0273-2289/92/3435—0811$02.00

Microbial Stabilization of Active-Sulfide Sludges

Scientific Note

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INTRODUCTION

In the petroleum industry, crude oil is often stored with water in water displacement tanks. Water is introduced into these tanks to float the oil, maintaining the oil level near the ceiling of the tank. When oil is added or withdrawn, the corresponding volume of displacing water is pumped out or injected into the tank. In this way, vapor space and therefore vapor losses are reduced.

In these tanks, both aerobic oil-oxidizing bacteria (OOB) and anaerobic sulfate-reducing bacteria (SRB) exist in the oil/water system (*1,2*). The SRB utilize sulfate as a terminal electron acceptor with reduction to sulfide. The SRB generally do not utilize hydrocarbons as carbon and energy sources. However, the OOB both create anaerobic conditions in these oil/water systems by removal of oxygen owing to respiration and produce organic acids that may act as substrates for the SRB. Therefore, the SRB begin to produce sulfide under anaerobic conditions after partial oxidation of components of the oil by the OOB (*3*).

Sulfides (H_2S, HS^-, S^{-2}) produced by the SRB result in an acceleration in the corrosion rate in steel tanks producing a variety of iron–sulfur compounds (*4*). Those compounds with iron:sulfide ratios of 1:2 (pyrite and marcasite) are pH and temperature stable. However, compounds with iron:sulfide ratios of 1:1 (mackinawite and pyrrhotite) and 3:4 (greigite and smythite) are soluble in mild acid with formation of H_2S (*5*).

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The fill and withdraw cyclic tank operation results in increasing numbers of SRB and increasing accumulation of iron sulfide solids in the tank. The potential for H_2S production makes these sludges a hazard in the work place and a disposal problem. At present, the active sulfides in these sludges are stabilized by oxidation with $KMnO_4$ or H_2O_2 producing either elemental sulfur (acidic conditions) or sulfate (alkaline conditions) (*6*). These reactions are highly exothermic, and have caused explosions and fatal injuries.

New technology is needed to oxidize these active-sulfide sludges under more moderate reaction conditions. This article describes a preliminary investigation of the anaerobic oxidation (with nitrate) of active-sulfide sludges by the chemoautotroph and facultative anaerobe *Thiobacillus denitrificans*. *T. denitrificans* utilizes reduced sulfur compounds as energy sources with oxidation to sulfate. Under anaerobic conditions, nitrate may be used as a terminal electron acceptor with reduction to elemental nitrogen. Sublette and Sylvester (*7–9*) and Sublette (*10*) have demonstrated that *T. denitrificans* may be readily cultured aerobically and anaerobically in batch and continuous reactors on gaseous H_2S under sulfide-limiting conditions. A microbial process for the removal of H_2S from a gas has been proposed based on contact of the gas with a culture of *T. denitrificans* (*7*). Immobilized *T. denitrificans* has also been used to remove and oxidize sulfides (up to 800 mg/L) in sour water containing H_2S, HS^- and/or S^{-2} in an upflow bubble column. These reactors have successfully operated continuously for up to 9 mo.

MATERIALS AND METHODS

Organism and Culture

A sulfide-resistant strain of *T. denitrificans* (strain F) was obtained by enrichment from cultures of the wild type (ATCC 23462) as described in detail elsewhere (*11*). Stock cultures of *T. denitrificans* strain F were maintained anaerobically in thiosulfate medium as previously described (*7*). In this medium, thiosulfate is the energy source, nitrate is the terminal electron acceptor, bicarbonate is the source of carbon, and ammonium ion is a source of reduced nitrogen. This medium also contains a phosphate buffer, a source of Mg^{2+}, Fe^{3+}, and trace elements. Stock cultures were transferred every 30 d and stored at 4°C until used.

Sludge Characteristics

The active sulfide sludge used in this study was obtained from a water displacement tank in which crude oil was stored at a West Coast refinery. A physical and chemical analysis of the sludge is given in Table 1. As noted in Table 1, very little soluble sulfide was detected in the filtrate when the sludge was filtered through a 0.45-μ membrane filter (Metricel

Table 1
Refinery Sulfide Sludge Analysis[1]

Total filterable solids (0.45-μ membrane filter)	0.2 g/mL	
Solids composition, wt% vacuum dried at 50°C[2]	C	2.3
	N[3]	2.51
	H	1.38
	Fe	31.1
	S	31.4 (ASTM D3177 method A)
Filtrate analysis	SO_4^{-2}	6.3 m*M*
	NO_3^-	2.6 m*M*
	NH_3	94.1 m*M*
	TOC	285 mg/L
	HS^-, S^{-2}	<0.01 m*M*
pH 9.0		

[1]Samples were purged with N_2 and sealed under an N_2 blanket. Typical refinery analysis based on whole sludge was 6% total sulfides and 450 mg/L reactive sulfides.
[2]Analysis provided by Warner Laboratories Division of Gould Energy (Cresson, PA).
[3]Reduced to 0.3% when solids were dried at 1 atm and 100°C.

