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Microbial Oxidation of Mixtures of Methylmercaptan and Hydrogen Sulfide

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ABSTRACT

Refinery spent-sulfidic caustic, containing only inorganic sulfides, has previously been shown to be amenable to biotreatment with *Thiobacillus denitrificans* strain F with complete oxidation of sulfides to sulfate. However, many spent caustics contain mercaptans that cannot be metabolized by this strict autotroph. An aerobic enrichment culture was developed from mixed *Thiobacilli* and activated sludge that was capable of simultaneous oxidation of inorganic sulfide and mercaptans using hydrogen sulfide (H_2S) and methylmercaptan (MeSH) gas feeds used to simulate the inorganic and organic sulfur of a spent-sulfidic caustic. The enrichment culture was also capable of biotreatment of an actual mercaptan-containing, spent-sulfidic caustic but at lower rates than predicted by operation on MeSH and H_2S fed to the culture in the gas phase, indicating that the caustic contained other inhibitory components.

Index Entries: Mercaptan; spent-sulfidic caustic; hydrogen sulfide; biotreatment.

INTRODUCTION

Sodium hydroxide (NaOH) solutions are used in petroleum refining to remove hydrogen sulfide (H_2S) from various hydrocarbon streams. Once H_2S reacts with the majority of NaOH, the solution becomes known as a spent-sulfidic caustic. Spent caustics typically have a pH > 12.0 and sulfide concentrations exceeding 2–3 wt%. Depending on the source, spent caustic may also contain phenols, mercaptans, amines, and other organic compounds that are soluble or emulsified in the caustic *(1)*.

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Although biological treatment can be an inexpensive disposal option, many refineries do not have the waste-water treatment capacity to treat the entire amount of spent caustic generated. Additionally, concerns regarding odors and toxicity frequently prohibit on-site treatment. Currently, most spent-sulfidic caustics generated by refineries are either sent off-site to commercial operations for recovery or reuse (pulp and paper mills, for example) or for disposal by deep-well injection.

Future regulatory changes could result in more stringent controls and increased cost for off-site management of spent caustic. In such an event, low-cost on-site treatment options would be desired. Even without regulatory changes, current off-site transportation and disposal costs warrant further investigation of on-site management alternatives. Wet-air oxidation (WAO) for on-site management is commercially available *(2)*, but can result in significant capital investment and high operating costs. WAO can be particularly expensive for spent-caustic streams from small- to medium-size refineries owing to an insufficient economy of scale.

We have previously reported an evaluation of the feasibility of biologically treating mercaptan-free refinery spent-sulfidic caustic using a bioreactor containing a microbial culture augmented with a sulfide-tolerant strain (strain F) of the chemoautotroph *Thiobacillus denitrificans*. It was envisioned that this process could be implemented either by augmenting an existing refinery-activated sludge unit so that it could handle higher concentrations of sulfides without toxicity or odor problems, or by using a relatively small bioreactor that would be specialized for treating spent-sulfidic caustic streams.

Mercaptan-free, spent-sulfidic caustic from two refineries (Table 1) was successfully biotreated at the bench scale (1.5 L) and pilot-scale (3.7 m^3) resulting in neutralization and removal of active sulfides *(3,4)*. Sulfides were completely oxidized to sulfate by *T. denitrificans*. Microbial oxidation of sulfides produced acid that at least partially neutralized the caustic. Mixed heterotrophs in the treatment culture acclimated to methyldiethanolamine (MDEA) present in these samples, resulting also in complete degradation of the amine. A preliminary economic analysis showed that the caustics could be treated for roughly 4–9¢/gal (1–2.3¢/L) plus the cost of any additional acid required to maintain a near-neutral pH over and above that produced by the microbial oxidation of sulfide *(5)*.

