopy (Zeiss Axiophot) with a Petroff-Hauser cell counting chamber.

Barium analysis. Twenty milliliters of sewage sludge and its filtered supernatant and 20 ml of bacterial cell suspension and its filtered supernatant were mineralized. Total barium was determined by atomic absorption spectroscopy with the furnace technique (Perkin-Elmer model 5000) by the U.S. Environmental Protection Agency standardized procedure (37). The detection limit was 4 μg · liter^{-1}, and the coefficient of variation in analyses of five replicates was 2.5%.

Solubilization of barium and accumulation in cells. Cultures of *D. desulfuricans* were grown in 50-ml borosilicate vials containing Postgate medium D amended with concentrations of $BaSO_4$ ranging from 0.01 to 2.5 g · liter^{-1}. The inoculum of each vial from an exponential-phase population of the strain was 1:50. The bacteria were incubated at 28°C for 3 days. Thirty milliliters of each *D. desulfuricans* culture was centrifuged for 15 min at 6,000 × *g*. The supernatant was filtered with a Minisart NML filter (Sartorius) (0.2-μm pore size), and the total barium in solution was determined as reported above.

To determine the quantity of barium in growing cells, the cell pellets were washed several times (more than five) with 10 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] buffer (pH 7.4) until barite was completely removed. The A_{600} of the cells resuspended in buffer was measured with a UV-visible spectrophotometer (Shimadzu model UV-160). The correlation between dried cell biomass and absorbance was as follows: $A_{600} = 0.023 + 1.63$ mg of cells (dry weight) · ml^{-1}; $n = 10$; $r^2 = 0.985$. The total amount of barium detected in the cells is reported as milligrams per gram of cells (dry weight).

Removal of barium from sewage sludge. In a side experiment under laboratory conditions, 500 ml of anaerobic (redox potential, −110 mV) sewage sludge from the Caccini treatment plant was distributed (250 ml each) in two 1-liter conical flasks. An average of 2.2 ± 0.008 μg of total barium · g^{-1} (dry weight) was determined in the suspended matter, which contained 0.0802 ± 0.0015 g of total solids · liter^{-1} when dried at 105°C. The duplicate sludge samples were aerated by stirring with a magnetic bar for 100 h at 20 ± 1.2°C and at pH 7.3 ± 0.21. An aliquot of 20 ml was taken from each flask and analyzed for barium content, pH, redox potential, and suspended matter. Evaporation of water during the experiment was avoided by refilling the flasks with barium-free double-distilled water 0.5 h before sampling.

Determination of free sulfide. Dissolved sulfides were measured by a modified iodometric method (1) with an automatic potentiometric titrator (Metrohom Titoprocessor model 536) in order to determine free sulfides. A volume of 5 ml of cell suspension was mixed with 0.5 ml of iodine solution (0.025 N) in a beaker and acidified with 0.4 ml of concentrated H_2SO_4 solution diluted 1:1 in double-distilled water. The residual iodine was assayed by potentiometric titration to determine the equivalence point. Replicate analyses of the same sample gave a coefficient of variation of 5.3%.

X-ray diffractometry analysis. The suspended aggregates were collected under an N_2 stream to maintain anaerobic conditions as much as possible. The sample was dried with absolute ethanol under N_2. Crystallographic analysis of microbial aggregates was performed with a Siemens D500 X-ray diffractometer. The spectrum of barite powder was based on identification of the highest peak of relative intensity (100%) at 3.45 Å and on the peaks 3.10 (100%) Å and 2.12 (80%) Å (1 Å = 0.1 nm). Other barium phases possibly present were not found in the composite samples by this method.

SCLM and image analysis. *D. desulfuricans* LS was grown in 1 liter of Postgate medium D supplemented with 0.20 g of barite · liter^{-1}. After 2 days of incubation, 0.5 ml of culture was sampled with a syringe and fixed with 0.5 ml of paraformaldehyde solution (5%); 0.1 ml was stained for 5 min with 0.050 ml of filtered acridine orange solution (0.5 mM) in the dark. Sixty microliters of sample was spread on a glass slide with a coverslip.

