Radioisotope Assay for the Quantification of Sulfate-Reducing Bacteria in Sediment and Water

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A radioisotope enrichment culture method was developed to estimate the physiologically active component of a population of sulfate-reducing bacteria in environmental water and sediment samples. Aliquots of water or sediment were added to 50-ml serum bottles filled with ³⁵S-sulfate broth incubated for approximately 30 h. After incubation, the disintegration rate per milliliter of spent medium was measured, and the percentage of loss of activity resulting from bacterial sulfate reduction was determined. This loss of sulfate from the medium was then translated to a specific number of Desulfovibrio desulfuricans cells that would reduce an equivalent amount of sulfate in the same incubation time. This comparison was done using a series of growth curves of D. desulfuricans covering a range of inoculum densities between 10² and 10⁷ cells. The radioassay was used to follow the effects of a pulp mill on a small anoxic river in Florida. The activity of the sulfate-reducing bacteria in the river was greatly suppressed when the mill was closed for annual maintenance. The initiation of waste treatment resulted in improved water quality in 1 week, but the river sediments required a month to show a 10-fold reduction in the population of sulfate-reducing bacteria.

Recent reports have indicated that dissimilatory sulfate reduction accounts for most of the sulfide found in natural anoxic waters. Deuser (1) determined that between 94 and 97% of the sulfide in the Black Sea is produced by bacterial sulfate reduction, whereas putrefaction can account for only about 5%.

Despite the acknowledged geochemical importance of sulfate-reducing bacteria, techniques for quantitatively studying their environmental activities have not been available. Enumeration of sulfate reducers is routinely performed by the most-probable-number dilution technique. We evaluated this procedure in preliminary studies of the microbial ecology of a river heavily polluted by pulp mill wastes. These population estimates indicated a uniform density of 10⁴ sulfate-reducing bacteria per ml along the entire length of the anoxic river (5). Total sulfide measurements, however, indicated that the sulfide concentration was not uniform but increased from 2 to 23 mg/liter along the course of the river. If the sulfide was bacteriologically produced, we would have expected the increase in sulfide concentration to be paralleled by an increase in the numbers of sulfate-reducing bacteria. The disparity we observed using most-probable-number techniques prompted the development of an assay that reflected the physiologically active component of a population of sulfate-reducing bacteria, or the functional potential of natural samples. We present our findings in this publication.

MATERIALS AND METHODS

Medium and culture preparation. The cultures used in this study were obtained from the American Type Culture Collection (ATCC) and included Desulfovibrio desulfuricans (ATCC 7757) and Pseudomonas fluorescens (ATCC 13042). A pure culture isolated from the Fenholloway River, Florida, designated culture FHR-3 was also used in this study.

D. desulfuricans and culture FHR-3 were carried as stab cultures on the following medium: peptone, 5.0 g/liter; beef extract, 3.0 g/liter; yeast extract, 0.2 g/liter; MgSO₄, 1.5 g/liter; Fe(NH₄)₂(SO₄)₂, 0.1 g/liter; glucose, 5.0 g/liter; agar, 15.0 g/liter; diluted with distilled water and adjusted to pH 7.

For all growth experiments, D. desulfuricans and culture FHR-3 were pregrown by inoculating a 50-ml serum bottle containing API sulfate broth (Difco Laboratories) with a fragment from a stab culture or 1 ml of an existing cell suspension. The serum bottle was filled to capacity, sealed with a rubber-serum stopper, and incubated as a stationary culture for 72 h at 35 C.

For experiments using inocula of specified size, the pregrown cultures were first enumerated by direct counting in a Petroff-Hauser chamber and then diluted to approximate densities of 10⁴, 10³, 10², 10¹,
and 10^7 cells/ml in API sulfate broth. Actual densities were then determined by plate counts on API sulfate agar using a GasPak anaerobic system (BBL).

The culture of *P. fluorescens* was carried on API sulfate agar and for experimental use was pregrown in API sulfate broth for 24 h at 35 C.

