

## Thermophilic Sulfate Reduction in Hydrothermal Sediment of Lake Tanganyika, East Africa

LARS ELSGAARD,<sup>1\*</sup> DANIEL PRIEUR,<sup>2</sup> GASHAGAZA M. MUKWAYA,<sup>3</sup> AND BO B. JØRGENSEN<sup>4</sup>

*Université Pierre et Marie Curie, Paris 6,<sup>1</sup> and Centre National de la Recherche Scientifique,<sup>2</sup> Station Biologique de Roscoff, F-29682 Roscoff, France; Centre de Recherche en Sciences Naturelles, Station d'Uvira, Uvira, Zaire<sup>3</sup>; and Max Planck Institute for Marine Microbiology, D-2800 Bremen 33, Germany<sup>4</sup>*

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**In environments with temperatures above 60°C, thermophilic prokaryotes are the only metabolically active life-forms. By using the <sup>35</sup>SO<sub>4</sub><sup>2-</sup> tracer technique, we studied the activity of sulfate-reducing microorganisms (SRM) in hot sediment from a hydrothermal vent site in the northern part of freshwater Lake Tanganyika (East Africa). Incubation of slurry samples at 8 to 90°C demonstrated meso- and thermophilic sulfate reduction with optimum temperatures of 34 to 45°C and 56 to 65°C, respectively, and with an upper temperature limit of 80°C. Sulfate reduction was stimulated at all temperatures by the addition of short-chain fatty acids and benzoate or complex substrates (yeast extract and peptone). A time course experiment showed that linear thermophilic sulfate consumption occurred after a lag phase (12 h) and indicated the presence of a large population of SRM in the hydrothermal sediment. Thermophilic sulfate reduction had a pH optimum of about 7 and was completely inhibited at pH 8.8 to 9.2. SRM could be enriched from hydrothermal chimney and sediment samples at 60 and 75°C. In lactate-grown enrichments, sulfide production occurred at up to 70 and 75°C, with optima at 63 and 71°C, respectively. Several sporulating thermophilic enrichments were morphologically similar to *Desulfotomaculum* spp. Dissimilatory sulfate reduction in the studied hydrothermal area of Lake Tanganyika apparently has an upper temperature limit of 80°C.**

Anaerobic oxidation of organic matter depends on microbial respiration processes, which may utilize, e.g., NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, or CO<sub>2</sub>, as a terminal electron acceptor (42, 53). In marine sediments, dissimilatory sulfate reduction to H<sub>2</sub>S is the quantitatively most important of these processes (30), but in freshwater sediments, a low sulfate concentration generally limits sulfate reduction rates, at least on an areal basis (4). Sulfate reduction is mediated by anaerobic sulfate-reducing microorganisms (SRM), which oxidize a range of low-molecular-weight organic compounds released by fermentation processes (46).

In geothermal environments, organic matter is mineralized by thermophilic prokaryotes (i.e., with optimum growth at temperatures above 45°C). Although they appear less taxonomically diverse, the thermophiles represent all major physiological categories found among the mesophiles (6, 20, 33, 34). The presently known thermophilic SRM are found within the genera *Desulfotomaculum*, *Thermodesulfobacterium*, *Thermodesulfovibrio*, and *Archaeoglobus* (23, 37, 47, 48). Because of endospore formation, thermophilic *Desulfotomaculum* spp. are widespread, and large numbers of spores may be found even in permanently cold sediments where temperatures enabling their growth never occur (26, 52). *Thermodesulfobacterium* and *Thermodesulfovibrio* spp. have been isolated from terrestrial and aquatic hot springs, while *Archaeoglobus* spp. have been isolated from submarine hydrothermal systems (23, 37, 39, 48, 54). The rates and temperature dependence of sulfate reduction in some of these environments have been studied (14, 32, 54). The highest temperatures at which microbial sulfate reduction occurs (102 to 110°C) thus have been found in sediments from the hydrothermal vent area at Guaymas Basin

(14, 31), which also were found to harbor the sulfate reducer *Archaeoglobus profundus*, with a maximum temperature for growth of 90°C (7).

In the present communication, we report the temperature dependence of sulfate reduction in hydrothermal sediment from a newly discovered hydrothermal site in Lake Tanganyika, East Africa. The influence of added substrates and pH on thermophilic sulfate reduction was assayed. Thermophilic SRM were enriched at 60 and 75°C, and the temperature dependence of sulfide production was tested at 40 to 90°C.

### MATERIALS AND METHODS

**Study site.** Meromictic Lake Tanganyika (3 to 9°S, 29 to 31°E) is the largest (34,000 km<sup>2</sup>) and deepest (maximum depth, 1,470 m; mean depth, 570 m) of the Great Lakes situated in the East African Rift Valley System (Fig. 1). The lake has been physically isolated over a long geological time (6 to 10 million years) and has developed a diverse, endemic fish population (11, 19). At present, the hydrology and ionic composition of the lake water are largely controlled by inflow from the Malagarasi River and the Ruzizi River and outflow through the Lukuga River (10–13). The lake water is alkaline (pH 8.5 to 9.2) and has a low salinity and a sulfate concentration of 30 to 50 μM (12, 40). Temperatures in the littoral part of the lake are 25 to 27°C throughout the year (11).