A scanning confocal laser (MRC-500; Bio-Rad Microscience Division) mounted on a Nikon Microphot microscope was used to obtain images of *D. desulfuricans* aggregates growing on barite. The analytical procedure for scanning confocal laser microscopy (SCLM) has been described in detail elsewhere (38). The recorded video images (512 × 768 pixels) were displayed on a 16-MHz black-and-white high-resolution 7-in. (ca. 18-cm) flat-screen video display (VM 1710; H. Lucius & Baer, Geretsried, Germany) and photographed with a Kodak F-301 camera with a 105-mm lens. Black-and-white photographs were taken with Tmax 100 ASA film (Kodak, Rochester, N.Y.). Image analysis of the sections was carried out with Comos Bio-Rad Software.

Aliquots of 60 μl (5 mg · ml^{-1}) of BaS (Johnson Matthey, United Kingdom), $BaSO_4$ (Fluka), and $BaCO_3$ (Fluka) standards were suspended in Postgate medium D and observed after 1 and 5 min by SCLM to detect dissolution halos of autofluorescent crystals.

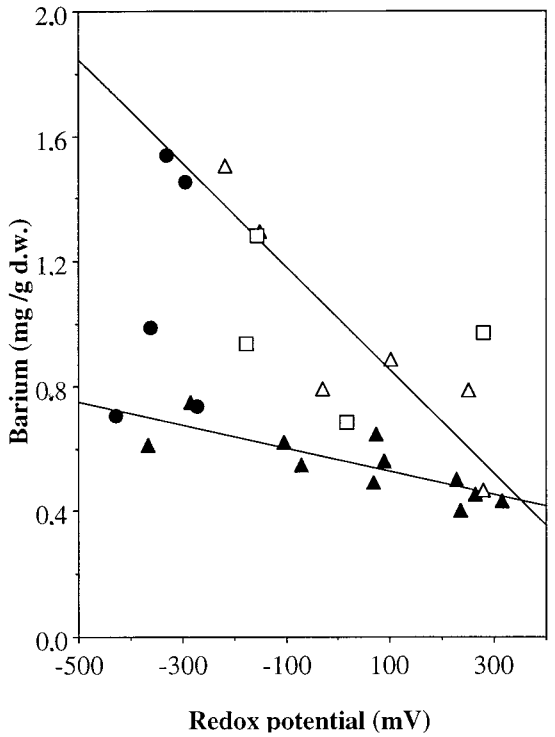

FIG. 1. Concentrations of total barium versus redox potential changes in sewage sludge from the digestor (△), settling pool (●), and aeration pond (□), in the Caccini treatment plant and from the settling pond in the Torre treatment plant (▲). d.w., dry weight.

RESULTS

The barium from the Caccini treatment plant consisted mainly of barite from hospital wastes (barium X-ray contrast medium) (21). The highest Ba concentration in suspended particulate matter (1.58 mg · g^{-1} [dry weight]) was found in sewage sludge from this treatment plant. At points with a more negative redox potential, significant negative correlation coefficients between barium content and redox potential were found: in the digestor at the Caccini treatment plant ($r = -0.808$; $n = 6$; $P < 0.05$) and in the settling pool at Torre ($r = -0.898$; $n = 11$; $P < 0.05$) (Fig. 1).

The concentrations of suspended-matter residues expressed as total dried solid ranged from 10.3 to 0.04 g · liter^{-1}. The pH varied from 8.9 to 7.2.

In the side experiment, in which a duplicate sample of sewage sludge was aerated for 100 h, 30% of the total barium disappeared from suspended matter after 24 h of stirring, and the redox potential rose from −110 to 250 mV after 100 h of