All experiments and activity measurements were conducted using API sulfate broth to which carrier-free ^35S-sulfate (^35S-API; New England Nuclear) was added to give an activity of approximately 8,000 dpm/ml of medium.

In an experiment to determine the effects of varying the total sulfate concentration, standard ^35S-API broth was used unaltered and, with the addition of unlabeled Na₂SO₄, to give final sulfate concentrations of 208.5, 228.5, 248.5, and 268.5 mg/liter.

**Standard sulfate reduction assay procedure.** Four 50-ml serum bottles were prepared for each sample or cell suspension to be assayed. Sufficient medium to completely fill all the bottles was prepared in advance. Each bottle was filled with 40 ml of medium and capped with aluminum foil, and the surplus ^35S-API broth was poured into a 1,000-ml flask and plugged with cotton. All vessels containing medium were then autoclaved at 15 lb/in² for 15 min, cooled in a cold water bath, and used immediately to minimize the re-solution of oxygen.

After cooling, 1 ml of sample, a suspension of either *D. desulfuricans*, FHR-3, or an environmental sample, was added to each of the four replicate bottles. Next, 1 ml of a suspension containing 10⁷ *P. fluorescens* cells was added to each bottle to assure anoxic conditions. One bottle of each sample was re-autoclaved to serve as a control. All bottles were then aseptically sealed with sterile rubber serum stoppers and filled to capacity with additional sterile medium.

To fill a sealed serum bottle, a 2-ml syringe without a plunger was first inserted so that the needle just penetrated the stopper. Sterile medium from the 1,000-ml flask was then injected using a sterilized repeating syringe until it flowed out into the 2-ml syringe. Both syringes were then withdrawn, the needles were flamed, and the process was repeated for each bottle. The use of a repeating syringe greatly reduced the time required to fill a large number of serum bottles while minimizing contamination. After inoculation the serum bottles were incubated at either 30 or 35 C on a gyratory shaker at 250 rpm. A temperature of 35 C was used to incubate samples collected in the river receiving a heated industrial effluent. An incubation temperature of 30 C was used for marine samples. After the desired incubation period, growth vessels were sacrificed in triplicate along with one control. When it was desired to generate growth curves, triplicate serum bottles were sacrificed when the first traces of ferrous sulfide precipitate appeared and at 4- to 5-h intervals thereafter. Next, 10 ml of the spent medium was passed through a 25-mm, 0.45-µm membrane filter in a Swinnex holder (Millipore Corp.). The 10-ml aliquots were bubbled with nitrogen for 5 min to purge dissolved hydrogen sulfide from the liquid. The disintegration rate of the remaining sulfate was measured by pipetting 1 ml of the filtrate into a liquid scintillation vial containing 15 ml of Aquasol (New England Nuclear) and counting, using the channels ratio method to correct for quench.

All controls for a given batch of medium were found to vary by no more than 5% and were therefore averaged. This average was used to calculate sulfate removal from the spent growth medium. The count rate of autoclaved sediment controls when compared to water controls showed that no sulfate was absorbed on the sediment. Uninoculated controls did not differ from re-autoclaved, inoculated controls. The variation between replicate samples when using the radioassay was consistent for a given operator and ranged between ±20% of the mean value when water samples were used. Sediments were found to be less variable with a range of ±10% of the mean.

Cells of *D. desulfuricans* were killed on membrane filters which were then used to inoculate growth vessels. A standard 47-mm glass filtration apparatus (Millipore Corp.) was assembled using a 0.45-µm filter and 25 ml of a sterile 1% sodium chloride solution poured into the funnel. To this was added 1 ml of a suspension of *D. desulfuricans*, and a vacuum of 7 lb/in² was applied. The filter was then used to directly inoculate a serum bottle in lieu of 1 ml of cell suspension. Survival of *D. desulfuricans* was determined by collecting 10⁴ cells on a series of membrane filters and exposing them to air for various periods of time in petri dishes with moist filter paper in the bottom. After air exposure, the filters were then used to inoculate a set of serum bottles. After 24-h incubation, sulfate removals were determined, converted to population estimates (see Discussion for details), and expressed as surviving fraction.