As noted above, many refinery spent-sulfidic caustics also contain mercaptans. Although mixotrophic strains of certain *Thiobacilli* have been reported *(6)*, *T. denitrificans* strain F is strictly autotrophic and incapable of using organic sulfur compounds as carbon and energy sources. Therefore, a microbial culture capable of oxidation of both inorganic sulfide and organic sulfur compounds, like mercaptans, will either be mixotrophic, or a coculture of a heterotrophic organism capable of mercaptan oxidation and an autotrophic, sulfide-oxidizer like *T. denitrificans*. We report here the development of an aerobic enrichment culture capable of oxidizing

Table 1
Characteristics of Spent-Sulfidic Caustic Successfully Biotreated at Bench and Pilot-Scale (3,4)

Sample	Sulfide, *M*	COD, mg/L	MDEA, wt%	OH, *M*
D1	1.06	82100	2.37	2.60
D2	1.05	113800	3.17	1.04
D3	1.06	107000	3.81	1.03
PC1	0.60	73300	2.08	2.46
PC2	0.58	40200	---	2.91

mercaptans and sulfides fed simultaneously. The culture was enriched for organisms capable of metabolizing mercaptans and sulfides using MeSH and H_2S as gas feeds. In this way, mercaptan and sulfide oxidation could be studied in the absence of other complicating factors related to the composition of sulfidic caustic. The enrichment culture was then used to biotreat an actual refinery spent-sulfidic caustic sample containing mercaptans.

MATERIALS AND METHODS

Organisms and Stock Cultures

Several species of *Thiobacilli* including *T. thioparus* (ATCC 23647), *T. versutus* (ATCC 25364), *T. thiooxidans* (ATCC 8085), and *T. neopolitanus* (ATCC 23638) were obtained from the American Type Culture Collection (Rockville, MD). *T. denitrificans* strain F was isolated as previously described *(7)*.

Stock cultures of these organisms were grown in partially-filled 10-mL culture tubes at 30°C in the thiosulfate mineral salts medium *(8)*. In this medium, thiosulfate is the energy source; bicarbonate is the source of carbon; and ammonium ion is the source of reduced nitrogen. The medium also contained a phosphate buffer and sources of Mg^{2+}, Ca^{2+}, Fe^{3+}, Mn^{2+}, and trace elements.

Development of Aerobic Enrichment Culture

Each of the above referenced organisms was cultured separately and aerobically in thiosulfate mineral-salts medium in a B. Braun Biostat M (Allentown, PA) (culture volume, 1.45 L) fermenter at 30°C. The pH for each of the cultures was maintained at the optimum for each organism as follows: *T. denitrificans*: 7.0; *T. thioparus*: 7.0; *T. neopolitanus*: 7.0; *T. versutus*: 7.5; *T. thiooxidans*: 5.0. The pH was controlled by addition of 10 *N* NaOH as needed. The cultures were maintained in a fed-batch mode with a gas feed of 300 mL/min of air + CO_2. The medium used was thiosulfate limiting

with respect to the growth of all five organisms. When thiosulfate was depleted, the agitation and aeration were terminated and the medium was centrifuged to recover the biomass which was stored at 4°C.

Activated sludge was obtained from a refinery aerobic waste-water treatment system (aerobic) and from an anaerobic industrial digester. The activated sludge from both the sources were washed free of organics with 0.10 *M* phosphate buffer (pH 7.0) prior to use.

Biomass from each of the *Thiobacillus* cultures were suspended together in a mineral-salts medium (Table 2) in a B. Braun Biostat M fermenter. About 200 mL of settled activated sludge from the refinery anerobic waste-water treatment system and approx 300 mL of activated sludge from the aerobic industrial digester were also added to the reactor. The volume was then made up to 1.45 L with mineral-salts medium and the mixed culture was maintained at 30°C and pH 7.0 with an aeration of 300 mL/min of air + 5% CO_2 to ensure that the medium would not become carbon limiting. The system was left under aeration overnight to ensure that there was no energy source remaining. Once these conditions were established, the culture began receiving a gas feed of 0.5% methylmercaptan (MeSH) in nitrogen (U.S. Specialty Gas, Tulsa, OK) at an initial rate of 10 mL/min. The outlet gas was passed to an 500-mL Erlenmeyer flask containing 300 mL of 0.3 wt% zinc acetate to trap any fugitive emissions of H_2S from the bioreactor. The MeSH gas was fed to the reactor during the normal working hours for approx 6–9 h/d and the outlet gas routinely monitored for MeSH. Samples of the supernatant (25 mL) were taken twice each day when feed was initiated and terminated and the culture was monitored for sulfate and ammonium-ion concentrations and soluble COD (sCOD). The culture medium was left under aeration overnight when not receiving MeSH feed.

