Biotreatment of Refinery Spent Sulfidic Caustic by Specialized Cultures and Acclimated Activated Sludge

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ABSTRACT

Sodium hydroxide solutions are used in petroleum refining to remove hydrogen sulfide from various hydrocarbon streams. The resulting sulfide-laden waste stream is called spent sulfidic caustic. Two microbial cultures have been investigated to compare their ability to biotreat refinery spent sulfidic caustic. One culture was a specialized flocculated culture of the autotrophic sulfide oxidizer, *Thiobacillus denitrificans* strain F. The other was an acclimated culture enriched from a refinery-activated sludge treatment system. Both cultures were capable of complete oxidation of caustic sulfides to sulfate at specific activities of 1.0–1.3 mmol sulfide/h/g mixed liquor suspended solids (MLSS). However, the enrichment culture exhibited less stable operation in terms of hydrogen sulfide emissions and was less tolerant of reduced temperatures. Use of caustic carbonates as the sole carbon source for sulfide oxidizers and the effect of refinery waste water on spent caustic treatment was also investigated.

Index Entries: Sulfide; hydrogen sulfide; sulfidic caustic; activated sludge; *Thiobacillus denitrificans*.

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 >13, sulfide concentrations exceeding 2–3 wt%, and a large amount of residual alkalinity. 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).

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. Biological treatment in the refinery wastewater treatment unit is an inexpensive disposal option. However, many refineries do not have the wastewater treatment capacity to treat the entire amount of spent caustic generated, and concerns regarding odors and toxicity frequently prohibit this practice.

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We have previously demonstrated that refinery spent sulfidic caustic can be successfully treated in a specialized bioreactor at the bench (1.5–L) and pilot (3800–L) scales resulting in neutralization of the caustic and complete removal of acid-labile sulfides (2,3). Sulfides were completely oxidized to sulfate by a sulfide-tolerant strain (strain F) of the chemoautotroph *Thiobacillus denitrificans*. Microbial oxidation of sulfide produced acid (H_2SO_4), which at least partially neutralized the caustic (pH 7.0).

As indicated above, some refineries dispose of spent caustic in their activated sludge treatment systems (ASU), blending caustic with refinery waste water. These ASUs represent a potential source of sulfide-oxidizing organisms, which can be used to develop an acclimated culture for treating spent caustic in a separate bioreactor where an ASU does not exist or an existing ASU has insufficient capacity to treat the entire amount of spent caustic generated. It is unclear whether these enrichments from activated sludge offer any advantages compared to specialized cultures, such as *T. denitrificans*.

We report here a comparison of two cultures for the biotreatment of refinery spent sulfidic caustic: (1) a flocculated culture of *T. denitrificans* strain F and (2) a sulfide-oxidizing culture enriched from a refinery ASU that receives spent caustic as a component of the feed to the unit. The objective of this work was to determine whether a specialized culture of *T. denitrificans* strain F offers any clear advantage over an enrichment culture from a refinery ASU in treating this waste stream.

MATERIALS AND METHODS

Organisms and Culture

T. denitrificans (ATCC 23642) was originally obtained from the American Type Culture Collection (Rockville, MD). A sulfide-tolerant strain (strain F) was isolated by enrichment as described previously (4). Stock cultures were grown anoxically in 10-mL culture tubes at 30°C in thiosulfate mineral salts medium (5). In this medium, thiosulfate is the energy source, nitrate the terminal electron acceptor, bicarbonate the carbon source, and ammonium ion the source of reduced nitrogen. The medium also contains a phosphate buffer and sources of Mg^{+2}, Ca^{+2}, Fe^{+3}, Mn^{+2}, and trace elements.

T. denitrificans was flocculated by aerobic coculture with floc-forming heterotrophs as previously described (6). The culture was harvested by centrifugation and resuspended in thiosulfate mineral salts medium (without thiosulfate or nitrate) prior to use.

The ASU enrichment culture (AS-1) was prepared using activated sludge taken fresh from the Sun Oil Company refinery in Tulsa, OK. At this refinery, the ASU occasionally receives spent caustic as a component of the feed to the unit. This system was, therefore, expected to contain sulfide-oxidizers. The activated sludge was washed three times with phosphate buffer (pH 7.0), harvested by centrifugation, and resuspended in mineral salts medium prior to use.