GN-6). Addition of concentrated HCl to the filtrate resulted in no odor of H_2S, and no H_2S could be detected by lead acetate paper. On the other hand, addition of concentrated HCl to the recovered solids produced a strong odor of H_2S and vapor that blackened lead acetate paper.

Oxidation of Active Sulfides by *T. denitrificans*

In a typical experiment, *T. denitrificans* strain F was grown in anaerobic thiosulfate medium at pH 7.0 and 30°C in an L. E. Marubishi MD300 fermenter (culture vol 1.4 L) to an optical density (460 nm) of about 1.0, as previously described (*7*). At this point, the culture density was about 10^9 cells/mL with cells actively growing in late exponential phase. Cells were then harvested by centrifugation at 4900×*g* at 25°C, resuspended in 1.3 L of fresh medium *without* thiosulfate, and transferred back to the fermenter. One hundred milliliters of sulfide sludge were than added to the culture, and incubation continued at 30°C and pH 7.0. In certain experiments, further additions of nutrients, cells, or sludge were made. Cultures were sampled periodically and analyzed for sulfate, acid-labile sulfide, ammonia, and viability.

Analytical

Sulfate was determined turbidometrically, and ammonia was determined by the Nessler's method as previously described (*7*). Acid-labile

sulfide was determined as follows. A 20-mL sample of culture mixed liquor was transferred to a 50-mL Erlenmeyer flask fitted with a three-hole stopper. Through one opening was added concentrated HCl dropwise. Through a second opening, the sample was sparged with nitrogen. Liberated $H_2S(g)$ was then transferred through the third opening to a second flask, and bubbled into 20 mL of 0.02% zinc acetate dihydrate and 0.2% acetic acid (v/v) to trap sulfide as ZnS. The resulting suspension was then analyzed for sulfide by the methylene blue method (*12*). A sulfide stock solution (2*M*) was prepared gravimetrically and standardized by titration with 0.100*M* lead perchlorate using an Orion Research Model 94-16 sulfide electrode to detect the end point. The stock solution was diluted to prepare sulfide standards.

Viability of *T. denitrificans* cells from culture samples was determined by inoculating 9.5 mL of thiosulfate anaerobic medium with 0.5 mL of the culture sample in 10-mL capped culture tubes. Viability was indicated by turbid growth of *T. denitrificans* after 2–3 d of incubation at 30°C. Total iron in the liquid phase of culture medium samples was determined by filtering samples through a 0.45-μ membrane filter (Metricel GN-6), and analyzing filtrates by atomic absorption spectroscopy using a Perkin Elmer Model 2380 Atomic Absorption Spectrometer and a 1000 mg/L iron standard (Fisher Scientific, Fair Lawn, NJ).

RESULTS AND DISCUSSION

When the active-sulfide sludge was added to suspensions of exponential-phase *T. denitrificans* in fresh medium containing no other reduced sulfur source, sulfate began to accumulate in the culture medium as shown in Fig. 1. However, the sulfate concentration typically leveled off at about 21–26 m*M*. No elemental sulfur was evident visually. (Interferences prevented colorimetric determination.) In a control experiment identical in all respects except for the absence of *T. denitrificans* cells, very little sulfate accumulation was seen (also shown in Fig. 1). Therefore, the accumulation of sulfate in the test cultures was biologically mediated. Since soluble sulfide concentrations in the active-sulfide sludge were < 0.01 m*M* (Table 1), these data indicate that *T. denitrificans* was able to oxidize some fraction of the insoluble sulfides in the solid phase. Presumably, the mechanism involved a reversal in the dissolution equilibrium with oxidation of sulfide occurring in the liquid phase.