Sublacustrine hydrothermal activity was discovered in the northern part of Lake Tanganyika in 1987 (44, 45) and was further explored via SCUBA diving during the Tanganyidro expedition in August to October 1991 (21, 41). Samples for the present study were collected at Cape Banza (4°3.07'S, 29°14.65'E), where the hydrothermal activity occurred at a sublacustrine plateau (300 by 40 m) situated at a water depth of 0 to 6 m. Seventy-two groups of aragonite chimneys and several patches of hot sediments (about 2 by 2 m) were identified in the area. Active chimneys ranged from 5 to 70 cm

\* Corresponding author. Present address: Danish Institute of Plant and Soil Science, Department of Soil Science, Research Centre Foulum, P.O. Box 23, DK-8830 Tjele, Denmark.

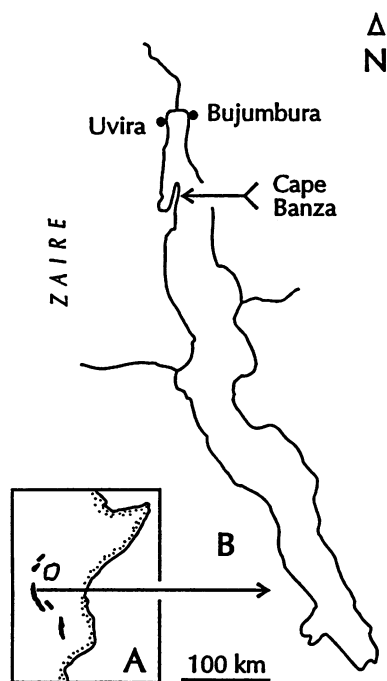


FIG. 1. Locations of Lake Tanganyika in East Africa (A) and the hydrothermal vent site at Cape Banza in the northern part of the lake (B).

in height and vented with flow rates estimated at 1 to several liters per second (41). Temperatures measured within the hydrothermal fluids ranged from 66 to 103°C, and white filamentous bacteria frequently occurred at the orifices of the chimneys (41). Hydrothermal sediments occurred adjacent to the hydrothermal chimneys, but no direct emission of hot water appeared to take place through the heated sediments.

**Sampling.** Hot sediment was sampled at two hydrothermal sediment patches, Banza I (site 27/28) and Banza II (site 65/68), located at a water depth of 2 to 3 m and spaced apart about 150 m. In situ temperatures within the upper 10 cm of the hydrothermal sediment were measured with a thermistor probe, and sediment with temperatures of between 60 and 101°C was sampled in acrylic cores (inner diameter, 26 to 36 mm; length, 20 cm). The sediment had a black, sandy, gravel-rich appearance and could be cored to a depth of 8 to 12 cm. Sediment for slurry experiments was transferred to 300-ml glass bottles prefilled with filter-sterilized (pore size, 0.2  $\mu\text{m}$ ; Millipore) lake water, chemically reduced with a sterile dithionite solution (final concentration, 100  $\mu\text{M}$ ). The bottles were completely filled with sediment from each site and were stoppered and screw capped. During the manipulations, sediment exposure to atmospheric  $\text{O}_2$  was very brief, and only slight dilution of the sediment occurred as the reduced lake water in the bottles was replaced by the sediment. Hydrothermal sediment (Banza I) and a hydrothermal chimney (5 cm in height; 2 to 3 cm in diameter) were collected for enrichment of thermophilic SRM. The samples were transferred to 50-ml serum bottles prefilled with filter-sterilized, reduced lake water (prepared as described above). The bottles were closed with butyl rubber stoppers and aluminum crimp seals. For the determination of physical parameters, sediment cores from Banza I were cut into 1- to 2-cm segments, which were transferred directly into 10 ml of 10%  $\text{ZnCl}_2$  (wt/wt) in

screw-cap vials. During the field expedition, samples were kept in the dark at ambient temperatures (20 to 25°C). Upon return to the laboratory, within 10 days after sampling, the samples were stored at 4°C. All experiments were initiated within 4 weeks after sampling.

**Medium and slurry preparation.** SRM medium was prepared from a basal salts solution, which contained (in grams per liter of distilled water) the following:  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.3;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.15;  $\text{NH}_4\text{Cl}$ , 0.3;  $\text{KH}_2\text{PO}_4$ , 0.2; and  $\text{Na}_2\text{SO}_4$ , 1.5 (for slurry experiments, the addition of  $\text{Na}_2\text{SO}_4$  ranged from 1 to 4 g liter<sup>-1</sup>). Upon autoclaving and cooling under an atmosphere of  $\text{N}_2$ , the medium was supplemented with nonchelated trace elements, a vitamin mixture, vitamin  $\text{B}_{12}$ ,  $\text{NaHCO}_3$ , and  $\text{Na}_2\text{S}$  as described by Widdel and Bak (49). To enhance the growth of SRM, a freshly prepared, filter-sterilized (pore size, 0.2  $\mu\text{m}$ ) dithionite solution was added to a final concentration of 100  $\mu\text{M}$  (49).

