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Biotreatment of Refinery Spent-Sulfidic Caustic Using an Enrichment Culture Immobilized in a Novel Support Matrix

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Abstract

Sodium hydroxide solutions are used in petroleum refining to remove hydrogen sulfide (H_2S) and mercaptans from various hydrocarbon streams. The resulting sulfide-laden waste stream is called spent-sulfidic caustic. An aerobic enrichment culture was previously developed using a gas mixture of H_2S and methylmercaptan (MeSH) as the sole energy source. This culture has now been immobilized in a novel support matrix, DuPont BIO-SEP™ beads, and is used to biotreat a refinery spent-sulfidic caustic containing both inorganic sulfide and mercaptans in a continuous flow, fluidized-bed column bioreactor. Complete oxidation of both inorganic and organic sulfur to sulfate was observed with no breakthrough of H_2S and <2 ppmv of MeSH produced in the bioreactor outlet gas. Excessive buildup of sulfate (>12 g/L) in the bioreactor medium resulted in an upset condition evidenced by excessive MeSH breakthrough. Therefore, bioreactor performance was limited by the steady-state sulfate concentration. Further improvement in volumetric productivity of a bioreactor system based on this enrichment culture will be dependent on maintenance of sulfate concentrations below inhibitory levels.

Index Entries: Spent-sulfidic caustic; hydrogen sulfide; methylmercaptan; BIO-SEP™, immobilization.

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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 to 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)*.

Although biological treatment can be an inexpensive disposal option, many refineries do not have the wastewater 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 (e.g., pulp and paper mills) 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 for on-site management is commercially available *(2)*, but can result in significant capital investment and high operating costs. Wet-air oxidation 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 (3,4)*. 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 caustics from two refineries were 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 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 cents/gal (1–2.3 cents/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 already noted, 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, such as mercaptans, will be either mixotrophic or a coculture of a heterotrophic organism capable of mercaptan oxidation and an autotrophic, sulfide-oxidizer such as *T. denitrificans*.

We recently reported the development of an aerobic enrichment culture capable of oxidizing mercaptans and sulfides simultaneously *(7)*. The starting material for the enrichment culture consisted of several *Thiobacilli* (*T. thioparus*, *T. denitrificans*, *T. thiooxidans*, and *T. neopolitanus*), activated sludge from a refinery aerobic wastewater treatment system, and sludge from an industrial anaerobic digester. The flocculated, suspended culture was enriched for organisms capable of metabolizing mercaptans and sulfides using methylmercaptan (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 containing mercaptans. However, treatment rates for refinery caustic were lower than predicted based on tolerance of the culture for combined gas feeds of H_2S and MeSH. These results suggested that the caustic treated contained other components inhibitory to the enrichment culture. The maximum observed volumetric productivity for biotreatment of the mercaptan caustic was 7.1 mmol of sulfide/(L·d) and 11.0 mmol of mercaptan/(L·d). In terms of sulfide oxidation, this volumetric productivity was about one tenth that observed in the biotreatment of mercaptan-free caustic by *T. denitrificans (8)*.

In this article, we report the immobilization of this enrichment culture in a novel support matrix containing powdered activated carbon (PAC), namely, DuPont (Wilmington, DE) BIO-SEP™ beads. If refinery spent-sulfidic caustic contains carbon-adsorbable species that are inhibitory to the mercaptan and sulfide-oxidizing organisms of the enrichment culture, the addition of PAC to the culture should provide a sink for these compounds, reducing culture sensitivity through a reduction in the bulk aqueous-phase concentration. The result should be an increase in the volumetric productivity of the culture for sulfide and mercaptan oxidation. It has previously been shown that immobilization of *T. denitrificans* in BIO-SEP beads increased the volumetric productivity for treatment of a mercaptan-free caustic by a factor of 10 *(9)*.

Materials and Methods

Culture

The enrichment culture used in the previously reported work *(7)* had been stored at 4°C in suspension for about 9 mo prior to the initiation of this work. The culture remained flocculated with no signs of significant cell lysis during storage. The culture was harvested by centrifugation