Start-Up of Bioreactors and Operation at 30°C

A sample of refinery spent sulfidic caustic was obtained from a refinery operated by a major oil company and characterized prior to use. The results of this characterization are given in Table 1. As shown in Table 1, the sample contained a relatively low concentration of sulfides and a high OH⁻ alkalinity. From this observation, it was predicted that the acid consumption required to maintain the pH in the bioreactor would be high, since there would be insufficient acid produced from sulfide oxidation to neutralize the high OH- concentration.

Table 1
Results of Analysis of Refinery Spent Sulfide Caustic

pH	13.0
COD	26,700 mg/L
Sulfide	5760 mg/L
Mercaptans	Not detected
Sulfate	420 mg/L
Nitrate	490 mg/L
Ammonium ion	Not detected
Total alkalinity	122,200 mg/L as $CaCO_3$
Carbonate	20.0 g/L
OH⁻ alkalinity	2.11N
MDEA/MEA	<1000 mg/L
TOC[a]	4080 mg/L
TDS[a]	130,000 mg/L
Total phenolics[a]	2753 mg/L

MDEA = methyl diethanolamine; MEA = methyl ethanolamine; TOC = total organic carbon; TDS = total dissolved solids.

[a]Results obtained from a commercial laboratory.

Figure 1 shows a schematic diagram of the testing apparatus used in this study. The caustic feed was conveyed to the bioreactors using a Harvard Apparatus syringe pump. A 50-mL syringe with a Teflon™ seal was used as the caustic source for each bioreactor. The caustic feed was introduced into each bioreactor at a point about 1 in. from the bottom, adjacent to the agitator impeller. Each bioreactor consisted of a B. Braun Biostat M fermenter with a working volume of 1.5 L. The agitation rate was 200 rpm, the temperature was controlled at 30°C, and the pH controlled at 7.0. The acid used for pH control was 5N HNO_3. This particular acid was used so that acid addition could be monitored by following the nitrate concentration in the culture medium. The cultures received a gas feed of 95% air + 5% CO_2 at a rate of 300–400 mL/min. The outlet gas from each reactor was passed to 500-mL Erlenmeyer flasks containing 400 mL of 0.3% zinc acetate to trap fugitive emissions of H_2S from the bioreactors. A tee connection was located between the bioreactors and the respective ZnAc traps for gas sampling.

At start-up the *T. denitrificans* reactor (R1) was filled to 1.5 L with a suspension of flocculated *T. denitrificans* (in mineral salts medium) at an initial concentration of mixed liquor suspended solids (MLSS) of 2900 mg/L. The AS-1 reactor (R2) was filled to 1.5 L with a suspension of washed refinery-activated sludge in mineral salts medium. The initial MLSS in this reactor was 3600 mg/L. Once these suspensions had equilibrated at 30°C and had been aerated for about 1 h, the caustic feeding procedure was initiated. At this time, a sample of mixed liquor was taken from each reactor for MLSS determination. A net of 40 mL of each culture was then removed, and the biomass recovered by centrifugation and returned to the appropriate reactor. A sample of the supernatant was retained for sulfate, nitrate, COD, and ammo-
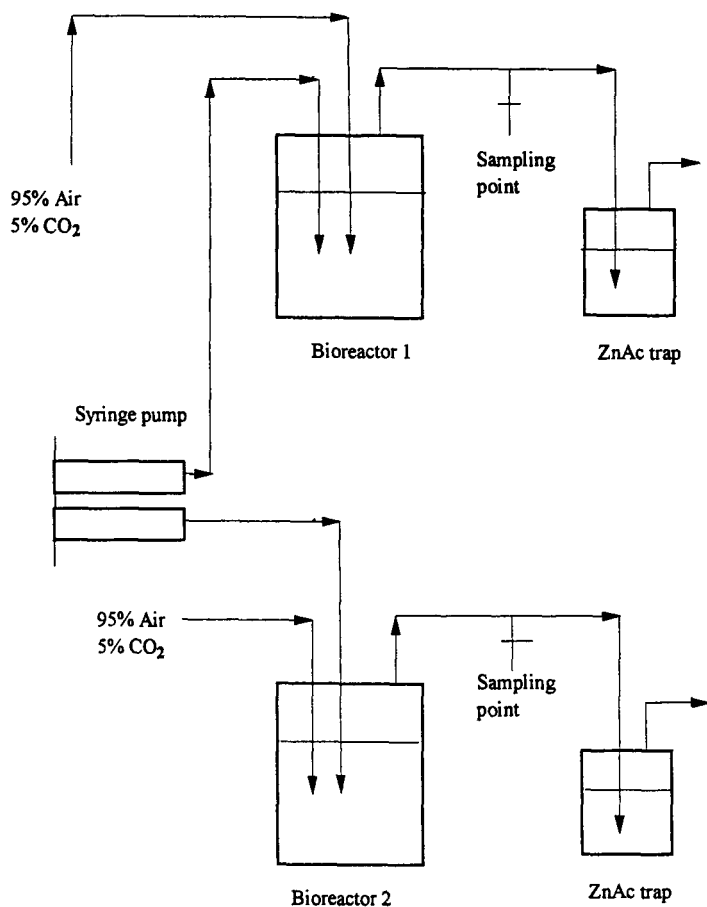
Fig. 1. Schematic diagram of bioreactor system for treating refinery spent sulfidic caustic.