A slurry of Banza I sediment was prepared from 600 ml of hydrothermal sediment in an  $\text{O}_2$ -free glove bag (Coy Laboratory Products, Inc.). The sediment was passed through a 1-mm-mesh-size sieve and mixed with SRM medium to a final volume of 1 liter. The suspension was stirred, and sand was allowed to settle before the slurry was transferred to a bottle with a bottom outlet for subsampling (the bottom outlet was connected to gas-tight Iso-Versinic tubing closed with a pinch-cock). The stoppered slurry bottle was taken out of the glove bag and equilibrated at 8°C under a headspace of  $\text{N}_2$ . The Banza I slurry had a density of 1.04 g ml<sup>-1</sup>, a pH of 7.9, and an  $\text{SO}_4^{2-}$  concentration of 12.6 mM. A slurry of Banza II sediment was prepared similarly from a 300-ml sediment sample that was mixed with 600 ml of SRM medium. The Banza II slurry had a density of 1.02 g ml<sup>-1</sup>, a pH of 7.4, and an  $\text{SO}_4^{2-}$  concentration of 4.7 mM.

**Sediment parameters.** Densities of core segments (grams cubic centimeter<sup>-1</sup>) were determined from the weights of known sediment volumes, while porosities (milliliters cubic centimeter<sup>-1</sup>) were calculated from the water loss after drying at 105°C for 24 h. Total organic matter was estimated as the weight loss of dried sediment samples after ignition at 450°C for 6 h. Sulfate concentrations were determined by nonsuppressed anion chromatography (Waters).

**Sulfate reduction in relation to temperature and substrates.** The temperature dependence of sulfate reduction was studied by incubation of samples in a stable temperature gradient that was established over 31 equidistant holes drilled in an insulated aluminum block (185 by 15 by 10 cm). The gradient ranged from 20 to 90°C and was controlled by a thermostat-regulated heating coil and a cooling bath attached to opposite ends of the block. The holes in the aluminum block had a diameter and a length so that the entirety of the 10-ml culture tubes could be subjected to temperature control.

Banza I slurry was homogenized by magnetic stirring and dispensed into three series of 10-ml culture tubes by use of slight  $\text{N}_2$  overpressure. All manipulations of the slurries were done under a flow of  $\text{N}_2$  gas (24) to avoid contamination with atmospheric  $\text{O}_2$ . The culture tubes each received 7 to 8 ml of suspension and were closed with Venocject stoppers. To one series of tubes, yeast extract and peptone (YP-mix) were each added to a final concentration of 0.2%, while another series of tubes was amended with a substrate mixture (C-mix) of acetate, formate, lactate, propionate, benzoate, and isobutyrate (each added to a 1 mM final concentration). With these electron donors added, the temperature response of SRM could be studied without substrate limitation and thus independently from the temperature response of the fermentative bacteria that normally provide the substrates for SRM (32, 46).

Carrier-free  $^{35}\text{SO}_4^{2-}$  ( $\sim 0.5$  MBq) was added to all tubes, which were then thoroughly mixed and incubated in the temperature block for 10 days (incubation of slurry samples at  $8^\circ\text{C}$  was done in a thermostat-controlled cold room rather than in the temperature block). During incubations, temperature measurements were taken at each end of the block, while temperatures were measured along the entire gradient before and after incubations. The temperatures in the gradient were stable to within  $\pm 0.5^\circ\text{C}$ . Incubations were terminated by injection of 1 ml of 20% zinc acetate into the samples that were mixed and cooled. This procedure prevented further microbial activity and served to fix the produced  $\Sigma\text{H}_2\text{S}$  as ZnS ( $\Sigma\text{H}_2\text{S} = \text{H}_2\text{S} + \text{HS}^- + \text{S}^{2-}$ ). Sulfate reduction rates were determined as described by Jørgensen et al. (29, 32). After centrifugation of each culture tube, the supernatant was recovered for analysis of  $^{35}\text{SO}_4^{2-}$ . The sediment precipitate was washed twice in tap water (to eliminate residual  $^{35}\text{SO}_4^{2-}$ ) and stored at  $4^\circ\text{C}$  until analysis of  $^{35}\text{S}_{\text{red}}$  ( $\text{S}_{\text{red}} = \Sigma\text{H}_2\text{S} + \text{FeS} + \text{S}^0 + \text{FeS}_2$ ) by a single-step chromium reduction procedure (17). In brief,  $\text{S}_{\text{red}}$  was liberated as  $\text{H}_2\text{S}$  and trapped in 10 ml of 5% zinc acetate as ZnS.  $^{35}\text{S}_{\text{red}}$ , rather than  $\Sigma\text{H}_2^{35}\text{S}$ , was quantified, as precipitation with iron and chemical isotope exchange may transfer the  $^{35}\text{S}$  from  $\Sigma\text{H}_2\text{S}$  to the other reduced sulfur pools (15, 18).  $^{35}\text{SO}_4^{2-}$  and  $^{35}\text{S}_{\text{red}}$  were quantified by liquid scintillation counting (Packard Tri-Carb 2200 CA apparatus) after gelling of 5 ml of diluted subsamples with 5 ml of scintillation fluid (DynaGel; Baker Chemicals). The rates of sulfate reduction (in micromolar day $^{-1}$ ) were calculated as follows:  $a \cdot (A + a)^{-1} \cdot [\text{SO}_4^{2-}] \cdot t^{-1} \cdot 1.06$ , where  $a$  is the radioactivity in the  $\text{S}_{\text{red}}$  pool,  $A$  is the radioactivity in the sulfate pool,  $[\text{SO}_4^{2-}]$  is the sulfate concentration (micromolar) at the time of tracer addition,  $t$  is the incubation time (days), and 1.06 is a correction factor for the kinetic isotope fractionation between  $^{32}\text{SO}_4^{2-}$  and  $^{35}\text{SO}_4^{2-}$  (29).