**Collection of environmental samples.** Water samples were collected from seven stations with a 3-liter Van Dorn sampler (GM Manufacturing) lowered in midstream, and the samples were collected approximately 6 inches (ca. 15 cm) off the bottom.

Sediment for bacterial and sulfide analysis was collected with an Ekman dredge. Subsamples (1 cm³) were obtained with sterile, 8-mm glass-core tubes calibrated to contain 1 cm³ of sediment. The tubes were inserted in the sediment, plugged with sterile corks, and stored on ice until assayed, which was never longer than 3 h. Sufficient cores were taken to inoculate the desired number of serum bottles.

Intersitial water for sulfide analysis was squeezed from the sediments by means of a nitrogen-operated nylon sediment press (4).

Sulfide was determined by the spectrophotometric method of Strickland and Parsons (6) using a Beckman model DK-2A spectrophotometer.

**RESULTS**

The assay for sulfate-reducing bacteria as used here is a highly reproducible enrichment culture method. This technique in no way is intended to simulate growth under in situ conditions, but rather to promote the rapid growth of dissimiatory sulfate-reducing bacteria in a consistent and reproducible manner. It
is known that, when a culture medium is inoculated, the duration of the lag phase of the growth cycle is inversely proportional to the logarithm of cell density in the inoculum (3). Once a culture begins to grow, the growth curves for different inocula sizes are identical, except that the curves are separated in time. This temporal displacement may be used to indicate the relative difference in cell density between a series of environmental samples. For use with sulfate-reducing bacteria, growth is followed by measuring the removal of labeled sulfate from the medium.

A family of growth curves was generated for D. desulfuricans over an inoculum range of \(10^3\) to \(10^7\) cells (Fig. 1). All curves enter the stationary phase after 90% sulfate removal and all have similar shapes and slopes, but each is displaced laterally with increasing lag period as the inoculum size decreases.

The temporal displacement of the growth curves, as measured by the time required to remove 50% of the sulfate in the medium, is shown in Fig. 2. For D. desulfuricans, the duration of the lag phase under the conditions of the ratio assay is inversely proportional to the logarithm of the cell density in the inoculum.

An experiment was performed to determine whether sulfate-reducing bacteria isolated from natural environments would behave in an analogous manner to the ATCC culture of D. desulfuricans. An experiment was performed to compare the shape of the growth curves and the duration of the lag phase for cultures of D. desulfuricans and FHR-3. Inocula of both organisms were prepared, and approximately \(10^6\) cells were added to all of the growth vessels. The growth curves obtained are shown in Fig. 3. The actual inoculum densities, determined by plate counting, were \(9 \times 10^4\) and \(2 \times 10^4\) cells for D. desulfuricans and FHR-3, respectively. The time required for 50% sulfate removal for the curves of Fig. 3 exactly fit data of Fig. 2, indicating that at least this one environmentally isolated culture behaves as does D. desulfuricans in the radioassay.

The question still remained whether an environmental sample with a mixed bacterial population would have the growth characteristics of D. desulfuricans. To resolve this question, water from an anoxic stream was used full strength and 10- and 100-fold diluted to inoculate a set of reaction vessels. The growth curves from this experiment are shown in Fig. 4 and have the same basic features as the pure culture experiments. Each of the three growth curves has the same slope and sulfate removal in the stationary phase. The curves are temporally displaced in proportion to the logarithm of the inoculum size, but the time interval between the growth curves is 5.2 h, as compared to 3.7 h obtained in the pure culture experiments.
FIG. 5. Comparison of two inoculating methods for the radioassay. Cells were either collected on membrane filters or added directly as a cell suspension to the reaction vessels.

FIG. 6. Sampling stations in the Fenholloway River. The river flows from station A to the Gulf of Mexico.