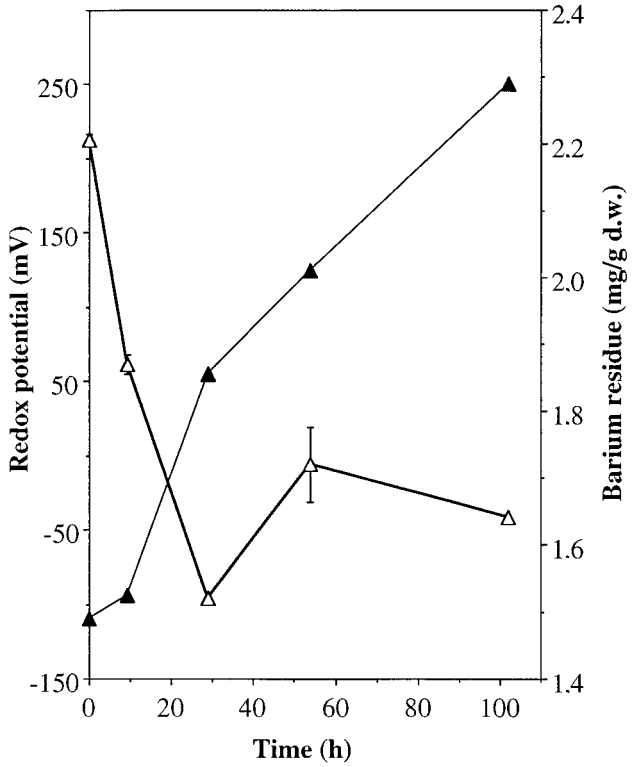

FIG. 2. Decrease in total barium (△) and increase in redox potential (▲) during aeration of anaerobic sewage sludge sampled from the treatment plant at Caccini. Aeration was performed in the laboratory at 20 ± 1.2°C and pH 7.32 ± 0.21. d.w., dry weight.

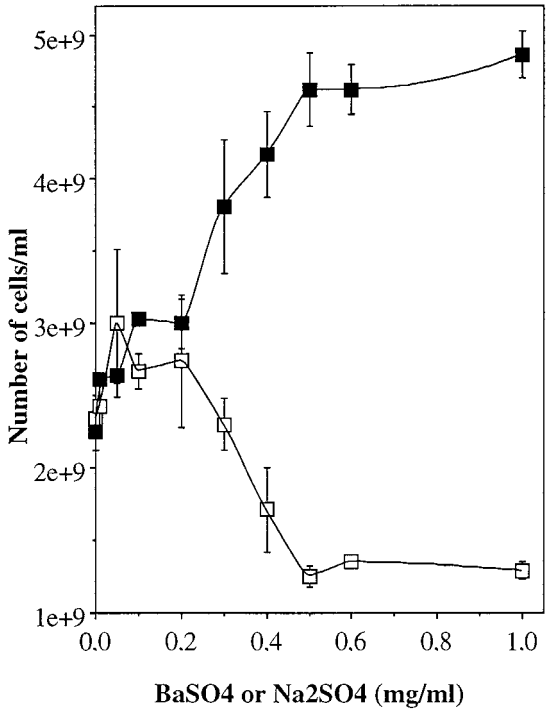

FIG. 3. Number of cells · milliliter^{-1} with standard deviations (bars) in relation to barium sulfate (□) and sodium sulfate (■) concentrations in Postgate medium D inoculated with *D. desulfuricans* LS and incubated for 3 days at 28°C.

aeration (Fig. 2). To explain the lower Ba concentrations in sewage sludges with positive redox values and the removal of Ba from suspended matter in the transition from anoxic to oxidized conditions, we refer to the literature which indicates strictly anaerobic sulfate-reducing bacteria as candidates for dissolving Ba from barite (4, 26, 31).

Sulfate-reducing bacteria in several sites in the treatment plants with different redox conditions were counted by the most-probable-number technique. At a redox potential of −355 mV, this group of bacteria numbered up to 1.6×10^6 cells · ml^{-1} in the settling pool at Caccini treatment plant. Even where the redox potential was slightly positive (+104 mV), as at Torre, 1.7×10^5 sulfate-reducing bacteria per ml were found.

The MIC of barite for *D. desulfuricans* LS was evaluated by counting cells in the presence of different concentrations of barium sulfate or sodium sulfate (Fig. 3). In the presence of barite, bacterial growth was inhibited at 0.3 g · liter^{-1} after 3 days of incubation. At low concentrations (≤0.3 mg · ml^{-1}) of $BaSO_4$ and $NaSO_4$, growth was similar. During the dissimilatory reduction of sulfate, Ba^{2+} from barite was partly taken up by the bacterial cells or was released into solution. When *D. desulfuricans* was exposed to different $BaSO_4$ concentrations up to 0.3 g · liter^{-1} (above this threshold microbial growth began to be inhibited in Postgate medium D), the cells assimilated Ba to 0.6 μg · mg^{-1} (dry weight) during 3 days of incubation at 28°C (Fig. 4).

Up to 2.5 g of barite · liter^{-1} dissolved in the presence of *D. desulfuricans*. This was almost three times more than in the uninoculated anaerobic sample (control) (Fig. 5A). The soluble Ba was closely correlated ($r^2 = 0.959$; $n = 7$) with free sulfides detected in the culture (Fig. 5B). From the relationship between soluble barium and free sulfides (soluble barium [y axis, in milligrams · liter^{-1}] = −0.22 + 0.048 free sulfides [x axis; in milligrams · liter^{-1}]), it can be calculated that in order to dissolve 1 mg of free ionic barium · liter^{-1} in 3 days, 25 mg of S^{2-} · liter^{-1} is required, which is equivalent to 185 mg of dissolved $BaSO_4$ · liter^{-1}.