**Time course and pH dependence of thermophilic sulfate reduction.** The time course of thermophilic  $^{35}\text{SO}_4^{2-}$  consumption was monitored for a 100-ml aliquot of Banza II slurry with added C-mix (final concentration, 1 mM). The sediment slurry was withdrawn under  $\text{N}_2$  into a 120-ml serum bottle, and  $\sim 0.5$  MBq of carrier-free  $^{35}\text{SO}_4^{2-}$  was added. The bottle was stoppered (butyl rubber) and incubated at  $60^\circ\text{C}$  for 15 days. During incubation, weighed subsamples ( $\sim 0.8$  ml) were withdrawn into  $\text{N}_2$ -washed syringes and precipitated with 0.5 ml of 20% zinc acetate. After centrifugation of the Zn-fixed subsamples, radioactivity remaining in the supernatant was determined by liquid scintillation counting and was attributed to  $^{35}\text{SO}_4^{2-}$ .

The effect of pH on sulfate reduction was assayed by use of a series of serum bottles (50 ml) that were filled with 30 ml of 50 mM Tris, with pH values adjusted in the range of 7.0 to 9.2. The buffer was autoclaved and cooled under an atmosphere of  $\text{N}_2$ . To each bottle was added 50  $\mu\text{l}$  of 0.5 M  $\text{Na}_2\text{S}$  (for reduction of the buffer),  $\sim 0.5$  MBq of carrier-free  $^{35}\text{SO}_4^{2-}$ , and a weighed amount of Banza II sediment slurry ( $\sim 15$  ml) with added C-mix (final concentration, 1 mM). The bottles were stoppered under  $\text{N}_2$  and incubated at  $60^\circ\text{C}$  for 5 days. Incubations were terminated by injection of 5 ml of 20% zinc acetate into the slurries, and sulfate reduction rates were determined as previously described. In all samples, the pH was stable or showed a slight increase ( $< 0.2$  unit) during incubations (the mean increase  $\pm$  standard deviation was  $0.06 \pm 0.06$ ;  $n = 13$ ). Sulfate reduction rates were correlated with the mean of pH values measured before and after incubation.

**Enrichment, sulfide production, and growth of thermophilic SRM.** Under an atmosphere of  $\text{N}_2$ - $\text{CO}_2$  (88:12), reduced SRM medium was distributed into 29-ml culture tubes in aliquots of

20 ml. The tubes were inoculated with hydrothermal sediment and chimney samples ( $\sim 1$  g) and sealed with butyl rubber stoppers and aluminum crimps. Enrichments were made at pH 7 to 8 with four combinations of added electron donors: lactate-acetate (10 mM each), benzoate-isobutyrate (2.5 and 5 mM, respectively), propionate-formate (10 mM each), and yeast extract-peptone (0.1% each). Enrichments with each substrate combination were incubated at 60, 75, 93, and  $103^\circ\text{C}$ . Sulfide production in the enrichments was assayed qualitatively after 14 days of incubation (9), and 5% inocula of the positive cultures were transferred to the corresponding fresh media. Enrichments grown on lactate-acetate and propionate-formate were also transferred to media with lactate, acetate, propionate, or formate as the sole electron donor. Subsequent transfers of positive enrichments were done at intervals of 4 to 7 days.

The temperature dependence of sulfide production and growth in SRM cultures, transferred three to five times, was tested with a temperature gradient ranging from 40 to  $90^\circ\text{C}$ . Three cultures were inoculated (5%) into SRM medium supplemented with 20 mM lactate in 0.5-liter bottles with a bottom outlet for subsampling. The inoculated medium was dispensed (7 to 8 ml) into sterile 10-ml culture tubes as described for sediment slurries and incubated in the temperature gradient for 2 to 4 days. Growth was monitored by inserting the culture tubes into a spectrophotometer (Spectronic 70; Bausch and Lomb) and measuring the increase in optical density at 562 nm. After incubation,  $\Sigma\text{H}_2\text{S}$  in the cultures was precipitated as ZnS by injecting 1 ml of 20% zinc acetate through the stoppers. The cultures were thoroughly mixed, and the ZnS was assayed spectrophotometrically by the methylene blue method (8).

In a control experiment, sterile SRM medium in culture tubes closed by black rubber stoppers (Sargent-Welch no. 00) rapidly lost  $\Sigma\text{H}_2\text{S}$  ( $> 0.18$  mM  $\text{h}^{-1}$ ) during incubation at a high temperature ( $> 50^\circ\text{C}$ ). This result most likely was due to the incorporation of sulfur into the black rubber (a mixture of styrene-butadiene and natural rubber). Butyl rubber stoppers and Venoject stoppers, used in the present experiments, both prevented high-level sulfide loss.

## RESULTS

The hydrothermal sediment at Cape Banza (Banza I) had a density of  $1.9 \pm 0.4$  g  $\text{cm}^{-3}$  in the upper 0 to 2 cm which increased to  $2.2 \pm 0.1$  g  $\text{cm}^{-3}$  at a depth of 5 to 7 cm. The porosities were  $40\% \pm 4\%$  in the upper 0 to 2 cm and  $35\% \pm 5\%$  at a depth of 5 to 7 cm (mean  $\pm$  standard deviation for five cores). The depth distribution of organic matter was variable and ranged from 2.5 to 4.1% of the sediment dry weight. Temperatures within the hydrothermal sediment patches showed a uniform depth distribution in the upper 10 cm but showed an extensive horizontal variation. Thus, on a decimeter scale, sediment temperatures ranged from a maximum of  $101^\circ\text{C}$  to about  $25^\circ\text{C}$  (i.e., ambient lake water temperature). No bacterial mats were observed at the hydrothermal sediment patches, and no smell of  $\text{H}_2\text{S}$  was noted during processing of the sediment.