nium ion determination. After sampling was completed, caustic feed was initiated at a rate of 40.3 mL/d to each reactor.

Caustic feed to the reactors continued until the nitrate concentrations in the reactor exceeded 10,000 mg/L. At this time, the caustic feed was terminated and the biomass allowed to settle under gravity. The biomass in each reactor concentrated to about 200 mL. The supernatants were then siphoned off and replaced with fresh mineral salts medium, and caustic feed reinstated at 40.3 mL/d. All other operating conditions remained unchanged. Ultimately (after 134 h of operation), the caustic feed rate was increased to 56.7 mL/d.

During the remainder of the experiment, the medium in both reactors was changed in this manner whenever the nitrate concentration exceeded 10,000 mg/L to avoid nitrate inhibition. Nitrate inhibition of *T. denitrificans* has been observed at these levels during growth on thiosulfate (data not shown).

The two reactors were ultimately operated at 30°C for a total of 250 h. As will be discussed below, at the end of this time, the two reactors were operating in an identical manner with respect to oxidation of sulfide in the caustic to sulfate and H_2S

emissions from the reactors. A series of experiments were then conducted for the purposes of distinguishing between the performance of the two cultures.

Effect of Reduced Temperature on Sulfide Oxidation

The operating temperatures of R1 and R2 were reduced in steps to 20°C while receiving a feed of 57.6 mL/d of caustic to each reactor. All other operating conditions remained unchanged. At the conclusion of this experiment both reactors were returned to 30 °C and received aeration only for 24 h. Caustic feed was then reinstated at 57.6 mL/d for 50 h. This period of operation was viewed as a recovery time for the cultures and to make sure the cultures were operating as expected before initiating the next phase of the experiments.

Specific Activity for Sulfide Oxidation

At the conclusion of the recovery period described above, the biomass from both reactors was allowed to settle under gravity to a volume of about 200 mL in each reactor. About one-half of the biomass from each reactor was removed and stored at 4°C to be used in other experiments. The remainder in each reactor was resuspended in fresh mineral salts medium and equilibrated at 30°C. The pH continued to be controlled at 7.0. After about 1 h of equilibration, a sample was taken for MLSS determination, and caustic feed was initiated at 28.8 mL/d. After about 16 h, the caustic feed rate was increased to 40.3 mL/d. The caustic feed rate was then increased about every 2 h while the H_2S concentration in the outlet gases from both reactors was monitored. The caustic feed rates investigated were 57.6, 80.6, 112.3, 158.4, 216.0, 302.4, and 432 mL/d.

These reactors systems can be expected to operate without large-scale production of H_2S as long as the sulfide is not fed to the reactors faster than the specific activity of the biomass for sulfide oxidation. Therefore, increasing the sulfide feed rate to the reactors until large-scale production of H_2S is observed allows estimation of the specific activity of the biomass in each culture. When significant H_2S production was observed, the aeration rate was increased (up to 1200 mL/min) to ensure that the upset was not owing to an oxygen limitation. An upset in terms of H_2S production that could not be reversed by an increase in the aeration rate was taken to be a result of exceeding the specific activity of the biomass for sulfide oxidation.

At the conclusion of this experiment, the medium in both reactors was changed to fresh mineral salts medium and the reactors aerated at 30°C for about 24 h. Caustic feed was then reinitiated at 40.3 mL/d to determine if the cultures would still operate as expected at this feed rate before proceeding to the next experiment.