FIG. 4. Effect of using serial dilutions of an environmental sample as inocula in the radioassay. The size of inoculum for the undiluted sample was $5 \times 10^6$ cells.

For many natural environments, exclusive of sediments, it would be unrealistic to find sulfate-reducing bacteria in appreciably high concentrations such that only 1 ml of sample would be sufficient to directly inoculate the reaction bottles. Membrane filtration was thus tested as a means of concentrating cells from water samples to serve as inocula in the radioassay. An experiment was performed using conventional glass Millipore apparatus to concentrate cells of *D. desulfuricans* on membrane filters, which then were used to inoculate growth vessels. The sulfate removals obtained using membrane filters were compared to those obtained by direct addition of a bacterial suspension of equivalent cell density. The results of this experiment (Fig. 5) indicated the cells collected on membrane filters can serve as suitable inocula in the radioassay.

In using membrane filtration, the collected cells are exposed to air when the filter is transferred to the growth vessel and possibly during the early stages of incubation. Since sulfate-reducing bacteria are obligate anaerobes, an experiment was performed to determine cell survival when exposed to air. A series of membrane filters was prepared such that each filter retained $10^5$ cells of *D. desulfuricans*. The filters were exposed to air. After the desired exposure period, triplicate filters were processed by the radioassay. Sulfate removals were converted to equivalent cell densities, and survival was expressed as the fraction surviving relative to the time zero population. For a period of up to 2 h, exposure of *D. desulfuricans* to air had no lethal effect.

The radioassay was used to measure the functional potential of sulfate-reducing bacteria in the Fenholloway River, an anoxic river polluted by pulp mill wastes (5). Samples were collected at various sites, designated A to G, along the river (Fig. 6). The pulp mill is located at B and the river flow is toward the Gulf of Mexico, 30 miles (ca. 48.3 km) below G.

Prior to waste treatment the river had high concentrations of sulfide (Fig. 7a). Above the mill (A) sulfide was undetectable. At B it was 1.4 mg/liter where the waste was introduced, and at E it reached a maximum concentration of 21.3 mg/liter. Further downstream the sulfide concentration dropped and was 13.6 mg/liter at G. Measurement of sulfate removal in the radioassay essentially mimicked the sulfide profile; very slight sulfate removals were observed at A and B, with a progressive increase in sulfate removal from C to E, followed by a decrease at F and G. The sulfate removals in the radioassay were used to estimate the density of sulfate-reducing bacteria in the sample material. The mechanics of this operation will be presented in the Discussion; however, the density of sulfate-reducing bacteria at the various sampling points along the river is also shown in
Fig. 7. Profiles of bacterial sulfate removal, sulfide concentration, and equivalent population density of sulfate-reducing bacteria in the water of the Fen holloway River. (a) Pulp mill operating at normal capacity with no waste treatment. Incubation time for radioassay was 39 h. (b) Pulp mill was completely closed for annual maintenance; incubation time for radioassay was 44. (c) Pulp mill operating at normal capacity 1 week after waste treatment facilities made operational; incubation time for radioassay was 39 h.

Fig. 7a. The maximum population of sulfate reducers was approximately 10^8 cells/ml at E.

When the mill was closed for 2 weeks for annual maintenance, a drastic reduction in the sulfide concentration was observed (Fig. 7b). The maximum sulfide concentration was 0.4 mg/liter at the mill and decreased to 0.14 mg/liter at G. The shape of the percent sulfate removal curve in the radioassay was similar to the sulfide profile. The greatest sulfate-reducing intensity was found at the mill, indicating that the waste-holding lagoon is the source of the sulfate-reducing bacteria.

The third condition studied was after waste treatment facilities were installed (Fig. 7c). The treatment facilities had only been in operation for 1 week, but marked changes in water quality were already evident. With the mill operating at normal capacity with waste treatment (Fig. 7c), the maximum sulfide concentration was 7.7 mg/liter, and high sulfide values were found only at locations D and E. The sulfide concentrations at F and G were equivalent to those measured when the mill was closed (cf. Fig. 7b and c). Sulfate removals in the radioassay ranged between 10 and 45%, with the exception of B. The approximate cell density at B was greater than 10^8 cells/ml, with densities less
than 100 cells/ml at C, D, E, and F. Station G exceeded the limit of detection without concentrating the cells on membrane filters.