**Sulfate reduction in relation to temperature and substrates.** Figure 2 shows the effect of temperature and substrate amendments on sulfate reduction in hydrothermal sediment from Cape Banza (Banza I). In nonamended slurries, sulfate reduction could be detected from 8 to  $80^\circ\text{C}$ , and maximal rates occurred at about 45 and  $65^\circ\text{C}$  (Fig. 2A). However, between the two optimum temperatures, the rates of sulfate reduction were also high. In slurries amended with a mixture of organic

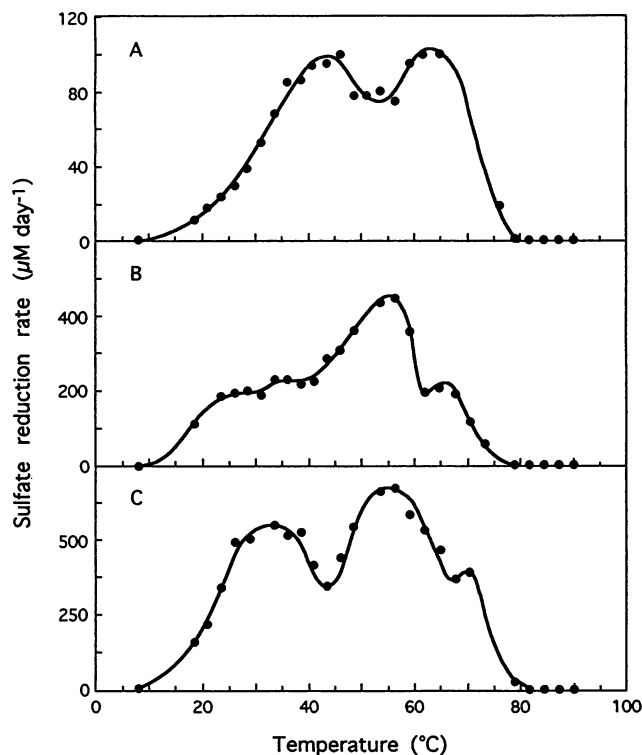


FIG. 2. Temperature profiles of sulfate reduction rates in hydrothermal sediment from Cape Banza (Banza I). Sediment suspensions were incubated with  $^{35}\text{SO}_4^{2-}$  for 10 days with no addition of electron donors (A), the addition of C-mix (B), and the addition of YP-mix (C).

acids (C-mix), mesophilic sulfate reduction showed no distinct temperature optimum but rather showed a plateau at 23 to 41°C (Fig. 2B). Thermophilic sulfate reduction showed an optimum at about 56°C and perhaps a smaller, second optimum at about 65°C (Fig. 2B). With YP-mix added as a substrate (Fig. 2C), maximal rates of sulfate reduction were found in broad peaks at about 34 and 56°C. In both substrate-amended slurries, the upper temperature limit for microbial sulfate reduction occurred at ~80°C, while a span of 24°C occurred between the optimum (56°C) and the maximum (80°C). This temperature span was larger than that usually observed for single bacterial populations (51) and agreed with the occurrence of different thermophilic populations suggested by the substructures at 65 to 70°C in the rate-versus-temperature curves (Fig. 2B and C).

Compared with the results for the nonamended slurries, the addition of substrate mixtures caused a substantial increase in sulfate reduction rates at all incubation temperatures (Fig. 2). Thus, the average levels of stimulation of sulfate reduction by C-mix and YP-mix were five- and ninefold, respectively. The stimulation was highest in the temperature range from 8 to 30°C, but sulfate reduction at 48 to 58°C also was preferentially stimulated. Maximal rates of thermophilic sulfate reduction in nonamended (~100  $\mu\text{M day}^{-1}$ ) and YP-mix-amended (~670  $\mu\text{M day}^{-1}$ ) sediment slurries corresponded to levels of total sulfate reduction of 1 and 6.7 mM, respectively, during the 10-day incubation period. Thus, as the initial sulfate concentration in the slurry was 12.6 mM, no sulfate limitation occurred during the experiment.

**Time course and pH dependence of thermophilic sulfate reduction.** Figure 3 shows the time course of  $^{35}\text{SO}_4^{2-}$  con-

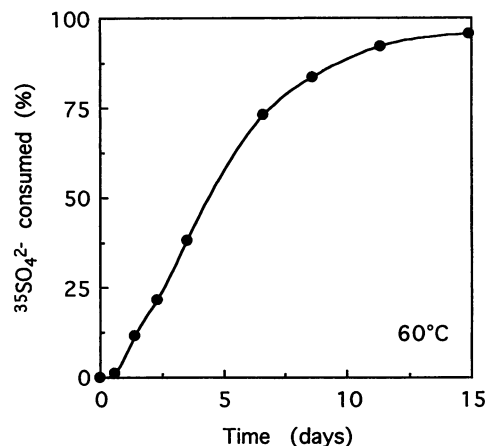


FIG. 3. Time course of thermophilic  $^{35}\text{SO}_4^{2-}$  consumption in hydrothermal sediment from Cape Banza (Banza II). The sediment suspension was incubated at 60°C with C-mix.