During incubation of inoculated samples, the vials with $BaSO_4$ were more turbid than those with $NaSO_4$, even when the number of cells was much smaller. The study using SCLM and image analysis showed that large aggregates formed in the cultures amended with $BaSO_4$. Specimens of aggregates observed in the light transmission mode (Fig. 6a) measured several tens of micrometers. The same spot on the specimen, when observed in the light reflection mode, showed microcrystals in the aggregate (Fig. 6b). X-ray diffraction analysis of the microbial aggregates demonstrated that the only crystal was orthorhombic barite with a main peak at 3.44 Å and other minor peaks. No $BaCO_3$ or BaS was observed in 3-day experiments with this technique.

D. desulfuricans emitted a strong fluorescence when the sample was stained with acridine orange (Fig. 7c). It formed clumps in close contact with the barite crystals as seen by merging the fluorescence and reflection mode photographs (Figure 7d). The dense material at the edge of the aggregate consisted of extracellular polysaccharides and was detected by adding ruthenium red solution (image not shown) to *D. desulfuricans* cultures (14).

After 3 days of incubation, several further cultures were observed in the light transmission mode. Several crystals per optical field showed a dissolution halo around the microcrystal (Fig. 7a). In the fluorescence mode, the dissolution halo showed autofluorescence, and a clump of microbes was associated with the crystal (Fig. 7b). The same dissolution halo was

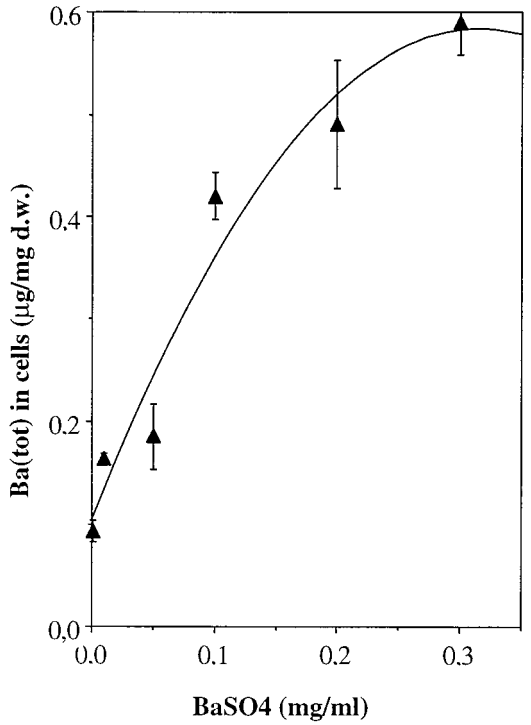

FIG. 4. Barium accumulation in cells of *D. desulfuricans* LS, expressed as total (tot) Ba with standard deviations (bars), after 3 days of incubation at 28°C with different concentrations of barium sulfate. d.w., dry weight.

found only around pure crystals of barium sulfide, which emitted autofluorescence when irradiated with the laser (488 nm) during SCLM observations (Fig. 7d). In the light transmission mode, BaS crystals in contact with air (see the air bubble in Fig. 7c and the dissolution halo in Fig. 7d) were seen to dissolve quickly, whereas other barium crystals, such as $BaCO_3$ and $BaSO_4$, did not dissolve.

The phase relations in the system HCl-H_2O-BaO-CO_2-H_2SO_4 can be understood in terms of a theoretical activity diagram or E_h-pH plot. However, under the given conditions of reduction potentials, pH values, and reactant activities, only the stability fields of $BaSO_4$ (barite) and $BaCO_3$ (witherite) occurred on the plot. The relevant reactions were the dissociation of barite and the protolysis of witherite, since the barium sulfide equilibria do not seem to be of significance (although they are thermodynamically feasible). The position and slope of the predominance field boundary between witherite and barite are determined by the stoichiometry of the minerals and the equilibrium constants of reactions representing the equilibrium.