The radioassay was applied to sediment samples collected 1 week (Fig. 8a) and 5 weeks (Fig. 8b) after the waste treatment facilities were brought on line. The sediment samples in Fig. 8a were collected on 30 January 1974, the same day as the water samples of Fig. 7c. There are no water data to correspond to the sediment analyses of 27 February 1974, as there was no detectable sulfide along the entire length of the river. No growth of sulfate-reducing bacteria was observed in the water samples, even with an extended incubation period of 48 h. The significant observations for the sediments are the low interstitial sulfide concentrations and the higher density of sulfate-reducing bacteria in the sediments relative to the water. The peaks found in the radioassay data in Fig. 8b reflect the presence of a sludge deposit at C and a thick deposit of decaying leaves at F. The texture of the material at F prevented collection of sufficient material for the sulfide analysis. Comparison of the cell densities (at C and D) in Fig. 8a and b indicate a 10- to 20-fold decrease in the numbers of sulfate-reducing bacteria with increasing time after the initiation of treatment operations.

**DISCUSSION**

In soils, much of the microflora has a dormant period which is regulated by environmental conditions or nutritional sources (2). Gray and Williams (2) state that: "Most soil bacteria do not produce resting cells obviously different in gross structure from vegetative cells, and many probably exist in soil for much of the time as vegetative cells in a reduced state of metabolic activity." Cultural methods such as plate counting or roll tubes do not differentiate reduced activity from viability and, in fact, provide conditions under which dormant cells can grow to produce a measurable colony. When dealing with sediments, plating methods are further complicated by cells adhering to sediment particles or by clumping. The presence of large quantities of detrital or sediment material

![Fig. 8. Profiles of bacterial sulfate removal, sulfide content of the interstitial water, and equivalent population density of sulfate-reducing bacteria in the sediments of the Fenlolloway River. (a) Samples collected 1 week after waste treatment was initiated and incubated for 27 h (compare with water data of Fig. 7c). (b) Samples collected 5 weeks after onset of waste treatment and incubated for 31 h. On 27 February, sulfate-reducing bacteria and sulfide were undetectable and oxygen was detected in the river water.](image-url)
in or on the agar substrate can make enumeration difficult, as we found in working with the sediments from the Fenholloway River.

To overcome some of the difficulties of cultural enumeration methods and to provide a measurement that reflects in situ activity of sulfate-reducing bacteria, we developed a liquid culture method in which growth is followed by the removal of $^{35}$SO$_4$-$^2$ from the spent medium.

When a liquid medium is inoculated, the length of the lag phase is influenced by the physiological age and the density of the inoculating culture (3). In general, the lag is greatest when the inoculum is taken from a stationary-phase culture or a population in the phase of decline. However, increasing the size of the inoculum decreases the duration of the lag (3). Taken together, these two parameters can be used to quantify a population of sulfate-reducing bacteria. The measurement obtained reflects the combined effect of cell density and physiological activity or the functional potential of the inoculating material.

The significant parameters of growth in the enrichment culture medium of the radioassay that make possible the quantification of the functional potential of sulfate-reducing bacteria are as follows. (i) The duration of the lag phase of growth is a function of the inoculum density. The more cells added to the growth flask, the shorter the lag period (Fig. 1 and 2). (ii) The percentage of sulfate removed from the growth medium, as a function of time, depends on the inoculum density. For cells in the same physiological state, a larger inoculum will remove a given amount of sulfate in less time than a small inoculum (Fig. 2). (iii) The percentage of sulfate removed in the stationary phase is independent of inoculum density, but for smaller inocula a longer incubation period is required to reach this final removal (Fig. 1 and 4). (iv) The inoculum may be added to the growth flasks either as a cell suspension or collected on membrane filters (Fig. 5). (v) Exposure of *D. desulfuricans* to air for periods up to 2 h has no effect on cell viability or the ability to grow in the radioassay medium.

