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Oxidation of Hydrogen Sulfide by an Enrichment from Sour Water Coproduced with Petroleum

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ABSTRACT

We have previously demonstrated that the chemoautotroph and facultative anaerobe *Thiobacillus denitrificans* may be readily cultured aerobically or anoxically in batch and continuous reactors on hydrogen sulfide under sulfide-limiting conditions. A sulfide-tolerant strain of *T. denitrificans* (strain F) was isolated by enrichment and recently used in a successful field test of a microbial process for the treatment of sour water coproduced with petroleum at an Amoco Production Co. site in Wyoming. Prior to the initiation of this field test, it was determined that the sour water at this site contained low concentrations of indigenous autotrophs, which could grow on thiosulfate as an energy source. Samples of this sour water have now been used to produce an enrichment culture for sulfide oxidizers. This enrichment has been characterized with respect to hydrogen sulfide oxidation, response to oxygen, pH and temperature optima, and sulfide tolerance. The enrichment was shown to be strictly aerobic and to grow on sulfide as an energy source with complete oxidation of sulfide to sulfate. The enrichment has a tolerance of sulfide comparable to that of *T. denitrificans* strain F. However, the enrichment has a higher optimum temperature (35°C) than strain F and was shown to oxidize sulfides over a much broader range of pH values (3.5–10).

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Index Entries: Hydrogen sulfide; sour water; petroleum; *Thiobacillus denitrificans*; enrichment.

INTRODUCTION

We have previously demonstrated that the chemoautotroph and facultative anaerobe *Thiobacillus denitrificans* may be readily cultured aerobically or anoxically in batch and continuous reactors on H_2S(g) under sulfide-limiting conditions with complete removal of H_2S from the gas and complete oxidation to sulfate (*1–4*). Maximum loading of the biomass was determined to be 5.4–7.6 mmol H_2S/h/g biomass under anoxic conditions and 15.1–20.9 mmol H_2S/h/g biomass under aerobic conditions.

A sulfide-resistant strain of *T. denitrificans*, strain F, was isolated by enrichment (*5*). Wild-type *T. denitrificans* is inhibited by sulfide concentrations of 0.1–0.2 m*M*. However, strain F is tolerant of sulfide concentrations in excess of 2.5 m*M*. Recently a microbial process for treatment of sour water coproduced with petroleum was field tested at the Amoco Production Co. Salt Creek Field near Midwest, WY utilizing this strain in a flocculated form. At the LACT-10 unit at this site, a 19,000 bbl (barrel) oil-skimming pit receives an average inlet flow of 5000 bbl/d of water containing 4000 mg/L total dissolved solids (TDS) and 100 mg/L (3.1 m*M*) sulfide at about 40°C. The pit was inoculated with 9–14 kg (dry wt) of flocculated *T. denitrificans* strain F, and the inlet sour water supplemented with NH_4NO_3 and P_2O_5 fertilizers to provide reduced nitrogen and phosphate to the organism. The first third of the pit was aerated with a blower with a capacity of 28 standard m^3/min. All other nutrients were provided by the produced water. In the absence of hydraulic upsets, sulfides were undetectable in the pit everywhere but at the inlet end of the pit, where the sulfide concentration averaged <2 mg/L during the 6-mo duration of the test. The temperature and pH in the pit ranged from 18–40.6°C and 7.0–8.6, respectively. This field test proved the validity of microbial treatment of sour water for sulfide removal (*6*).

Prior to inoculation of the pit with *T. denitrificans* strain F, the pit was sampled to determine the number of indigenous potential sulfide oxidizers using the most probable number method and a thiosulfate medium (*1*). Approximately 10^3 cells/mL were found compared to MPN counts of 10^7–10^8/mL after inoculation with *T. denitrificans* strain F. Samples of produced water taken from the LACT-10 pit prior to inoculation with strain F have now been used to produce an enrichment culture for sulfide oxidizers. This enrichment has been characterized with respect to H_2S oxidation, response to oxygen, pH and temperature optima, and sulfide tolerance to determine if the enrichment offers advantages over *T. denitrificans* strain F in the oxidation of H_2S for treatment of sour water.