DISCUSSION

The total barium concentrations decreased from negative to positive redox potential values in the sewage sludge. The low concentration of total barium in the suspended matter in aerobic areas of the same treatment plant suggests that barium is somehow removed when the sewage sludge is oxidized. This was confirmed by a side experiment, in which a sample from a Ba-polluted point in the Caccini treatment plant with a low redox potential (−110 mV) was aerated in the laboratory. This experiment demonstrated that there was an "oxidizable" fraction of barium compounds in the sewage sludge, which explains the finding of lower dissolved Ba concentrations in oxidized suspended matter. It is difficult to explain how mere oxidation can cause the release of barium from suspended matter.

The mechanism by which barium becomes soluble has hitherto been unclear, and its cycle has not been investigated (39). Obvious questions, however, are if barium compounds are so insoluble, why is barium contaminating drinking water (2, 30, 35) and food (5, 17, 33, 34), and where does it enter the food chain? Answers to these questions emerge from the present indications of the role of bacteria in dissolving Ba from barite.

The strictly anaerobic sulfate-reducing bacteria are good candidates for mobilizing barium from barite because they use the counterion sulfate as an electron acceptor. There have been reports that sulfate-reducing bacteria grow in the presence of $BaSO_4$. In 1965 Römer and Schwartz (31) demonstrated that these bacteria grew with barium sulfate as the sole sulfur source and reprecipitated barium as witherite ($BaCO_3$) after 2 weeks of incubation. McCready et al. (25), studying sulfur isotope fractionation by *Desulfovibrio vulgaris* with $BaSO_4$ as an electron acceptor, reported that the quantity of soluble barium measured (5.3 mg of Ba · liter^{-1}) was much less than expected (188 mg · liter^{-1}). The soluble barium concentration was 5.3 mg of Ba · liter^{-1}, whereas the amount of

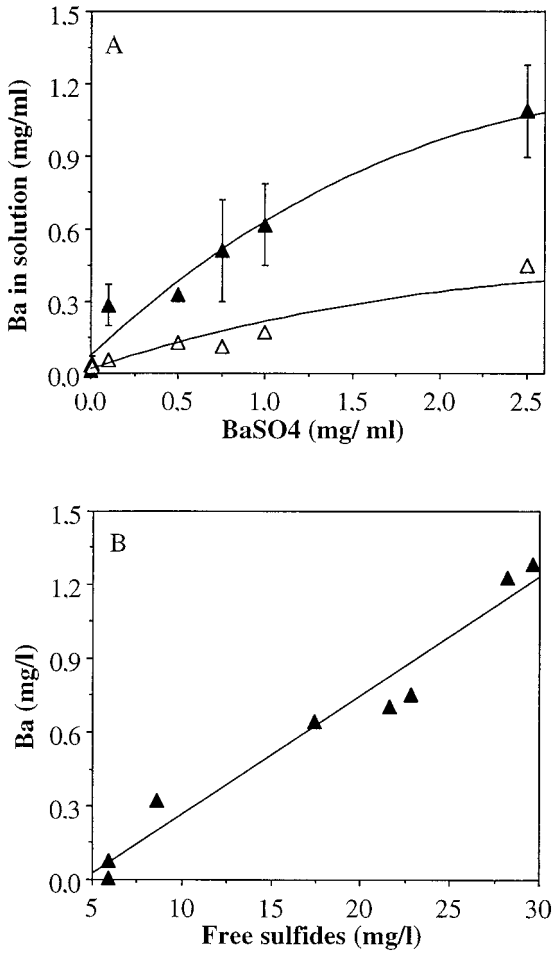

FIG. 5. (A) Barium solubilization in cell cultures of *D. desulfuricans* (▲) (with standard deviations [bars]) and in uninoculated samples (△) of Postgate medium supplemented with different barium sulfate concentrations, after three days of incubation. (B) Correlation between soluble barium in the supernatant of *D. desulfuricans* cell suspensions and free sulfides formed at different barium sulfate concentrations by dissimulative reduction.