Use of Caustic Carbonates as Sole Carbon Source

At the conclusion of the recovery period described above, the biomass in each reactor was harvested by centrifugation, resuspended in mineral salts medium without sodium bicarbonate, and returned to the appropriate bioreactor. Temperature and pH were maintained at 30°C and 7.0, respectively, and the cultures were aerated with air without the CO_2 supplement used in all previous experiments. When the cultures temperatures reached 30°C caustic feed was initiated to both reactors at 40.3 mL/d. After 42.7 h, the rate was increased to 57.6 mL/d. Caustic was fed to the reactors for a total of 112 h with one change of medium. The purpose of this experiment was to determine whether carbonates in the caustic would provide sufficient carbon for sulfide oxidizers in the cultures.

Effect of Refinery Waste Water on Caustic Treatment

Potentially the capacity of an existing ASU for treatment of spent caustic as a component of the waste-water influent can be increased by augmenting the activated sludge culture with greater numbers of sulfide oxidizers. An experiment was conducted to compare the performance of the *T. denitrificans* culture and the enrichment culture as a source of sulfide oxidizers for this type of application.

At the conclusion of the previous experiment, the biomass in both reactors was allowed to settle to a volume of about 200 mL. The supernatant was then drained off and discarded. To each reactor was then added 200 mL of settled activated sludge from a refinery that does not receive spent caustic feed. Activated sludge, feed, and effluent from the ASU had been shipped cold to the laboratory by overnight delivery and stored at 4 °C on arrival. This experiment was initiated within 48 h of the arrival of these materials. After addition of activated sludge the volume in each reactor was made up to 1.5 L with ASU effluent supplemented with all of the nutrients of the mineral salts medium, except sodium bicarbonate. A feeding procedure designed to give a hydraulic retention time of 29–30 h was then initiated as follows. From each reactor was removed 600 mL of mixed liquor. The biomass was recovered by centrifugation and returned to the appropriate reactor. At this time, a feed of "ASU feed" supplemented with mineral salts nutrients (without bicarbonate) was initiated at 0.8 mL/min. Caustic feed was also initiated at 57.6 mL/d. Each reactor was sparged with 300–400 mL/min of air without a CO_2 supplement. With these two liquid feeds, about 600 mL were delivered to each reactor in 12 h. Every 12 h, this feeding procedure was repeated until the 3 L of ASU feed obtained from the refinery were exhausted. Therefore, each reactor was operated at this feed rate for one hydraulic retention time.

Analytical

The hydrogen sulfide concentrations in the outlet gas of cultures in the B. Braun Biostat M reactors were estimated using GasTec Analyzer tubes (Yokohoma, Japan). The range of the analyzer tubes was 2.5–60 ± 2 ppm using 100-mL samples.

Total aqueous sulfide (H_2S, HS^-, and S^{-2}) was determined colorimetrically using the methylene blue method (7). Sulfides were precipitated with 0.3 wt% zinc acetate as ZnS prior to analysis. Sulfate was determined turbidometrically following precipitation with $BaCl_2$ (8). Elemental sulfur was determined by reaction with cyanide to produce thiocyanate, which was quantitated as $Fe(SCN)_6^{-3}$ (9). Ammonium ion was determined by the Nesslers method without distillation (8). Nitrate was determined by the cadmium reduction method. Chemical oxygen demand (COD) was determined using Hach Chemical Co. (Loveland, CO) premeasured reagents. The MLSS concentration was determined by filtering known volumes (5 mL or 10 mL) of the culture medium through tared, prewashed Whatman GF/C glass fiber filters (8).

OH^- alkalinity was determined by titration with standardization HCl and phenolphthalein as indicator (8). Total alkalinity was determined by titration with standardized HCl to pH 4.5 (8). Carbonate alkalinity was calculated from the total and OH^- alkalinities (8).