and resuspended in 1.5 L of mineral salts medium (1.2 g/L Na_2HPO_4, 1.8 g/L KH_2PO_4, 0.4 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L NH_4Cl, 0.03 g/L $CaCl_2$, 0.02 g/L $MnSO_4$, 0.033 g/L $FeCl_3$, 1.0 g/L $NaHCO_3$, 15 mL/L trace metals *[3]*, and 50 mL/L mineral water), transferred to a B. Braun Biostat M (Allentown, PA) fermentor, and the pH and temperature maintained at 7.0 and 30°C, respectively. The culture was aerated with 300 mL/min of air plus 5% CO_2. The culture was maintained under these conditions for 24 h to allow diffusion of waste products from the cells. Then the culture was again harvested by centrifugation and resuspended in fresh medium under the same conditions. At this time a gas feed of 0.5% MeSH in nitrogen was initiated in addition to aeration. The MeSH (g) feed rate was increased gradually in steps until the culture was reacclimated to 50 mL/min of 0.5% MeSH as the only carbon and energy source with accumulation of sulfate in the culture medium. Prior to immobilization of the enrichment culture, approx 1 g wet wt of *T. denitrificans* strain F *(10)* grown on thiosulfate was added to the culture to ensure the sulfide-oxidizing capability in the process culture.

Caustic Biotreatment System

Figure 1 presents a schematic diagram of the caustic biotreatment system. The system consisted of a fermentor, fluidized-bed bioreactor, and peristatic pump for hydraulic circulation. The fermentor was a New Brunswick Scientific Bio Flow II (New Brunswick Scientific, Edison, NJ) with a liquid capacity of 3.0 L. The fermentor was equipped with agitation, pH control (via addition of 5 *N* NaOH or HCl), and temperature control. The fluidized-bed bioreactor housed the immobilized culture and consisted of a 5.1-cm id Plexiglas tube 100 cm long. The column widened at the top to a diameter of 10 cm to decrease the vertical fluid velocity. Inside the column was a 3.8-cm id draft tube that ran the length of the smaller diameter section of the column. Air or air + 5% CO_2 was introduced at the base of the draft tube using an high-performance liquid chromatography sparger with a 0.2-μ pore size. The smaller diameter section of the bioreactor was jacketed for temperature control. Reactor medium was introduced at the bottom of the bioreactor delivered by a high-capacity (0–5 L/min) peristaltic pump. Caustic feed was introduced into the medium feed line just before entering the bioreactor using a syringe pump (Harvard Apparatus, Holliston, MA). Medium and entrained gases exited the fluidized-bed bioreactor through a port in the top plate of the bioreactor and returned to the fermentor by gravity flow. Gas exited the system through a condenser on the fermentor vessel and was conveyed to a zinc acetate (0.5 wt%) trap. Dissolved oxygen was measured using an Ingold (Wilmington, MA) dissolved oxygen probe at the top of the fluidized-bed bioreactor.

Immobilization of Enrichment Culture in BIO-SEP Beads

DuPont BIO-SEP beads used in this study were 3–4 mm in diameter and consisted of a composite of 25 wt% polymer and 75 wt% pAC. The bulk

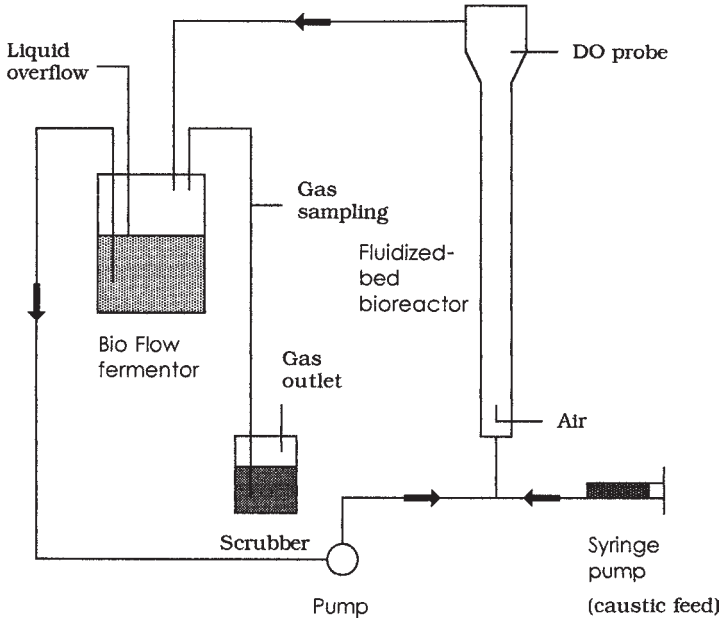

Fig. 1. Schematic diagram of refinery spent-sulfidic caustic biotreatment system.

density of the beads was approx 0.16 g/cm^3. As measured by mercury intrusion porosimetry, the beads had a porosity of 75%, a total intrusion volume of 2.3 mL/g, and a median pore diameter of 19 μ. Large marco pores (>20 μ) exist inside the beads.