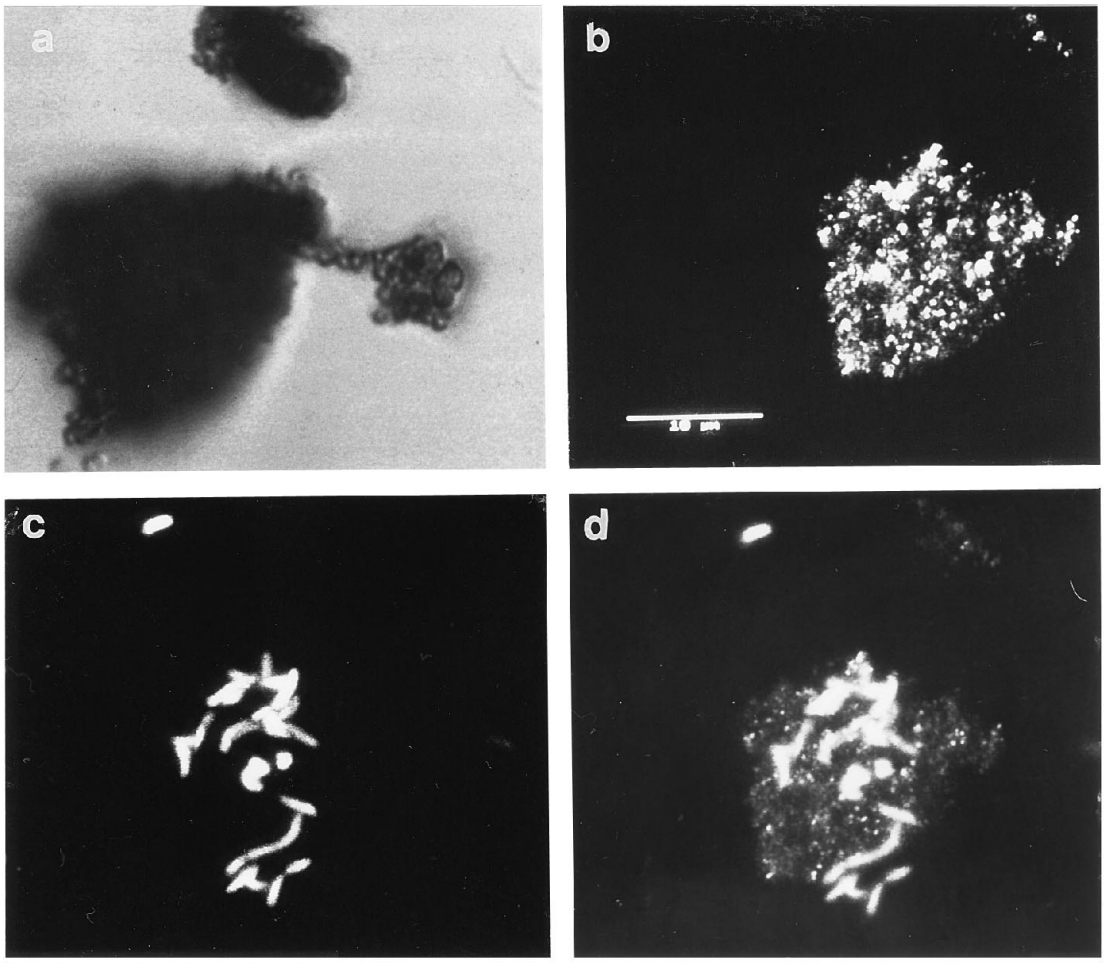

FIG. 6. (a) Transmission light microscope image of a floating aggregate from a *D. desulfuricans* culture in Postgate medium with 0.2 g of barium sulfate · liter^{-1} after 2 days of incubation at 28°C. (b) Reflection confocal laser microscope image of the same aggregate spot, showing light reflected from barite (bar, 10 μm). (c) Fluorescence of confocal laser microscope image digital reconstruction from the sum of 15 scanned *xy* sections (0.6 μm) of the same aggregate spot, showing cells of *D. desulfuricans* LS stained with acridine orange. (d) Merged images of reflection and fluorescence images of the aggregate.

sulfide evolved in the 19-day experiment was 43 mg · liter^{-1}, equivalent to 320 mg of dissolved $BaSO_4$ · liter^{-1}. This underdetection of soluble barium was explained by the presumed formation of witherite ($BaCO_3$) from CO_2 evolved by oxidation of organic compounds (31).

The same order of magnitude of barium dissolved from barite by *D. desulfuricans* LS was found in the present study. With regard to free sulfide formation, we expected 185 mg of barite · liter^{-1} to be dissolved, equivalent to 109 mg of Ba · liter^{-1}, instead of the 1.2 mg of Ba · liter^{-1} obtained in 3 days of incubation at 28°C. We did not detect witherite, and neither did Bolze and coworkers (4), who found similar low soluble Ba concentrations (1.8 to 3.8 mg · liter^{-1}) from barite in a mixed culture of sulfate-reducing bacteria after 17 days of incubation. In our study, Ba uptake by *D. desulfuricans* cells was negligible in the mass balance, because it accounted for only 0.58 μg of Ba per mg of cells (dry weight). This intracellular Ba concentration was enough to inhibit microbial growth.