The Sun Oil Co. refinery-activated sludge treatment system clearly contained sufficient sulfide-oxidizing organisms to allow the R2 culture to acclimate to the caustic at the feed rates used in this study. In order to demonstrate that these cultures were distinctly different in terms of the dominant populations of sulfide
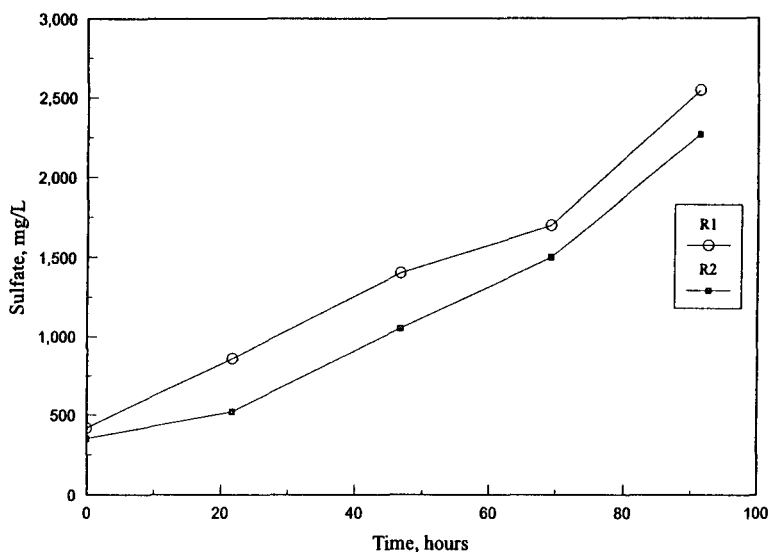
Fig. 2. Sulfate accumulation in *T. denitrificans* (R1) and ASU enrichment (R2) cultures during start-up at 30°C. Caustic feed rate = 40.3–56.7 mL/d.

oxidizers, samples of mixed liquor from both reactors were removed from R1 and R2 just prior to the initiation of the temperature reduction experiment described above. These samples were used to inoculate completely filled, 10-mL culture tubes containing anoxic thiosulfate mineral salts medium with nitrate as the terminal electron acceptor. These cultures were serially diluted, incubated at 30°C, and a most probable number (MPN) of denitrifying, sulfide-oxidizing organisms obtained.

RESULTS AND DISCUSSION

Start-Up and Operation at 30°C

Within 2 h of start-up of the reactor system, trace amounts of H_2S were detected in the outlet gas of the AS-1 reactor (R2). The H_2S concentration in the outlet gas from R2 increased to 75 ppm after 7 h, and then declined to zero over the next 20 h. The activated sludge in the AS-1 reactor acclimated to the caustic during this period and began to oxidize the sulfide to sulfate (Fig. 2). During this start-up period, no H_2S was detected in the outlet gas of the *T. denitrificans* reactor (R1). Figure 2 shows more sulfate accumulation in R1 than in R2 as expected, since all of the sulfide fed to R1 was oxidized to sulfate, whereas during start-up, some of the sulfide fed to R2 was stripped as H_2S. After H_2S breakthrough in R2 ceased, the rate of sulfate accumulation in the two reactors was approximately the same as shown in Fig. 2. Figure 3 shows the COD concentration in the two reactors during this time. In this figure, Dil refers to the COD concentration that would be expected in the bioreactors if no oxidation of caustic components takes place. These values were estimated based on simple dilution of the caustic.

After this initial start-up period, the reactors were operated for an additional 160 h corresponding to three medium change cycles. No H_2S emissions were detected
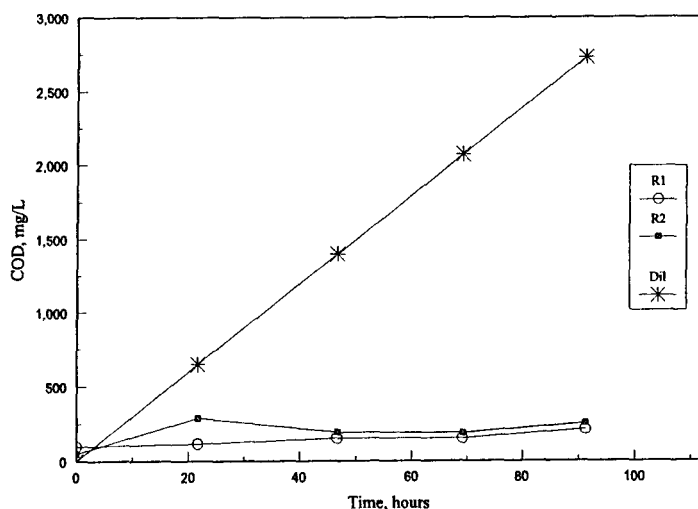
Fig. 3. COD concentrations in *T. denitrificans* (R1) and ASU enrichment (R2) cultures during start-up at 30°C. Caustic feed rate = 40.3–56.7 mL/d. Dil refers to the COD concentration that would result in either culture from simple dilution of the caustic feed.

from either reactor during this time by gas analysis, and no ZnAc was detected in the ZnAc trap. Sulfate accumulated in the both culture media as caustic was fed to the reactor. Sulfur balances (Tables 2 and 3) indicated complete oxidation of caustic sulfide to sulfate in both reactors. We have previously demonstrated that about 1 mol of acid is produced/mol of sulfide oxidized by *T. denitrificans* in the microbial treatment of spent sulfidic caustic *(3)*. Therefore, it was anticipated that the amount of acid required to maintain the pH at 7.0 would be approximately equal to the equivalents of OH⁻ alkalinity less the moles of sulfide feed. This would give an HNO_3/OH⁻ ratio <1. This ratio was actually about one or greater (1.04–1.09) in this first series of experiments. This suggests that there was some other sink for acid in these cultures other than the OH⁻ alkalinity in the caustic.