Figure 2 shows the concentration of acid-labile sulfides in both the test and control reactors described above. From the control plot, it is seen that the acid-labile fraction of sludge sulfide increased with time. Apparently, agitation and interaction of sludge solids with medium components made more of the sulfide accessible and/or more reactive with time. Less acid-labile sulfide was found in the test culture in the early phases of incu-

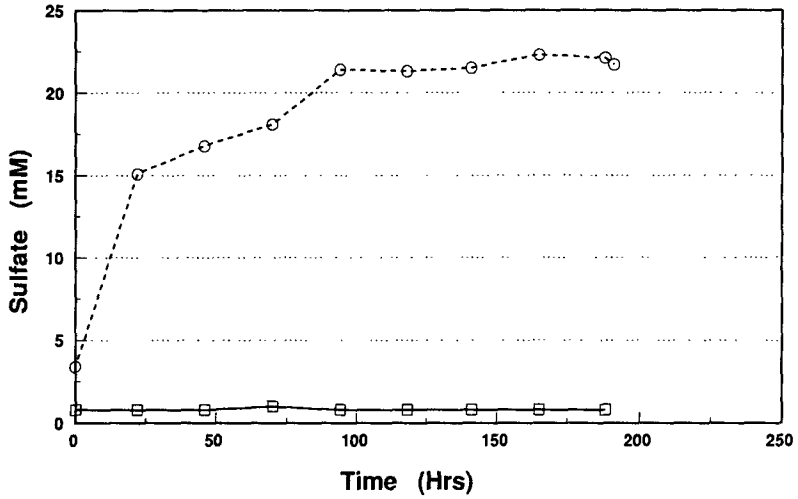

Fig. 1. Sulfate concentrations in test culture and control with feed of active-sulfide sludge. --○-- Test culture with cells. —□— Control without cells.

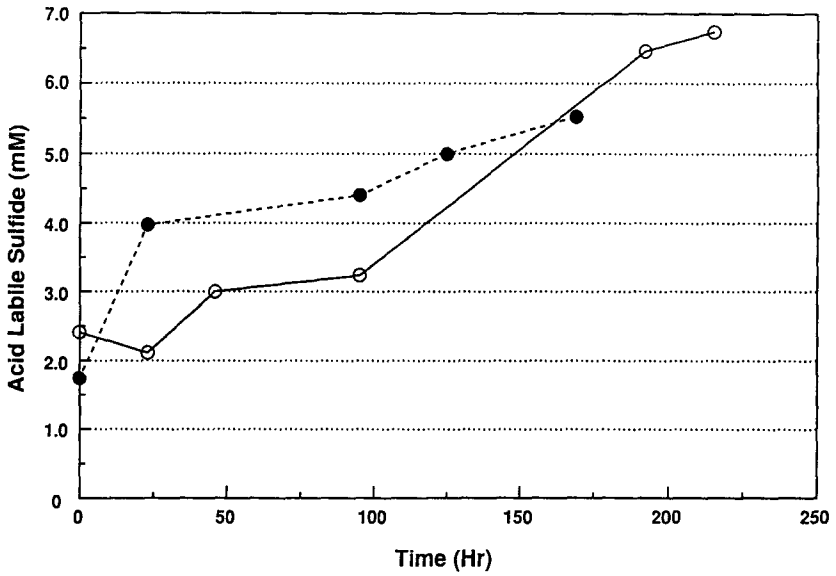

Fig. 2. Concentrations of acid-labile sulfide in test culture and control with feed of active-sulfide sludge. —○— Test culture with cells. --●-- Control without cells.

bation than in the control. This is the same period of time in which sulfate was observed to accumulate. No mass balance was attempted. However, these observations indicate that the acid-labile fraction of the sludge was susceptible to microbial oxidation.

Figure 2 also indicates that the oxidation of acid-labile sulfide was incomplete. One possible explanation was the depletion of a key nutrient for *T. denitrificans.* Nitrate was present in the cultures at an initial concentration of 50 m*M*. However, some component(s) of the sludge interfered

with nitrate analysis by the cadmium reduction method (*13*). Therefore, the nitrate concentration could not be determined. However, addition of more nitrate (as KNO_3) or fresh medium to these cultures after sulfate accumulaton had ceased did not produce additional sulfate accumulation or depletion in acid-labile sulfide.

Another possible explanation was a loss of viability in the culture. When the sulfate concentration in the test culture described by Figs. 1 and 2 leveled off, the total solids (biomass and sludge solids) in the culture were recovered by centrifugation and resuspended in fresh medium (without thiosulfate). No further sulfate production was observed during 100 h of incubation at pH 7.0 and 30°C. After 100 h, $Na_2S_2O_3$ and KNO_3 were added to the culture. No sulfate production was observed, indicating complete inhibition of *T. denitrificans* or complete loss of viability.