The radioassay may be used to quantify the functional potential of sulfate-reducing bacteria in two ways. The first method measures the percentage of sulfate removed from the growth medium after a fixed incubation period. An inoculum with a larger cell density, or a more physiologically active population, will remove more sulfate in a fixed time than will a smaller or less active inoculum (Fig. 1 and 2). The percentage of sulfate removed from the spent medium is thus a relative measure of the density or physiological activity of sulfate-reducing bacteria. The radioassay was used in this fashion in an anoxic river and the results are shown in Fig. 7. The significant point to note is that changes in sulfide concentration in the river show corresponding changes in sulfate removal from the radioassay medium. This correlation between sulfide content and the results of the radioassay is to be expected only if the assay reflects bacterial function or activity. When most-probable-number techniques were tested, no correlation with sulfide concentration was found, and sulfate-reducing bacteria were found in a uniform density over the entire course of the river (5).

The second method for using the radioassay introduces time as an additional variable and thereby extends the range of the technique.

In looking at Fig. 1, it is seen that for growth flasks incubated for a fixed time the radioassay can only differentiate between inocula with cell densities that differ at most by a factor of 50. This range can be extended by incubating cultures with smaller inocula for longer periods of time. The formation of a black precipitate can be used as a visual index of culture development. When the duration of incubation is varied, the percentage of sulfate removed from the growth medium can no longer be used as a measure of bacterial density or function. All cultures, with sufficient time, will reach a given percentage of sulfate removal and so appear as equivalent if time is ignored. For example, an inoculum with $10^8$ cells will remove 50% sulfate in 26 h, whereas an inoculum of $10^9$ cells will reach this same removal in 35 h. To resolve this problem, the family of growth curves of Fig. 1 is used to translate incubation time and percentage of sulfate removed into a population equivalent. The cell density so obtained is not the actual population density contained in the sample, but rather it is the equivalent inoculum density of the standardizing *D. desulfuricans* culture that will reduce an equivalent amount of sulfate in the same incubation time. A large population of less active sulfate reducers and a smaller, more physiologically active one could reduce the same amount of sulfate and therefore be interpreted as equal populations. Numerically the populations are not equal but their ability to reduce sulfate is.

The use of the curves of Fig. 1 to arrive at a population assumes that all species of sulfate-reducing bacteria will behave in a manner analogous to *D. desulfuricans* under the conditions of the radioassay. The data indicate that one pure culture isolate behaved exactly as did *D. desulfuricans* (Fig. 3), but one environmen-
of a water sample and used to inoculate a set of growth flasks, the resultant growth curves had a temporal displacement of 5.2 h for each 10-fold dilution, rather than 3.7 h as was obtained with *D. desulfituricans* (Fig. 4). The difference in temporal displacement will, in effect, reduce the apparent size of the sulfate-reducing population when the curves of Fig. 1 are used to estimate population equivalents. If a closer approximation to the actual population density is desired, rather than an estimate based on relative physiological activity, the differences in the displacement of the experimental growth curves relative to the standard curves in Fig. 1 can be corrected for. To make this correction, a set of growth curves in which the cell densities differ by factors of 10 and 100 is generated, as was done for the sample in Fig. 4. The time interval between each 10-fold dilution is then determined and used to derive an adjusted incubation time that conforms to the standard curves in Fig. 1.

The procedure is illustrated using the data of Fig. 4 as follows. The sample incubation period is 36 h, the time interval between successive 10-fold dilutions of sample is 5.2 h, and the time interval between successive 10-fold dilutions of standard curves (Fig. 1) is 3.7 h. The adjusted incubation period \( = 3.7/5.2 \times 36 = 25.6 \) h. Using the standard curves (Fig. 1) we obtain a population estimate of \( 10^5 \) cells/ml for an unadjusted incubation period of 36 h and a population density of \( 6 \times 10^4 \) cells/ml when the adjusted incubation period of 25.6 h is used. The population density as determined by anaerobic plate counts was \( 5 \times 10^4 \) cells/ml.