In each cycle, the MLSS was seen to increase (about 0.5 g/L increase in each cycle) as the cultures grew at the expense of sulfide oxidation in the caustic. However, the amount of increase in MLSS in either reactor cannot be accounted for strictly in terms of accumulation of biomass based on previous measurements of biomass yield from aerobic growth of *T. denitrificans* on H_2S *(10)*. Some increase in COD was observed in both reactors. However, this increase in COD was only 1–6% of the COD expected from simple dilution of the caustic. The ammonium ion concentration was seen to increase (from 160 to 210 mg/L) in both reactors as caustic was fed to the cultures. Ammonium ion is a source of reduced nitrogen for the cultures and would be expected to decrease in concentration with time in the absence of a mechanism to generate ammonium. It appears that the amines present in the caustic were metabolized by the heterotrophs in both cultures liberating ammonium ion *(2)*. This may also account for the greater biomass yields.

Effect of Reduced Temperature on Sulfide Oxidation

At the conclusion of the series of experiments described above, the medium was changed in both reactors to fresh mineral salts medium and caustic feed

Table 2
Sulfur Balances R1

Experiment	Sulfide in		Sulfate accumulated		SO_4^{-2}/S^{-2}
	mg	mmol	mg	mmol	mol/mol
First feed at 30°C					
Cycle 1	611	19.1	1882	19.6	1.03
Cycle 2	838	26.2	2473	25.8	0.98
Cycle 3	627	19.6	1949	20.3	1.04
Temperature reduction					
Cycle 1	838	26.2	2378	24.8	0.95
Cycle 2	752	25.3	2488	25.9	1.02
Temperature reduction					
recovery	851	26.6	2598	27.1	1.02
Overload					
recovery	557	17.4	1800	18.8	1.08
w/o CO_3^{-2}					
Cycle 1	903	28.2	2605	27.1	0.96
Cycle 2	778	24.3	2094	21.8	0.90

Table 3
Sulfur Balances R2

Experiment	Sulfide in		Sulfate accumulated		SO_4^{-2}/S^{-2}
	mg	mmol	mg	mmol	mol/mol
First feed at 30°C					
Cycle 1	611	19.1	1979	20.6	1.08
Cycle 2	838	26.2	2243	23.4	0.89
Cycle 3	627	19.6	1728	18.0	0.92
Temperature reduction					
Cycle 1	838	26.2	275	2.9	0.11
Cycle 2	752	25.3	1606	16.7	0.66
Temperature reduction					
recovery	851	26.6	2367	24.7	0.93
Overload					
recovery	557	17.4	1579	16.4	0.94
w/o CO_3^{-2}					
Cycle 1	903	28.2	2374	24.7	0.88
Cycle 2	778	24.3	1863	19.4	0.80

restarted at 57.6 mL/d. Both reactors were operated at 30°C for 4.9 h. At this time, the temperature was reduced in both cultures to 26.5°C by lowering the temperature of the jacket cooling water. Within 3–4 h, the AS-1 reactor (R2) was producing 900 ppm H_2S. No H_2S was found in the outlet gas of the *T. denitrificans* reactor (R1). At this point, the temperature in both reactors was increased to 30°C. The H_2S

concentration in the outlet gas from R2 then dropped sharply. The reactors were operated overnight at this temperature while continuing to receive a caustic feed at 57.6 mL/d.