In duplicate experiments, viability tests conducted with samples taken after sulfate accumulation ceased produced no growth in medium supplemented with thiosulfate. No effort was made to dilute these cultures out more than described above. In other experiments, concentrated suspensions of fresh cells (with and without additional nitrate) were added after sulfate accumulation ceased. No stimulation in sulfate production was observed, although acid-labile sulfide remained.

These results suggest that some component of the sludge or a byproduct (either chemical or physical) of the oxidation of acid-labile sulfides in the sludge has a biocidal effect on *T. denitrificans*. As seen in Table 1, the sludge contained 94.1 m*M* ammonia. Including the NH_4Cl normally present in the medium, the total ammonia nitrogen concentration in the test cultures was 15.3 m*M* as NH_3. However, in separate experiments, it was shown that *T. denitrificans* exhibited growth comparable to controls in media supplemented with 50 m*M* $(NH_4)_2SO_4$. The concentration of sulfate in this growth experiment also exceeded the ultimate sulfate concentration in test cultures with sulfide-active sludge. In fact, *T. denitrificans* has been shown to be resistant up to 250 m*M* sulfate (unpublished results).

Analysis of filtered culture medium samples for iron showed an iron concentration of only 3–6 mg/L at all times during the course of the experiment. Therefore, there was no excessive iron accumulation resulting from oxidation of acid-labile sulfides. This concentration is sufficient to maintain viability in *T. denitrificans* (*14*).

CONCLUSIONS

It has been demonstrated that a sulfide-tolerant strain of *T. denitrificans* (strain F) can oxidize under moderate conditions at least part of the acid-labile sulfide solids in a refinery sulfide sludge resulting from the costorage of petroleum with water. Sulfate was the final oxidation product. However, some as yet unidentified byproduct of the process is apparently

biocidal, resulting in lost culture viability before oxidation of the acid-labile sulfide solids was complete. Future work will focus on identifying the biocidal agent and diminishing its effect on *T. denitrificans* in order to effect the complete oxidation of the acid-labile sulfides in these active-sulfide sludges.

ACKNOWLEDGMENT

This work was funded by ABB Environmental Services (Portland, ME), formerly C-E Environmental Services.

REFERENCES

1. Gilbert, P. D., Steele, A. D., Morgan, T. D. B., and Herbert, B. N. (1983), *Microbial Problems and Corrosion in Oil and Oil Product Storage,* Hill, E. C., ed., Institute of Petroleum, London.
2. Moosavi, A. N. and Hamilton, W. A. (1987), *Microbial Problems in the Offshore Oil Industry,* Hill, E. C., Shennan, J. L., and Watkinson, R. J., eds., Proceedings of the Institute of Petroleum, London (1), pp. 13–26.
3. Morgan, T. D. B., Steele, A. D., and Gilbert, P. D. (1983), *Microbial Corrosion,* NPL Teddington, The Metals Society, London, pp. 66–73.
4. Hardy, J. A. (1983), *Microbial Problems and Corrosion in Oil and Oil Product Storage,* Centre-Point Colour Ltd., London, pp. 57–63.
5. Garrett, R. L., Clark, R. K., Carney, L. L., and Grantham, C. K. *J. Petroleum Tech.,* 787–796.
6. FMC Corp. (1978), *Industrial Waste Treatment with Hydrogen Peroxide,* FMC Corp. Industrial Chemical Group, Philadelphia, PA.
7. Sublette, K. L. and Sylvester, N. D. (1987), *Biotechnol. Bioeng.* **29(2),** 249.
8. Sublette, K. L. and Sylvester, N. D. (1987), *Biotechnol. Bioeng.* **29,** 753–758.
9. Sublette, K. L. and Sylvester, N. D. (1987), *Biotechnol. Bioeng.* **29(6),** 759–761.
10. Sublette, K. L. (1987), *Biotechnol. Bioeng.* **29,** 690–695.
11. Sublette, K. L. and Woolsey, M. E. (1989), *Biotechnol. Bioeng.* **34,** 565.
12. Tanner, R. S. *J. Microbiological Methods,* submitted.
13. American Public Health Association (1976), *Standard Methods for the Examination of Water and Wastewater,* 14th ed., APHA, New York.
14. Baalsrud, K. and Baalsrud, K. S. (1954), *Arch. Mikro.* **20,** 34.