It was anticipated that in the early phases of the enrichment there would be a significant amount of cell death and lysis among bacteria that were not capable of utilizing the mercaptan as a sole carbon source. This lysis would provide substrates for some of the heterotrophs in the culture causing a "bloom" that could deplete the medium of other nutrients. Therefore, the medium was changed every 15 d, to replenish nutrients. To change the medium, agitation, aeration, and mercaptan gas feed were terminated and the biomass allowed to settle under gravity. The supernatant liquid was then discarded and replaced with fresh mineral-salts medium. This process also selected for retention of flocculated biomass.

In the early stages of the development of the culture the ammonium-ion concentration was seen to decline quite rapidly. Initially, when the ammonium ion was depleted, the medium was changed as described above. Subsequently, when the culture became more tolerant of MeSH, NH_4Cl was added directly to the reactor when depleted to give a concentration of approx 0.2 mg/mL and the medium was replenished only when

Table 2
Mineral Salts Medium

Component	Per Liter
Na_2HPO_4	1.2 g
KH_2PO_4	1.8 g
$MgSO_4 \cdot 7H_2O$	0.4 g
NH_4Cl	0.5 g
$CaCl_2$	0.03 g
$MnSO_4$	0.02 g
$NaHCO_3$	1.0 g
Trace mineral solution (8)	15.0 mL

the sulfate concentration exceeded 10,000 mg/L. The enrichment culture was operated with a MeSH feed at increasing feed rates for 4 mo until a target feed rate of 1.2 mmoles MeSH/h (100 mL/min of 0.5% MeSH) was achieved with minimal MeSH breakthrough (<5 ppmv).

The caustic chosen for treatment in this study (Table 3) contained both sulfides and mercaptans. Therefore, once mercaptan oxidation by the enrichment culture had been demonstrated, H_2S (g) was blended with the MeSH feed gas to the culture to see if the enrichment culture was capable of oxidizing both compounds simultaneously. The enrichment culture proved to be intolerant of even low levels of H_2S (10 mL/min of 1.0% H_2S, balance N_2) indicating that the enrichment culture was incapable of oxidizing both organic and inorganic sulfur. At this point, a known sulfide-oxidizing bacterium, *Thiobacillus denitrificans* strain F, was added to the enrichment culture a second time. The flocculated organism was grown separately and harvested by centrifugation as described by Ongcharit, et al., *(9)*. About 2 g of wet-packed cells of flocculated *T. denitrificans* strain F were added to the mercaptan-oxidizing culture reactor and the reactor was kept under aeration for 24 h before initiating a combined MeSH and H_2S feed.

Ultimately a combined feed of 1.2 mmoles/h MeSH and 0.64 mmoles/h H_2S could be achieved with no breakthrough of H_2S and little breakthrough (< 5 ppmv) of MeSH in the reactor outlet gas. These MeSH and H_2S feed rates corresponded to a caustic (Table 3) feed rate of 40 mL/d in terms of mercaptan and sulfide components only. Operation of the enrichment culture initially on gas feed of MeSH and H_2S provided a benchmark

Table 3
Results of Analysis of Mercaptan-Containing Caustic Used as Feed to the Aerobic Enrichment Culture

Parameter	Value
pH	13.1
COD	104,800 mg/L
Sulfide	0.485 M
Sulfate	281 mg/L
Ammonium ion	0.4 mg/L
Nitrate	306 mg/L
Mercaptans	0.758 M as MeSH
OH^- alkalinity	3.75 N (142.8 g/L as NaOH)
Total alkalinity	212,800 mg/L as $CaCO_3$

for caustic treatment. Treatment of caustic at lesser mercaptan and sulfide feed rates would indicate inhibition of the enrichment culture by other caustic components.

Biotreatability of Spent-Sulfidic Caustic Containing Mercaptans

The objective of this part of the study was to evaluate the ability of the acclimated enrichment culture described above to treat a selected spent-sulfidic caustic containing mercaptans. Samples of a refinery spent-sulfidic caustic were shipped from a major refinery and were sealed until used. Samples were analyzed for sulfides, mercaptans, OH^- alkalinity, sulfates, COD, nitrate, ammonium ion, and carbonates (Table 3).