The temperature of both reactors was decreased to 25°C the next day, some 21 h after the initiation of caustic feed. R2 responded by producing H_2S at concentrations of up to 30–40 ppm for 6-8 h followed by occasional breakthrough of H_2S production at lower concentrations thereafter. No H_2S emissions were detected from R1 at this temperature. After a total of 38 h of operation at 25°C, the temperature of both reactors was lowered to 20°C. Almost immediately R2 produced small amounts of H_2S (5–30 ppm) while no H_2S was produced from R1. After 12 h at this temperature (overnight), R2 was producing over 200 ppm H_2S. R2 had apparently been producing H_2S for most of the night as indicated by ZnS in the ZnAc trap. R1 continued to operate normally with no H_2S detected in the outlet gas or ZnAc trap. At this time, the caustic feed was terminated to R2 and the reactor continued to receive aeration at 20°C. After an additional 24 h of operation at 20°C, elemental sulfur was observed in R1, indicating stress in the culture. However, no H_2S was detected in the R1 outlet gas, and no sulfide had precipitated in the ZnAc trap.

When elemental sulfur was detected in R1, the caustic feed to R1 was terminated and the operating temperatures of both R1 and R2 were increased to 30°C. Both reactors continued to receive aeration at this temperature for about 24 h. The elemental sulfur in R1 began to disappear visibly when the temperature was increased to 30°C and was gone within 2 h.

Sulfate accumulation was reduced in R2 compared to R1 because of stripping of sulfide as H_2S in R2. This was especially true during the first cycle of operation where high concentrations of H_2S were observed in the R2 outlet gas. During the first cycle of operation at reduced temperatures, very little acid addition was noted in R2. The pH during this time was maintained constant at 7.0. It is unknown how the OH^- was neutralized during this time. COD accumulated at low concentrations in both reactors as caustic was fed to the reactors. The exception was R2 during the first cycle of operation when large amounts of H_2S were produced. The COD in R2 increased from 50 to almost 400 mg/L. It is postulated that this was caused by some cell death and lysis in the culture. Ammonium ion concentrations were seen to increase in both reactors again, suggesting degradation of the amines.

Sulfur balances (Table 2) indicated complete oxidation of sulfides to sulfate in R1 during this temperature reduction experiment. Not surprisingly, molar SO_4^{-2}/S^{-2} ratios (Table 3) in R2 were significantly <1 because of stripping of H_2S. Acid utilization in R1 was lower ($HNO_3/OH^- = 0.84$) at these reduced temperatures compared to results obtained at 30°C.

After 24 h at 30°C with aeration only, the medium was changed to fresh mineral salts medium and caustic feed reinitiated at 57.6 mL/d. Both reactors were operated at this condition for 50.4 h. This period of operation was viewed as a recovery time for the cultures to make sure that the cultures were operating as expected before initiating the next phase of the experiments. Both cultures operated as expected based on earlier results obtained at 30°C. Hydrogen sulfide was occasionally detected at low concentrations in R2. No H_2S was detected in the outlet gas from R1 during this period. Sulfur balances (Tables 2 and 3) indicated complete oxidation of sulfide to sulfate in both reactors during this time.

Specific Activity for Sulfide Oxidation

At the conclusion of the previous experiment the caustic feed rate to both reactors was increased as described above. No H_2S was detected in the outlet gas of either reactor until the caustic feed rate reached 302.4 mL/d. At this time, the aeration rate was increased to about 800 mL/min resulting in 80–90% reduction in the H_2S concentrations in the outlet gases. This reduction suggested that the upset induced at a caustic feed rate of 302.4 mL/d may have been the result of oxygen limitation and not of exceeding the specific activity of the biomass for sulfide oxidation. The caustic feed rate was then increased to 432 mL/d. Both reactors immediately produced large amounts of H_2S, even when the aeration rate was again increased to over 1200 mL/min. The caustic feed to each reactor was then terminated, the medium changed to fresh mineral salts medium, and the cultures aerated at 30°C. It is interesting to note that the supernatant in R2 following gravity settling of the biomass, which preceded the change of medium, was very turbid, suggesting significant cell lysis.

The highest caustic feed rate achieved in this experiment without major upset was 302.4 mL/d. Based on the initial MLSS concentration in each reactor, the maximum specific activities observed were 1.3 mmol sulfide/h/g MLSS in R1 and 1.1 mmol sulfide/h/g MLSS in R2. In this laboratory, we have previously measured the specific activity of flocculated *T. denitrificans* for aerobic sulfide oxidation in the treatment of sour water containing up to 800 ppm sulfide in a well-mixed, up-flow bubble column. That specific activity was 1.0 mmol sulfide/h/g MLSS *(7)*.