In the radioassay, the activity of sulfate-reducing bacteria is determined by measuring changes in the specific disintegration rate per milliliter of the radioactive sulfur in the spent growth medium. Additions of stable sulfur with the inoculating material would dilute the radioactive sulfate and reduce the apparent rate of \( ^{35} \text{SO}_4 \) removal. The API sulfate broth contains 208.5 mg of anhydrous sulfate per liter (Difco Laboratories), and experiments in which 20, 40, and 60 mg of stable sulfate per liter were added indicated no appreciable effect between the range of 20 to 80% sulfate removal.

The radioassay was used to determine the polluting effects of a pulp mill on a small river in north Florida. A detailed description of the area has been presented elsewhere [15]. The Fenholloway River is approximately 30 miles (ca. 48.3 km) long, with average flow rates of 80 ft\(^3\) (2.2 m\(^3\))/s in the winter and 2 ft\(^3\) (0.056 m\(^3\))/s in the summer. The pulp mill discharges 55 million gallons (ca. 208 million liters) of waste material per day which constituted about 54% of the river flow in the winter and almost 100% of the summer flow. The river was anoxic from the pulp mill to the Gulf of Mexico.

During the course of the study, the mill was closed for repair, and at the end of the survey waste treatment facilities were installed. The effects of the mill were easily seen in comparison to those times when mill activity was curtailed. The principal findings when the radioassay was applied to the Fenholloway River are as follows. (i) Bacterial sulfate reduction (sulfate removal) curves mimicked the sulfide concentrations in the river under all conditions of mill operation (Fig. 7). In contrast, the use of most-probable-number techniques suggested a constant density of sulfate-reducing bacteria (5). (ii) When the mill was not functioning the activity of the sulfate-reducing bacteria and the sulfide concentration decreased between 10- and 100-fold. (iii) When the treatment facilities were installed, they effectively reduced the waste levels entering the stream to the point where anoxic conditions prevailed over only a portion of the river after 1 week. Within 5 weeks oxygen was detected along the entire river.

The radioassay was applied to sediment samples collected when the treatment facilities were brought on line and 5 weeks later. The sediments, like the water samples, showed signs of improvement in quality between the two sampling periods; however, the rate of change was much slower than was observed for the water. The sediments contained approximately \( 10^4 \) times the maximum bacterial density of the water (cf. Fig. 7a and 8a), but the population in the sediments decreased by a factor of 10 after waste treatment facilities had been functional for 5 weeks (Fig. 8a and b). In spite of the larger population of sulfate reducers in the sediments, the sulfide concentration of the interstitial water was generally lower than that found in the overlying water. This difference probably reflects the formation of FeS in the sediments.

It is of particular interest to note the effects of mill operations on the quality of the river and the rate of recovery when treatment facilities were installed. When the mill was closed for 2 weeks for maintenance, the river water showed almost complete recovery from its anoxic state, as indicated by the reductions in sulfide concentration and activity of sulfate-reducing bacteria (Fig. 7a and b). When the mill resumed operations, a grossly polluted state similar to Fig. 7a was resumed. Within 2 months, waste treatment effectively reduced the effects of the mill effluent to the point where oxygen was detecta-
ble throughout the river. Dissolved oxygen has long been the primary means of studying water quality and for determining the efficacy of waste treatment. The application of the radioassay to a polluted river indicated the effects of mill operations and waste treatment weeks before oxygen was detectable in the stream. Sediments were slower to recover than the overlying water column, but the radioassay was effective in depicting changes in benthic deposits. Measurements of microbial activity may well be a more suitable indicator of changes in polluted environments than the conventional measurements of dissolved oxygen and biochemical oxygen demand.

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