MATERIALS AND METHODS

Production of LACT-10 Enrichment Culture

Samples of water from the LACT-10 oil-skimming pond were obtained in sterile sample cups and stored at 4°C until used. An enrichment for potential sulfide oxidizers was produced by inoculating 2 L of thiosulfate medium in a Marubishi MD 300 fermenter with 100 mL of produced water. Thiosulfate medium has been previously described (*1*). This medium contains thiosulfate as an energy source, ammonium chloride as a source of reduced nitrogen, bicarbonate as a carbon source, a phosphate buffer, $MgSO_4$ and trace minerals. Nitrate (50 m*M*) was also added to the medium to induce denitrifying enzymes if any facultative sulfide oxidizers were present. The culture was aerated with 0.35 L/min of air + 5% CO_2. The CO_2 supplement was used to ensure a continuous availability of a carbon source. The culture was maintained at 30°C and pH 7.0. After 72 h, the thiosulfate had been depleted, and the optical density at 460 nm was >1.0. This enrichment was used to inoculate stock cultures in 10-mL capped culture tubes containing 5 mL of thiosulfate medium with nitrate.

Characterization of LACT-10 Enrichment

Response to Oxygen

The response to oxygen was investigated by inoculation of thiosulfate medium, with and without 50 m*M* nitrate, with LACT-10-enrichment stocks followed by incubation of 30°C. Aerobic cultures consisted of 5 mL of thiosulfate medium (without nitrate) in loosely capped 10-mL culture tubes, whereas anoxic cultures consisted of thiosulfate medium (with 50 m*M* nitrate) in completely filled 10-mL culture tubes. The initial pH was 7.0. Growth was monitored by following the optical density at 460 nm.

Response to Temperature

The response to temperature was also investigated using thiosulfate medium (without nitrate) in half-filled, loosely capped 10 mL culture tubes inoculated with LACT-10 enrichment stocks. The initial pH was 7.0. Culture tubes were incubated at 22, 30, 35, and 40°C. Growth was monitored by following the optical density at 460 nm.

Response to Sulfide

Sulfide tolerance was investigated in a similar manner in thiosulfate medium (without nitrate) supplemented with initial sulfide (Na_2S) concentrations of 0, 0.94, 1.57, 1.90, 2.70, 3.13, and 4.70 m*M*.

Stoichiometry of H_2S Oxidation

Batch growth of the LACT-10 enrichment on H_2S(g) as an energy source was investigated using a Marubishi MD 300 (culture volume 2 L)

bench-scale fermenter. In a typical batch experiment, the LACT-10 enrichment was first grown in thiosulfate medium (without nitrate) at 30°C and pH 7.0. Aeration was provided at a rate sufficient to maintain 60–100 μM O_2 in the culture medium. Air was supplemented with 5 mol % CO_2. The purpose of this prior cultivation on thiosulfate was to develop a sufficient concentration of biomass in the reactor so that an appreciable rate of H_2S could be fed to the reactor without exceeding the sulfide biooxidation capacity of the biomass. Otherwise, potentially toxic levels of sulfide could accumulate in the culture. Sulfide has been shown to be an inhibitory substrate for *T. denitrificans* (*1*).

The reactor was autoclaved at 121°C and 205 kPa for 30 min prior to inoculation with 20 mL of the LACT-10 stock culture. When the optical density at 460 nm reached 0.4–0.5, cells were harvested aseptically by centrifugation, washed, and resuspended in fresh medium without thiosulfate prior to the initiation of a H_2S feed. The purpose of this medium change was to eliminate sulfate produced by thiosulfate oxidation so the sulfate produced from H_2S oxidation could be more accurately quantitated. Following the medium change, the culture received two gas feeds: 0.9–1.1 mol% H_2S, 5 mol% CO_2, and balance nitrogen and air supplemented with 5 mol% CO_2. The aeration rate was typically 0.35 L/min. Foaming was controlled by the periodic addition of 0.5 mL of a one-tenth dilution of General Electric AF93 silicone antifoam emulsion, which had been previously autoclaved at 121°C and 205 kPa. Periodically during growth on H_2S, the culture medium was sampled to determine biomass, ammonium ion, and sulfate concentrations, and the outlet gas was monitored for H_2S.

Specific Activity for H_2S Oxidation

The specific activity (mmol H_2S oxidized/h/g biomass) of LACT-10-enrichment cultures was determined by increasing the H_2S feed rate to cultures with known biomass concentrations until sulfide began to accumulate in the culture medium, resulting in H_2S breakthrough in the outlet gas and the elemental sulfur formation. At this point, the mass flow rate of H_2S exceeded the rate at which the biomass in the reactor could oxidize the sulfide to sulfate (*1*).