sumption at 60°C in a substrate-amended sediment slurry (Banza II). Sulfate consumption proceeded at a constant rate after a lag phase of 12 h (Fig. 3). The rate slowed down after 7 days, apparently as the sulfate pool became increasingly exhausted. The consumption rate during days 0.5 to 7 was 12.0% of the added  $^{35}\text{SO}_4^{2-}$  day $^{-1}$ . Calculated from the initial sulfate concentration in the slurry (4.7 mM), the  $^{35}\text{SO}_4^{2-}$  consumption rate was equal to a sulfate reduction rate of 564  $\mu\text{M day}^{-1}$ .

The pH dependence of sulfate reduction at 60°C in a hydrothermal sediment slurry is shown in Fig. 4. The rates of thermophilic sulfate reduction were highest at pH 7.0 to 7.5 and then decreased gradually as pH increased. Sulfate reduction ceased at pH 8.8 to 9.2; thus, despite the alkalinity of Lake Tanganyika water (pH 8.5 to 9.2), thermophilic sulfate reduction was clearly neutrophilic rather than alkaliphilic.

**Enrichment, sulfide production, and growth of thermophilic SRM.** The results of enrichment of sulfide-producing cultures are shown in Table 1. Hydrothermal sediment and chimney samples both yielded  $\text{H}_2\text{S}$ -producing cultures at 60°C, whereas only chimney samples yielded  $\text{H}_2\text{S}$  production at 75°C. All four

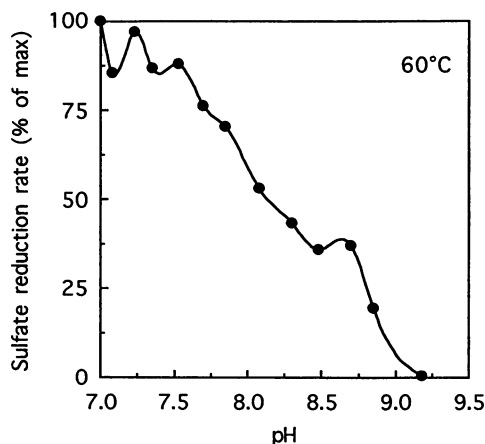


FIG. 4. Effect of pH on thermophilic sulfate reduction in hydrothermal sediment from Cape Banza (Banza II). The sediment suspension was incubated at 60°C for 5 days with C-mix. max, maximum.

TABLE 1. Sulfide production in enrichments from the hydrothermal Cape Banza area

Culture code <sup>a</sup>	H <sub>2</sub> S production <sup>b</sup> in the following samples at the indicated temp (°C):			
	Chimney		Sediment	
	60	75	60	75
LA 7.4	–	++	++	–
LA 7.9	++	++	++	–
BI 7.4	++	–	–	–
PF 7.3	+	++	++	–
PF 7.9	+	+	+	–
YP 7.1	++	+	++	–
YP 7.6	++	+	+	–

<sup>a</sup> Designations indicate the electron donors and pH in the media: LA, lactate plus acetate; BI, benzoate plus isobutyrate; PF, propionate plus formate; YP, yeast extract plus peptone.

<sup>b</sup> ++, +, or –, good, poor, or no sulfide production.

substrate combinations tested gave rise to sulfide production and thus were utilized by thermophilic SRM. No sulfide production or increase in turbidity was observed at 93 or 103°C. When transferred to media with single electron donors, sulfide-producing cultures grown on lactate-acetate and propionate-formate proliferated on lactate and formate but not on acetate and propionate (2 weeks of incubation). Sporulating cells (1 by 3 to 5 μm) with rod- to vibrio-shaped morphology predominated in the lactate-grown enrichments at 60°C. Enrichments grown on lactate or formate at 75°C were dominated by rod-shaped cells (1 to 1.5 by 3 to 6 μm) with no apparent sporulation. These cells often occurred in pairs or in chains of three to five. Enrichments grown on YP-mix often showed a rapid turbidity increase (within a few hours) without accompanying sulfide production. However, upon further incubation (3 to 5 days), substantial amounts of sulfide (>4 mM) were formed in these cultures. This result indicated that the substrates for thermophilic sulfate reduction were produced by preceding thermophilic fermentations. In all cultures grown on YP-mix, several bacterial morphologies were observed, and sulfide production may not have been due to the morphologically dominant cell types.

The temperature responses of sulfide production in two lactate-grown (20 mM) enrichment cultures are shown as Arrhenius plots in Fig. 5. The two cultures, LC60 and LS60, were enriched at 60°C from chimney and sediment samples, respectively, and showed an almost identical temperature dependence of sulfide production. The optimum temperature for sulfide production was 63°C, and the maximal ΣH<sub>2</sub>S concentrations in the cultures were 8.1 mM for LC60 and 7.1 mM for LS60. In the lactate-grown culture LC75, enriched at 75°C from a chimney sample, growth and sulfide production occurred concurrently in a narrow temperature range from 60 to 75°C (Fig. 6). The optimum temperature for growth and sulfide production was 71°C, and the maximal ΣH<sub>2</sub>S concentration was 6.7 mM. In agreement with the slurry experiments, the results of the enrichment studies indicated the occurrence of overlapping temperature groups of SRM in the hydrothermal environment of Lake Tanganyika.