A further explanation for the underdetection of Ba^{2+} could be the formation of another crystal, barium sulfide. SCLM and X-ray diffraction clearly indicated that the microbial aggregates initially consisted only of barite and bacteria. After 3 days of incubation, several crystals became autofluorescent under laser irradiation at 488 nm, and they dissolved quickly under the coverslip in contact with air. The presence of a dissolution halo around the crystals suggested that this compound was unstable in air. The same halo was observed with pure crystals of commercial barium sulfide. Obviously, other types of minerals can also emit fluorescence, but in our controlled system the only significant products were barium compounds. The only fluorescent mineral of barium is witherite, which is quite insoluble (K_s = 10 mg · liter^{-1}). Only when irradiated with higher-frequency rays does it emit light; however, the light is at wavelengths that cannot be detected by SCLM. The finding of decomposable and light-autofluorescent crystals in the *D. desulfuricans* aggregates suggested that the compound was barium sulfide. At low concentrations (parts per million), and because it tends to decompose, this compound cannot be measured by direct X-ray diffractometry.

The biochemical reaction triggered by sulfate-reducing activity in the presence of sodium lactate as an electron donor and $BaSO_4$ as an electron acceptor at pH 7 and ≥ -110 mV is as follows. Under anaerobic conditions, Na-lactate + $BaSO_4$ = biomass + $BaCO_3$ + BaS + CO_2 + H_2S. Likewise, hydrogen sulfide reduces barite to BaS ($BaSO_4 + 2H_2S = BaS + SO_2 + 2H_2O + S$), while under aerobic conditions (15), $BaS + 2H^+$