As indicated above, at the conclusion of the upset experiment, the medium in both reactors was changed to fresh mineral salts medium, and the reactors aerated at 30°C for about 24 h. Caustic feed was then reinitiated at 40.3 mL/d to determine if the cultures would still operate as expected at this feed rate before proceeding to the next experiment. Both cultures operated as expected based on earlier results obtained at 30°C. No H_2S was detected in the outlet gas from either R1 or R2 during this period. Sulfur balances (Tables 2 and 3) indicated complete oxidation of sulfide to sulfate in both reactors during this time.

Use of Caustic Carbonates as Sole Carbon Source

At the conclusion of the recovery period described above, the biomass in each reactor was harvested by centrifugation, resuspended in mineral salts medium without sodium bicarbonate, and returned to the appropriate bioreactor. During this period, no H_2S was detected in the outlet gas from R1. Hydrogen sulfide was occasionally seen in the R2 outlet gas at low concentrations (2–3 ppm). Both cultures operated in a manner similar to cultures supplemented with CO_2. Clearly, the cultures were both capable of deriving all of the carbon required for growth of sulfide oxidizers from the caustic.

Sulfur balances (Table 2) indicated complete oxidation of sulfide to sulfate in R1. Sulfate/sulfide ratios were a bit low in R2 (Table 3), possibly indicating incomplete oxidation of sulfides to sulfate under these conditions. The H_2S emissions were too low to account for the discrepancy, and no elemental sulfur was detected in the reactor. Therefore, the reason for the reduced ratios is unknown. Acid utilization calculations show that acid consumption (relative to the amount of OH⁻ alkalinity introduced into the reactors) was lowest in the absence of supplemental CO_2 (HNO_3/OH⁻ = 0.68–0.77).

Effect of Refinery Waste Water on Caustic Treatment

During the 30 h of operation in the presence of refinery-activated sludge and with a refinery waste-water feed, sulfate accumulated in each culture medium, and no H_2S was detected in the outlet gas of either reactor or precipitated in the ZnAc trap. Initial sulfate concentrations were quite high compared to previous experiments. In all respects, the reactors seemed to operate in a manner comparable to those operated in the absence of any refinery waste water. Only modest increases in COD concentration were observed. The final COD concentrations in both R1 and R2 were significantly lower than would be predicted based on simple dilution of the ASU feed. Therefore, oxidation of some of the ASU feed COD by heterotrophs in both reactors is indicated.

Enumeration of Denitrifying Sulfide Oxidizers

R2 was shown to contain only 10^6–10^7 cells/mL of denitrifying sulfide oxidizers. Therefore, the predominant sulfide oxidizers in the AS-1 culture were incapable of sulfide oxidation under anoxic conditions. In comparison, R1 contained over 10^8 cells/mL of denitrifying sulfide oxidizers. Of course, *T. denitrificans* is capable of oxidation of reduced sulfur compound under denitrifying conditions. Clearly, the population dynamics of the two cultures were distinctly different.

CONCLUSIONS

Both flocculated *T. denitrificans* and acclimated activated sludge (AS-1) from the Sun Oil Co. refinery were capable of treating the caustic with complete oxidation of sulfide to sulfate at specific activities of 1–1.3 mmol/h/g MLSS. An abiotic control in which caustic was fed to aerated mineral salts medium without *T. denitrificans* or acclimated activated sludge resulted in immediate breakthrough of H_2S in high concentrations and accumulation of elemental sulfur in the medium.

The acclimated activated sludge was more sensitive to reductions in temperature than the *T. denitrificans* culture, resulting in large-scale production of H_2S and apparent cell lysis at temperatures of 20–25°C. The *T. denitrificans* culture eventually accumulated elemental sulfur at 20°C, however, no H_2S emissions were observed at this temperature. Both cultures recovered quickly when the temperature was raised to 30°C.

The *T. denitrificans* culture produced H_2S emissions only when excess caustic was fed to the reactor and the specific activity of the biomass for sulfide oxidation was exceeded. However, the ASU enrichment culture occasionally produced H_2S at unpredictable times in the absence of reduced temperature or excess caustic feed. These concentrations were often only 2–3 ppm; however, in a large-scale application, this would be enough to cause significant odor problems.

The caustic contained enough inorganic carbon to support autotrophic sulfide oxidation in both the *T. denitrificans* culture and the acclimated activated sludge. MPN counts in the two cultures for denitrifying sulfide oxidizers showed that the cultures were distinctly different populations. The acclimated activated sludge appears to have contained predominately aerobic sulfide oxidizers, whereas the *T. denitrificans* culture contained large numbers of denitrifying sulfide oxidizers as expected.

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