Response to pH

The response to the LACT-10 enrichment to pH was determined using cultures growing in the Marubishi fermenter where pH could be monitored and controlled. A LACT-10-enrichment culture growing on H_2S was developed at pH 7.0 as described above. The pH of the culture medium was then adjusted upward by 0.5 U every 18–30 h. After 28 h of operation at pH 10, the pH was adjusted back to 7.0, and the culture maintained at this pH with H_2S feed for 118 h. At the end of this time, the pH was adjusted to 6.0, 5.0, 4.0, 3.5, and 3.0 operating for 18–30 h at each pH value. The reactor was closely monitored for an upset condition (H_2S breakthrough and elemental sulfur formation) during this time.

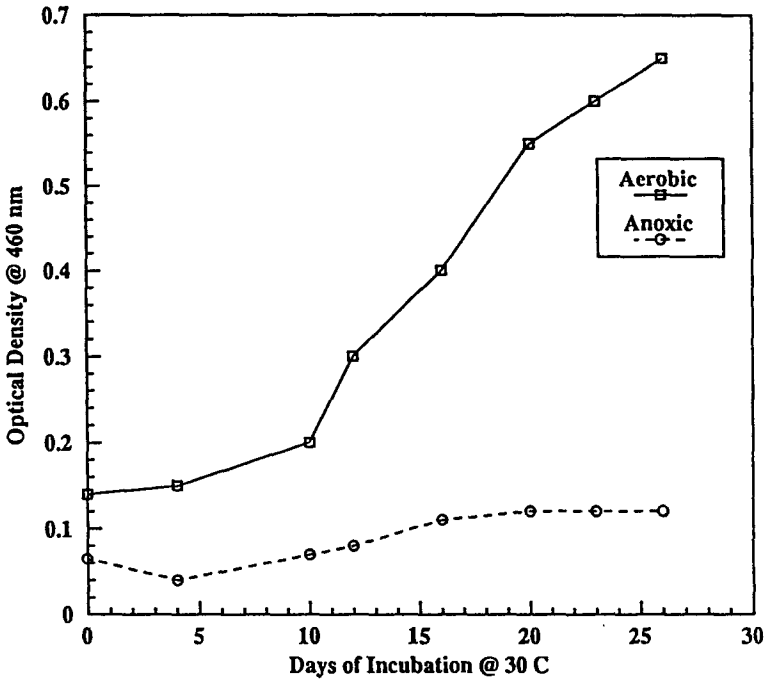

Fig. 1. Growth of the LACT-10 enrichment on thiosulfate under aerobic and anoxic conditions.

Analytical

Thiosulfate, sulfate, elemental sulfur, sulfide, and ammonium ion were determined in culture medium samples as previously described (*1*). Total biomass concentration was determined using a correlation with optical density at 460 nm developed for *T. denitrificans* (data not shown). Hydrogen sulfide in the reactor outlet gases was monitored using Gas Tech (Yokahoma, Japan) chromophoric tubes for H_2S (0–60 ppm range).

RESULTS AND DISCUSSION

Response to Oxygen

Growth of the LACT-10 enrichment on thiosulfate under aerobic and anoxic conditions is illustrated by Fig. 1. Each data point in Fig. 1 represents the average of the optical density in four culture tubes, which received the same inoculum and were incubated in parallel. As shown in Fig. 1, growth was observed only under aerobic conditions (without nitrate). No growth was observed under denitrifying conditions. These aerobic cultures were then used as inocula for a second series of tests under aerobic and anoxic conditions (with nitrate). Similar results were obtained in this second series with no growth observed in the absence of oxygen. These