At start-up the bioreactor (B. Braun Biostat M) was filled to 1.45 L with a suspension of the enrichment culture in mineral-salts medium at an initial mixed liquid suspended solids (MLSS) concentration of approx 3000 mg/L. After the suspension had equilibrated at 30°C and had been aerated for approx 1 h, caustic feed was initiated. The caustic feed was conveyed to the bioreactor using a Harvard Apparatus (Cambridge, MA) syringe pump with a 50-mL syringe with a Teflon seal. The caustic feed was introduced into the reactor at a point approx 2.5 cm from the bottom adjacent to the agitator impeller. The agitation rate was maintained at 200 rpm. The temperature was controlled at 30°C and pH was maintained at 7.0. To control the pH, 5 *N* HNO_3 was used. This particular acid was chosen to permit the monitoring of acid addition in the bioreactor by following the nitrate concentration. The culture was aerated with 300 mL/min air

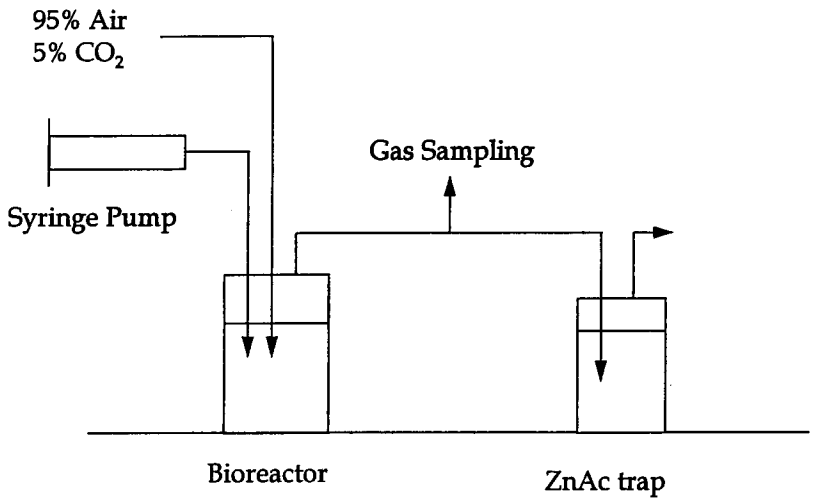

Fig. 1. Schematic diagram of equipment used in the biotreatment of spent-sulfidic caustic.

+ 5% CO_2. The outlet gas from the bioreactor was passed to a 500-mL Erlenmeyer flask containing 350 mL of 0.3% zinc acetate to trap any fugitive emissions of H_2S from the bioreactor. A tee connection was located between the bioreactor and the zinc acetate trap for gas sampling. A schematic diagram of the equipment setup is shown in Fig. 1.

Caustic feed was initiated at a rate of 3.8 mL/d and the H_2S and MeSH gases in the outlet monitored periodically. The reactor was successfully operated without upset (MeSH or H_2S breakthrough) at this rate. Over the next 2 mo, the caustic feed rate was increased in steps of 2–3 mL/d to 20.4 mL/d. The culture received caustic feed during normal working hours and only aeration without feed overnight.

Analytical

Thiosulfate, sulfide, sulfate, ammonium ion, nitrate, MLSS, sCOD, hydroxide alkalinity, carbonate alkalinity, and total alkalinity in culture medium samples or caustic were determined as previously described *(3,4)*. Soluble COD was determined on the supernatant of culture medium samples after centrifugation at 5000*g* for 10 min. Total mercaptan in the caustic was determined by titration with standard 0.10 *M* lead perchlorate using an Orion Model 94–16 (Cambridge, MA) sulfide/silver electrode to detect the endpoint.

The hydrogen sulfide and methylmercaptan concentrations in the outlet gas of the bioreactor were analyzed using Gastec Analyzer tubes (Gastec, Ayase-City, Japan) with a minimum accuracy of ± 25%. Various ranges of the analyzer tubes in the concentration of 2.5 to 5000 ppmv were used with 100-mL samples. No effect of H_2S as an interferent in MeSH analysis was observed up to H_2S concentration of 500 ppmv.