## DISCUSSION

The microbiology of sediments and hydrothermal activity in Lake Tanganyika has not received previous attention. At deep-sea hydrothermal vents, chemoautotrophic bacteria provide a basis for the establishment of diversified animal com-

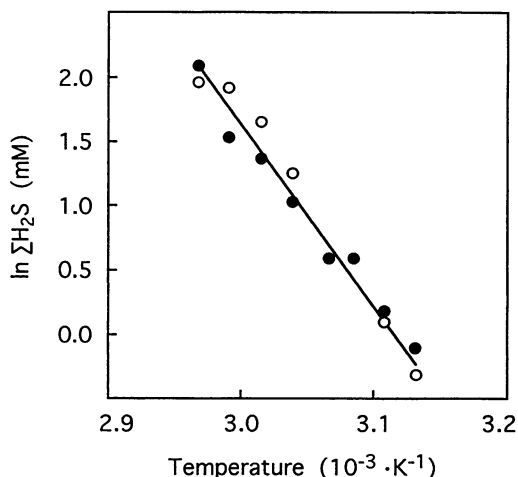


FIG. 5. Temperature dependence (Arrhenius plots) of sulfide production in lactate-grown enrichment cultures from the Cape Banza area. Cultures LC60 (●) and LS60 (○) were enriched from hydrothermal chimney and sediment samples, respectively.

munities, including invertebrates with symbiotic prokaryotes (27). Filamentous *Beggiatoa*-like bacteria frequently were found in association with hydrothermal discharges in Lake Tanganyika, but no specific macrofauna were observed in association with the shallow hydrothermal activity that was investigated down to a depth of 46 m (21, 41). Deeper sublacustrine springs in Lake Tanganyika have been indicated (43–45), but the existence of oxygen-dependent, chemoautotrophic communities at a great depth seems to be excluded, as the lake is anoxic and sulfidic below a depth of 100 to 200 m (10, 12).

**Sulfate reduction and temperature.** Meso- and thermophilic sulfate reduction in the sediment slurries generally occurred at similar high rates. The significance of mesophilic sulfate reduction in the hydrothermal sediment is not clear, however, as our sampling procedure may have caused a proliferation of meso-

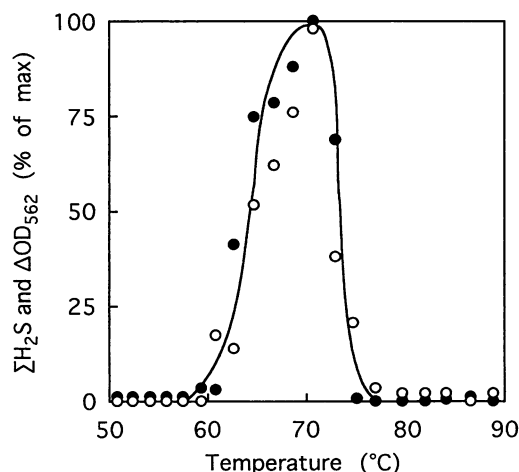


FIG. 6. Temperature dependence of sulfide production (ΣH<sub>2</sub>S) (●) and growth (change in optical density at 562 nm [ΔOD<sub>562</sub>]) (○) in the lactate-grown culture LC75 enriched from a hydrothermal chimney sample at Cape Banza. max, maximum.

philic SRM during transport to the laboratory. On the other hand, the sampling and storage procedures did not promote the growth of thermophilic sulfate reducers. Also, it has been found that even some non-spore-forming thermophilic SRM may survive for 1 year at 27°C (23). In a time course experiment done at 60°C (Fig. 3), it was shown that thermophilic sulfate consumption followed a linear course after a lag phase of 12 h. This result suggested the presence and activity of a stable thermophilic SRM population without significant growth upon incubation. The high rate of sulfate reduction ( $564 \mu\text{M day}^{-1}$ ) observed upon incubation for 12 h indicated a large population of thermophilic SRM which must have been present in situ at the time of sampling.

Thermophilic sulfate reduction occurred with maximal rates at 56 to 65°C, while the upper limit for microbial sulfate reduction was 80°C in all experiments. These results were similar to the temperature characteristics found for the endospore-forming *Desulfotomaculum* spp., for which optimum temperatures of 54 to 65°C and maximal temperatures of 56 to 85°C have been reported (33, 35, 47). The upper limit for microbial sulfate reduction, 80°C, is the highest which has been measured in freshwater lake sediments. For terrestrial hot springs of Yellowstone National Park, Zeikus et al. (54) found a sulfate-reducing activity that could be detected at up to 83°C. Thermophilic sulfate reduction was attributed to the presence of *Thermodesulfobacterium commune*, which was isolated from the hot springs and which had an upper temperature limit for growth of 85°C. Higher growth temperatures for SRM in pure cultures have been found only for *Archaeoglobus* spp., which grow at up to 90 to 95°C (37, 39). Microbial sulfate reduction at 110°C, however, has been demonstrated in deep-sea sediment from the Guaymas Basin hydrothermal vent area (31), although microorganisms with this capacity have not yet been cultivated.