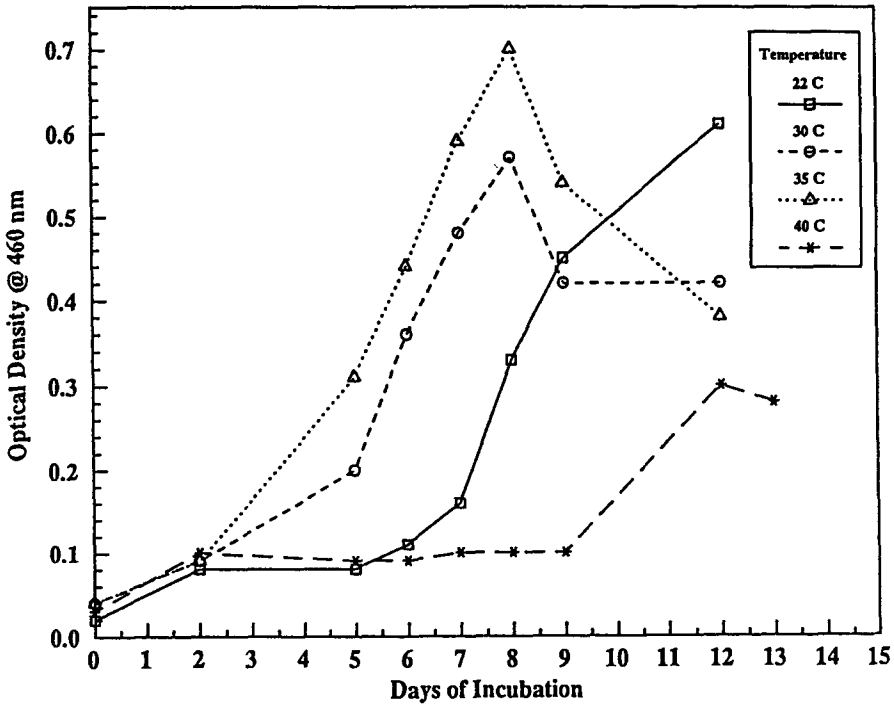

Fig. 2. Effect of temperature on aerobic growth of the LACT-10 enrichment on thiosulfate.

experiments clearly distinguish the LACT-10 enrichment from *T. denitrificans,* which is capable of anoxic growth on thiosulfate with nitrate as the terminal electron acceptor (*1*).

Response to Temperature

Aerobic growth of the LACT-10 enrichment on thiosulfate at 22–40°C is illustrated in Fig. 2. Best growth was observed at 35°C, 5°C higher than the optimum temperature of *T. denitrificans* (*1*).

Response to Sulfide

Aerobic growth of the LACT-10 enrichment on thiosulfate in the presence of initial sulfide concentrations of 0–4.7 m*M* is illustrated in Fig. 3. As shown in Fig. 3, good growth was observed at initial sulfide concentrations as high as 3.1 m*M*. This tolerance of sulfide is comparable to that of the sulfide-tolerant strain of *T. denitrificans* and some 30 times higher than that of wild type *T. denitrificans* (*7*).

Stoichiometry of H_2S Oxidation

Hydrogen sulfide was introduced into cultures of the LACT-10 enrichment previously grown on thiosulfate at initial loadings of 0.9–2.1 mmol H_2S/h/g biomass. Hydrogen sulfide was immediately metabolized with

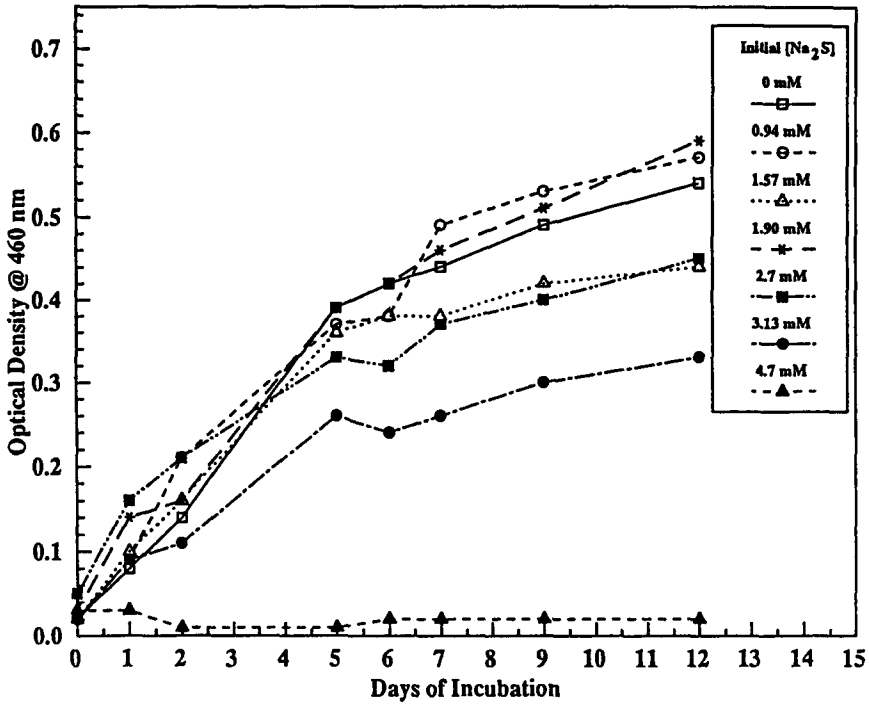

Fig. 3. Effect of sulfide (as Na_2S) on aerobic growth of LACT-10 enrichment on thiosulfate.