RESULTS AND DISCUSSION

Development of Aerobic Enrichment Culture

The enrichment culture was operated with 0.5% MeSH (g) feed for a total of 139 h, ultimately achieving a feed rate of 104 mL/min without significant breakthrough of MeSH in the outlet gas. During this time sulfate accumulated in the culture medium as MeSH was removed from the feed gas. A sulfur balance (Table 4) indicated complete oxidation of mercaptan sulfur to sulfate. Ammonium ion was also observed to be utilized as the culture grew on MeSH as a carbon and energy source. During 139 h, of operation the soluble COD was observed to increase to 240 mg/L. This increase in COD has been attributed to the accumulation of cell lysis products from organisms incapable of using MeSH.

As noted above, once mercaptan oxidation had been demonstrated, hydrogen sulfide was blended with the MeSH feed gas to the culture to demonstrate simultaneous removal and oxidation of both compounds. Ultimately, a combined feed of 100 mL/min of 0.5% MeSH and 25 mL/min of 1.0% H_2S was achieved without significant breakthrough of either sulfur compound in the outlet gas. This combined mercaptan and sulfide feed rate was equivalent to 40 mL/d of caustic feed (Table 3). This feed condition was maintained for over 25 h of operating time. During this time, sulfate was observed to accumulate in the culture medium and ammonium ion was consumed as MeSH and H_2S were removed from the feed gas. A sulfur balance showed that at least 87% of MeSH and H_2S sulfur was recovered as sulfate. No other forms of sulfur were detected in the culture medium or outlet gas. It should be noted that when operating with a combined MeSH and H_2S feed, it was important to initiate the H_2S feed before or at the same time that MeSH feed was initiated. If the culture was first exposed to MeSH, H_2S could not be tolerated. However, in this case cessation of MeSH and H_2S feed and overnight aeration of the culture resulted in a renewed capability to treat both simultaneously when initiated in the right sequence.

Biotreatability of Spent Sulfidic-Caustic Containing Mercaptans

Caustic feed was initiated to the reactor at a rate of 3.8 mL/d. During this start-up period, neither H_2S nor MeSH were detected in the outlet gas of the reactor. The caustic feed rate was gradually increased periodically in increments of 2–3 mL/d. Ultimately, it was possible to increase the feed rate to 20.4 mL/d without significant emission of MeSH or H_2S (< 3 ppmv).

As caustic was fed to the culture there was a corresponding increase in the sulfate concentration (Fig. 2). A mass balance on sulfur indicated at least 90% conversion of MeSH sulfur and sulfide from the caustic to sulfate. Ammonium-ion concentrations in the reactor medium fell in response to caustic feed indicating microbial utilization of NH_4^+ as the nitrogen source

Table 4
Sulfur Balance in the Aerobic MeSH Enrichment Culture with a Gas Feed of 0.5% MeSH in Nitrogen

MeSH Feed Rate (mL/min)	MeSH Feed Rate (mmol/h)	MeSH In (mmol)	MeSH Out (mmol)	Sulfate Produced (mmol)	SO_4^{-2}/ MeSH
58-104	0.7-1.0	144.3	3.8	133.1	0.95
74	0.89	68.2	5.3	58.2	0.93

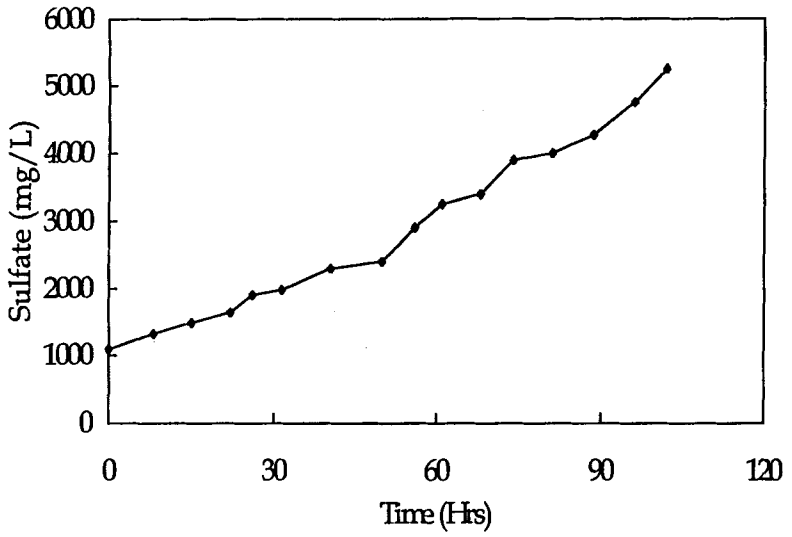

Fig. 2. Sulfate accumulation in enrichment culture during caustic feed.