**Role of substrates.** The organic carbon content found in the hydrothermal sediment at Cape Banza was in a range comparable to that for many estuarine and coastal sediments. Thus, high rates of sulfate reduction potentially could be supported in the hydrothermal sediment if sulfate were not limiting. Lake water sampled outside the hydrothermal vent area had a sulfate concentration of 39 to 42  $\mu\text{M}$ , while the mean concentration of sulfate in the hydrothermal fluids ( $n = 8$ ) was  $430 \pm 151 \mu\text{M}$  (5). It has been shown for other freshwater sediments that the sulfate uptake system of SRM is well adapted to low sulfate concentrations, and low apparent half-saturation constants ( $K_m$  values) in the range of 35 to 68  $\mu\text{M SO}_4^{2-}$  have been reported (4, 36). As the  $\text{SO}_4^{2-}$  concentration in the hydrothermal sediment was likely influenced by the composition of the hydrothermal fluids, the in situ rates of thermophilic sulfate reduction at Cape Banza may not have been limited by the sulfate concentration.

Suitable organic substrates for SRM in pure cultures are primarily low-molecular-weight molecules, although the utilization of long-chain ( $\text{C}_{12}$  to  $\text{C}_{20}$ ) alkanes was recently shown for some SRM (1, 50). In the sediment studied, the addition of YP-mix caused a twofold-higher average stimulation of sulfate reduction rates than did the addition of low-molecular-weight organic acids (C-mix). However, the temperature dependencies of sulfate reduction were similar with either of the two substrate solutions added. This result indicated that the added YP-mix was fermented by a range of thermophilic bacteria with temperature characteristics similar to those of the SRM. One such example was a newly isolated fermentative, endospore-forming anaerobe that grew on YP-mix at an optimum temperature at 60°C (16).

**Role of pH.** Sulfate reduction and SRM in natural environ-

ments have been reported to occur within a pH range of 3.8 to 9.9 (2, 22). However, only neutrophilic SRM have been isolated so far (46). At the Cape Banza site, the pH of the lake water was 8.6 to 8.9, while the pH of the hydrothermal fluids ranged from 7.7 to 8.7 ( $n = 8$ ), with a mean pH of  $8.2 \pm 0.4$  (5). Thermophilic sulfate reduction in the sediment slurries was neutrophilic and was completely inhibited at pH 8.8 to 9.2. Thus, thermophilic sulfate reduction showed a pH optimum that was closer to the pH of the hydrothermal fluids than to the pH of the ambient lake water.

**Enrichment, sulfide production, and growth of thermophilic SRM.** Thermophilic sulfide-producing enrichment cultures that were morphologically similar to *Desulfotomaculum* spp. were obtained from hydrothermal sediment and chimney samples during incubation at 60°C. From chimney samples, sulfide-producing cultures could also be enriched at 75°C, but these cultures showed no apparent sporulation. However, the morphology of vegetative cells was comparable to that of *Desulfotomaculum* spp. rather than to that of *Thermodesulfobacterium* or *Thermodesulfovibrio* spp., which are characterized by their small size ( $<1.5 \mu\text{m}$ ) (23, 48). Although the in situ temperatures in the hydrothermal samples were up to 101°C, sulfide-producing enrichments were not obtained at 93 or 103°C. Likewise, no sulfate-reducing archaea were obtained from other parts of the hydrothermal Cape Banza site (38). These results are in agreement with those of the slurry experiments (Fig. 2), in which an upper temperature limit for sulfate reduction was found at 80°C. The results suggested that sulfate-reducing archaea were absent in the hydrothermal environment (rather than lost during storage and cultivation), as it has been found that the long-term (2 to 5 years) survival of hyperthermophilic archaea in sediment and smoker wall samples may be very high (28).

The temperature dependence of sulfide production in the enrichment cultures LC60 and LS60 was similar and (below the optimum temperature) fitted the logarithmic form of the Arrhenius equation:  $\ln k = \ln A - (E_a/RT)$ , where  $k$  is the process rate,  $A$  is the Arrhenius constant,  $E_a$  is the apparent activation energy,  $R$  is the gas constant, and  $T$  is the absolute temperature (Fig. 5). The temperature dependence indicated an apparent activation energy of  $114 \text{ kJ mol}^{-1}$ , corresponding to a temperature coefficient ( $Q_{10}$ ) of 3.3 (calculated for the interval from 60 to 70°C). Studies of mesophilic SRM showed that  $Q_{10}$  values calculated for sulfide production and growth were equivalent (3), and the present results were similar to the temperature response for the growth of and sulfate reduction by thermophilic *Desulfotomaculum kuznetsovii* P60 (25, 26).

In the thermophilic enrichment culture LC75, sulfide production and growth occurred within a temperature span of 15 to 20°C (Fig. 6). This temperature interval was smaller than that observed for thermophilic sulfate reduction in the sediment slurries. This result could have been due to a lower temperature tolerance for the same bacteria in highly enriched cultures as compared with sediment slurries or alternatively to the activity of diverse populations in the sediment slurries. The uniform morphology and the agreement between the turbidity increase and sulfide production in the LC75 enrichment culture (Fig. 6) indicated that a single SRM population was active in this culture.

In conclusion, microbial thermophilic sulfate reduction at up to 80°C has been demonstrated in hydrothermal sediments of Lake Tanganyika. Cultures of SRM enriched from the hydrothermal environment were morphologically similar to the sporulating *Desulfotomaculum* spp. and had optimum temperatures for sulfide production of 63 and 71°C.

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