no apparent lag. No H_2S was detected in the outlet gas, and <1 μM of sulfide was found in the culture medium; however, sulfate accumulated in each reactor medium as H_2S was removed from the feed gas (Fig. 4). No elemental sulfur was detected in the culture medium at any time. Hydrogen sulfide oxidation to sulfate was accompanied by growth as indicated by a decrease in the NH_4^+ concentration and an increase in the biomass concentration in the reactor medium (Fig. 4). The reaction was acid-producing, as indicated by the consumption of OH^- equivalents. The observed stoichiometry of H_2S oxidation by the LACT-10 enrichment in batch reactors is given in Tables 1 and 2. As seen in Tables 1 and 2, virtually all H_2S was oxidized to sulfate in these cultures. The stoichiometry of aerobic H_2S oxidation by the LACT-10 enrichment was comparable to that reported for aerobic oxidation of H_2S by *T. denitrificans* (4).

Specific Activity of the LACT-10 Enrichment for H_2S Oxidation

When the H_2S feed rate was increased stepwise to LACT-10-enrichment cultures produced as described above, an upset condition was indicated by H_2S breakthrough in the outlet gas and the accumulation of elemental sulfur in the culture medium. (Breakthrough of H_2S and accumulation of elemental sulfur have been observed in aerobic and anoxic cultures of *T. denitrificans* when the maximum specific activity of the

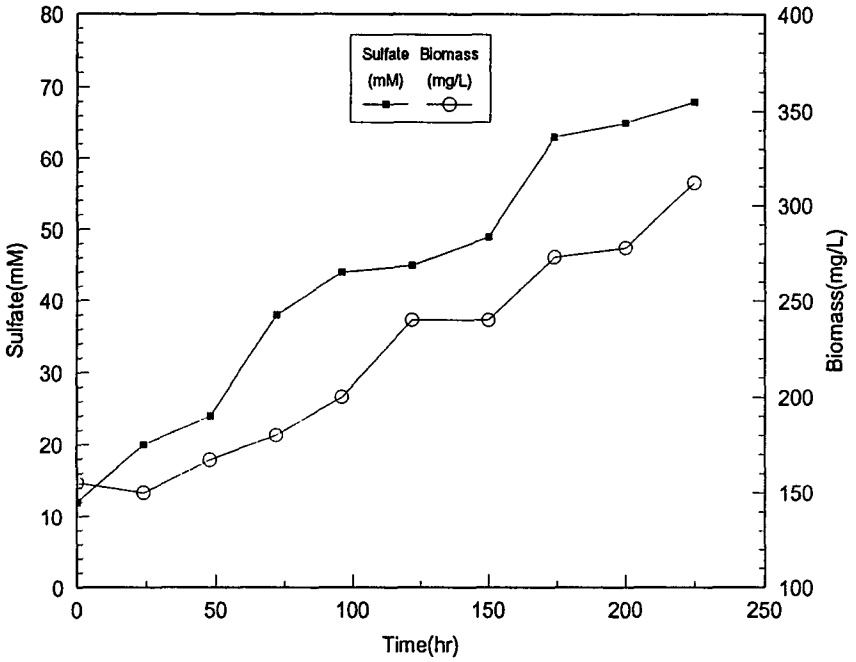

Fig. 4. Sulfate and biomass accumulation in a batch aerobic culture of LACT-10 enrichment growing on H_2S. Average H_2S feed rate was 0.35 mmol/h.

Table 1
Stoichiometry of H_2S Oxidation in Batch Cultures of the LACT-10 Enrichment

Exp. no.	mmol H_2S oxidized	mmol SO_4^{-2} produced	mmol NH_4^+ used	mmol OH^- used	mg Biomass produced
E1	86.7	84.3	6.0	ND	276
E2	45.3	46.9	ND*	90.6	143
E3	113.6	109.1	ND	260.3	ND

*ND = not determined.