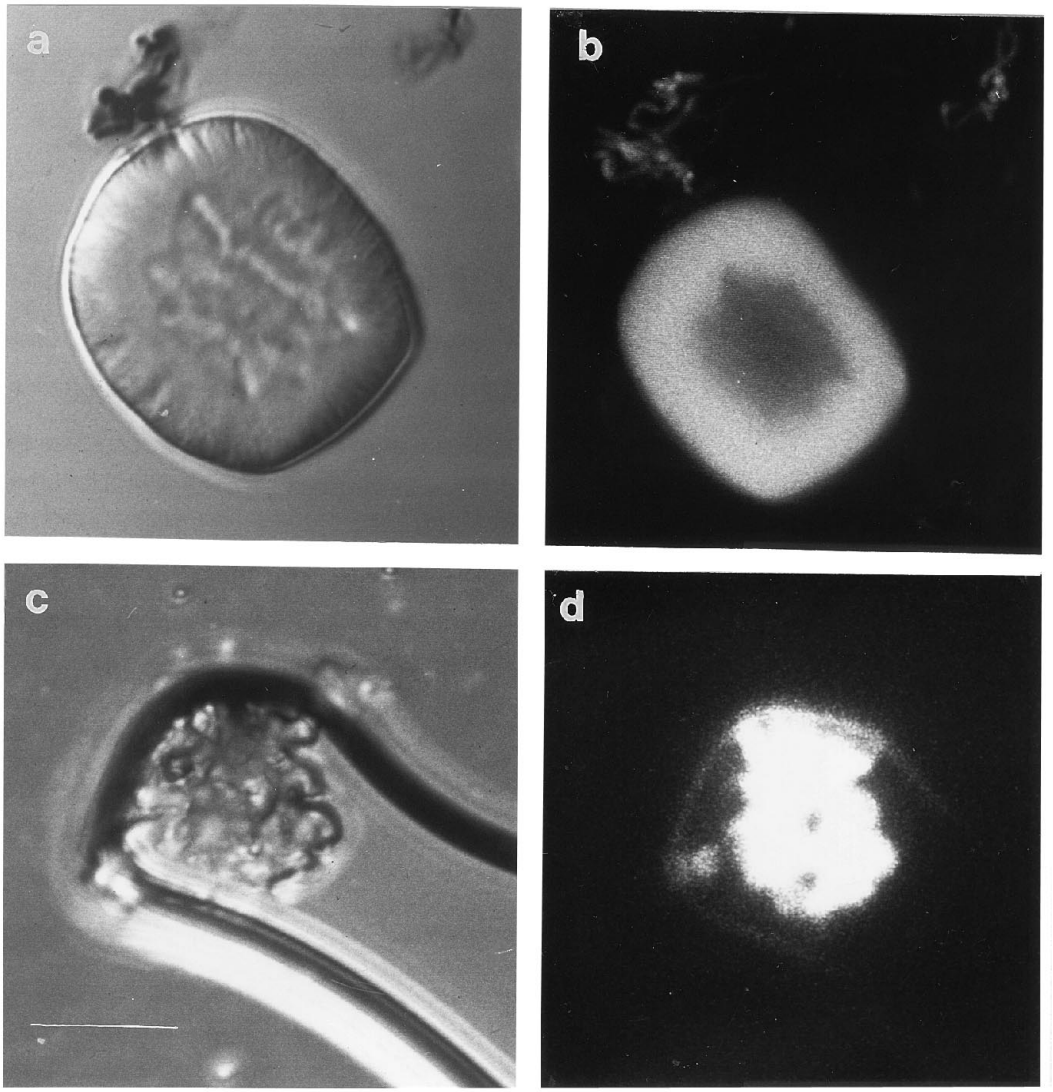

FIG. 7. (a) Transmission light microscope image of a floating crystal with dissolution halo associated with a clump of bacteria in a culture of *D. desulfuricans* LS in Postgate medium with 0.2 g of barium sulfate · liter^{-1} after 5 days of incubation. (b) Fluorescence confocal laser microscope image of the same crystal. The dissolution halo is photoluminescent. (c) Transmission light microscope image of pure commercial barium sulfide crystals exposed to air for 3 min; note air bubble (bar, 25 μm). (d) Fluorescence image of the commercial barium sulfide crystal with the photoluminescent dissolution halo.

$= Ba^{2+} + H_2S$. Another possible side reaction is the formation of barium oxide (15): $BaS + 3BaSO_4 = 4BaO + 4SO_2$. Under aerated conditions, BaS decomposes to Ba^{2+} and H_2S and probably other intermediates, such as barium hydroxide and the hydrosulfide of barium [Ba(HS)2H_2O] (24, 28). Many barium polysulfides and hydrosulfides have nonstoichiometric compositions, making Ba-H_2O-H_2S-CO_2 systems very sensitive to O_2, CO_2, and H_2S fugacities, with an array of metastable sulfidic phases. Even though barium sulfide may form in the process of barite reduction and dissolution, it could not be positively identified in the solid phase. Despite its instability in aqueous solution, we believe that BaS is a reactive transient species stabilized on a colloidal or solid phase. Polysaccharide complexes which frequently form organic coatings on suspended matter may account for barium sulfide stabilization, especially when involved in colloid formation. These microenvironments could be associated with biological debris or microbial aggregate microniches. These observations seem to be in agreement with the findings of Falkner et al. (13) on the redox behavior of Ba in marine environments.

The laboratory experiment may therefore explain some aspects of the barium-oxidizable fraction in sewage sludge treatment plants near Florence. Under anaerobic conditions, part of the barite may react with H_2S from sulfate reduction to form the unstable BaS, which is oxidized as soon as O_2 is pumped into the sewage sludge (from aeration pools and digestors). The rapid changes in redox conditions at various points of the treatment plants could cause a partial removal of barium from suspended matter. Activity and fugacity diagrams, representing equilibria between mineral phases and aqueous solutions, afford a convenient reference frame for predicting mineral reactions. This is true both for laboratory experiments and for natural biogeochemical processes. They also facilitate predictions of mass transfer in biotic and abiotic systems. In our study, analysis of the equilibrium relationships between witherite and barite made it possible to compare their activity

ratios with those in the effluent waters of the treatment plant. It is interesting to consider the concentrations of dissolved Ba in marine sediment pore water, which is generally around or above saturation with respect to $BaSO_4$, while seawater is undersaturated (8, 19). A similar situation in the sewage sludge and effluent water would maintain a concentration gradient across the solid-liquid interface, which would result in a remobilization of Ba and a diffusive flux from the sludge to the overlying water. Another possibility for barite solubilization is complexation with dissolved or colloidal organic matter in the sludge. Other possible Ba carrier phases are Fe and Mn oxyhydroxides (13) or celestite ($SrSO_4$), provided that the Sr budget of the system is sufficiently high. It is not clear whether diagenetic reactions in the sludge column produce higher Ba concentrations in the overlying water. If barite is the predominant solid phase of particulate Ba, sulfate reduction by *D. desulfuricans* is a thermodynamically and kinetically favored process.

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