as the organisms used mercaptans and sulfides in the caustic as energy sources. During caustic feeding there was a small increase in the soluble COD (to 180 mg/L) in the culture suggesting the possible accumulation of some material from the caustic in the culture medium. However, this COD accumulation was very small compared to simple dilution of the caustic in the culture medium (Fig. 3). The MLSS concentration was seen to increase as the cultures grew at the expense of mercaptan and sulfide oxidation in the culture from 3000 mg/L when caustic feed was initiated to 4600 mg/L after a total of 180 h of operation on a caustic feed.

Attempts to increase the flow rate beyond 20.4 mL/d resulted in large emissions of MeSH. The preliminary conclusion from these observations was that the caustic (Table 3) contained some component(s) that was (were) inhibitory to the MeSH-oxidizing organisms in the culture since the culture could tolerate combined feeds of MeSH and H_2S in the gas phase that greatly exceeded the molar feed rates of MeSH and H_2S represented by

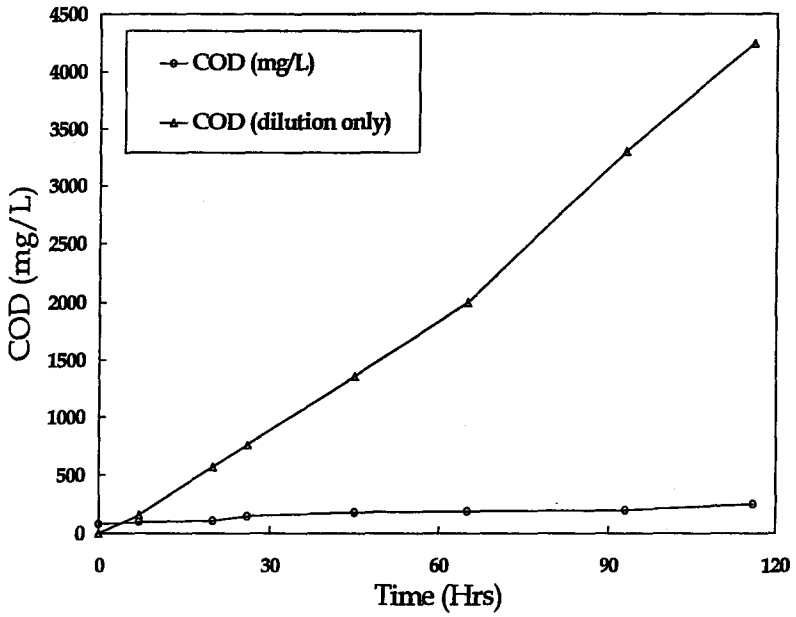

Fig. 3. Accumulation of sCOD in enrichment culture during caustic feed.

these caustic feed rates. In equivalent molar terms the culture should have been able to treat 40 mL/d of this caustic.

Based on the sulfide and mercaptan analysis of the spent caustic given in Table 3, and assuming an average MLSS of 4.0 g/L, the highest specific activities for oxidation of sulfide and mercaptan in spent caustic observed in these experiments were 0.102 mmoles sulfide/h/g MLSS and 0.170 moles mercaptan/h/g MLSS, respectively. In terms of sulfide oxidation this is approximately one-tenth the specific activity for sulfide oxidation observed previously in the treatment of mercaptan-free caustic by *T. denitrificans* strain F *(10).*

CONCLUSIONS

An aerobic enrichment culture has been developed that is capable of biotreatment of refinery spent caustic containing both inorganic sulfide and mercaptans. Treatment rates of refinery caustic were lower than predicted based on tolerance of the culture for combined gas feeds of MeSH and H_2S used to simulate spent-sulfidic caustic feed. These results suggest that the selected caustic contains other components inhibitory to the process culture.

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