Table 2
Stoichimetric Ratios Relative to H_2S
Oxidized by the LACT-10 Enrichment in Batch Cultures

Exp. no.	SO_4^{-2}/H_2S	NH_4^+/H_2S	OH^-/H_2S	Biomass/H_2S
E1	0.97	0.07		3.2
E2	1.04		2.0	3.2
E3	0.96		2.3	
	Avg. 0.99			

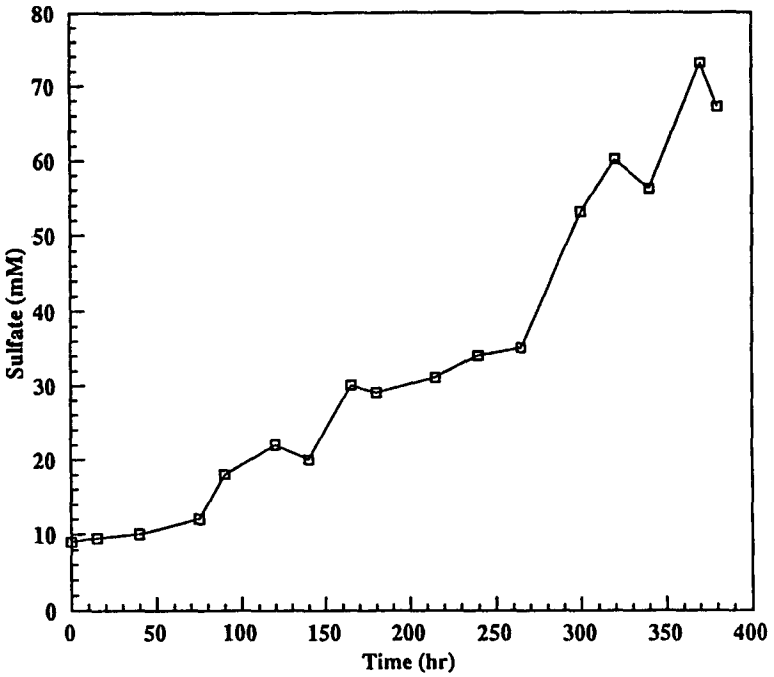

Fig. 5. Sulfate accumulation in a batch aerobic culture of LACT-10 enrichment growing on H_2S at pH 3.5–10. Average H_2S feed was 0.30 mmol/h; biomass concentration was 130–230 mg/L.

biomass for H_2S oxidation is exceeded [*1,4*]). If the upset could not be reversed by an increase in the aeration rate, the upset was attributed to exceeding the maximum specific activity of the enrichment for H_2S oxidation. This maximum specific activity for H_2S oxidation was found to be 6.0 ± 1.0 mmol H_2S/h/g biomass. This is about one-third the maximum specific activity reported for *T. denitrificans* for H_2S oxidation under aerobic conditions (*4*).

Response to pH

As described above, the effect of pH on the LACT-10 enrichment was determined using cultures actively growing on H_2S (loadings of 1.3–2.0 mmol H_2S/h/g biomass). A specific pH was taken to be inhibitory if H_2S breakthrough in the outlet gas and elemental sulfur accumulation in the culture medium were observed. As the pH of these cultures was increased, no effect on H_2S oxidation by the LACT-10 enrichment was observed at pH values as high as 10. Cultures were also successfully operated at pH values as low as 3.5 (up to 23 h). However, after 24 h at a pH of 3.0, H_2S breakthrough (50 ppmv) and elemental sulfur production were observed. Increasing the pH to 7.0 resulted in the disappearance of H_2S in the outlet gas and elemental sulfur in the culture medium. During the entire course of this experiment, sulfate accumulated in the culture medium as H_2S was removed from the feed gas (Fig. 5). The pH range of *T. denitrificans*

strain F is 6.0–8.8 (data not shown). Clearly, the LACT-10 enrichment is capable of H_2S oxidation over a much broader range of pH values.

CONCLUSIONS

The LACT-10 enrichment offers both advantages and disadvantages compared to *T. denitrificans* strain F as a source of sulfide-oxidizing organisms for sour water remediation. The LACT-10 enrichment is strictly aerobic, limiting its use to environments that receive sufficient oxygen or can be efficiently aerated. *T. denitrificans* strains F can be utilized in anoxic environments (the subsurface, for example) with nitrate as the terminal electron acceptor.

Under aerobic conditions, the LACT-10 enrichment offers the advantages of a higher optimum temperature and a wider pH range compared to *T. denitrificans* strain F. The LACT-10 enrichment and *T. denitrificans* strain F have a comparable tolerance of sulfide. However, the LACT-10 enrichment has a lower maximum specific activity for sulfide oxidation. Therefore, for the same sulfide loading, more LACT-10 biomass would be required than *T. denitrificans* strain F biomass to remediate a sour water or gas stream effectively.

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