Immobilization of the enrichment culture began with loading 3.0 L of mineral salts medium into the fermentor vessel and 3.2 L of medium and 200 g of BIO-SEP beads into the fluidized-bed bioreactor. Recirculation was established at 1.0 L/min and pH and temperature were stabilized at 7.0 and 30°C, respectively. At this time, approx 30 mL of wet-packed cells from the enrichment culture were added directly to the fermentor vessel. The fluidized-bed column bioreactor was aerated with air + 5% CO_2 at a rate of 1.0 L/min.

After 3 d of incubation and circulation under these conditions, a caustic feed was introduced at an initial rate of 3.6 mL/d. The caustic used in this study was obtained from a major refinery. Table 1 gives an analysis of the caustic. The system was operated in a fed-batch mode for 510 h. The caustic feed rate was increased in steps to 7.2–10.1 mL/d after about 100 h of operation. During this time, medium samples were removed from the fermentor vessel for analysis. Gas samples were taken from the off gas of the fermentor vessel. After initiation of caustic feed, aeration was performed with air alone at a sufficient rate to maintain >40–100% of air saturation. Temperature was controlled at 30°C in the fermentor vessel using the Bio Flow II (New Brunswick Scientific) control system and in the fluidized-bed bioreactor by circulating water at 30°C through the jacket. The pH was controlled in the fermentation vessel at 7.0 ± 0.2.

Table 1
Results of Analysis
of Refinery Caustic Used as Feed
to the Immobilized Enrichment Culture

Parameter	Value
pH	>13
COD	105,000 mg/L
Sulfide	350 m*M*
Sulfate	2.9 m*M*
Ammonium	0.02 m*M*
Mercaptans	660 m*M* as MeSH

After 510 h of operation, the caustic feed was stopped and all medium from the system was removed and replaced with fresh mineral salts medium. Caustic feed was reinitiated and all previous operating conditions were re-established. Caustic feed was maintained in a fed-batch mode for an additional 930 h with a gradual increase in the feed rate as biomass accumulated in the column bioreactor. Ultimately, the caustic feed rate reached 28.8 mL/d.

Periodically, BIO-SEP beads were removed from the fluidized-bed bioreactor, cut in half with a razor blade, fixed and coated, and examined at ×10,000 by scanning electron microscopy (SEM) to determine whether and to what extent the culture was populating the interior of the beads.

Continuous Operation of Caustic Biotreatment System

At the end of the second period of fed-batch operation, SEM analysis of BIO-SEP beads in the system indicated that the beads were heavily populated with bacteria. At this time, all the medium in the system was once again removed and replaced with fresh mineral salts medium. Caustic feed was then initiated at 24.4 mL/d in a continuous mode with continuous removal of system medium from the fermentor vessel. The caustic feed rate was increased periodically in an effort to determine the maximum volumetric productivity of the system for caustic biotreatment. As discussed subsequently, the principal indicator of excessive caustic feed was MeSH breakthrough exceeding 20 ppmv.

Sulfate accumulation in the system appeared to limit the performance of the system as indicated by excessive breakthrough of MeSH. At this time, an additional aqueous feed of mineral salts medium was added to reduce the sulfate concentration in the system while operating at higher caustic feed rates. The system was ultimately operated in a continuous mode with a caustic feed of 49 and 490 mL/d of mineral salts medium. At the conclusion of an 840-h operating period under these conditions, BIO-SEP beads and wall growth were removed from the column bioreactor for microbial analysis.

Analytical Methods

The soluble chemical oxygen demand (sCOD), ammonium ion, and sulfate concentrations in the caustic feed and system medium were determined as described previously *(8)*. The sulfide concentration in the caustic feed was determined using the methylene blue method *(11)*. The total mercaptan concentration in the caustic feed was determined by titration with $Pb(ClO_4)_2$ *(7)*.

The concentrations of H_2S and MeSH were determined in the fermentor vessel off gas using Gas Tech (Yokohama, Japan) chromophoric analysis tubes.

Microbial Analysis

Samples of immobilized enrichment culture in BIO-SEP beads and wall growth from the column bioreactor were packed in ice and shipped by overnight delivery to Microbial Insights (Rockford, TN). Samples were subjected to two types of analyses: phospholipid fatty acid analysis (PLFA) and denaturing gradient gel electrophoresis (DGGE). DGGE bands were excised with 16S rDNA extracted and sequenced for identification.

Results and Discussion

Immobilization and Fed-Batch Operation

During the first phase of immobilization and fed-batch operation (520 h), caustic feed rates averaged 7.2–10.1 mL/d. This corresponds to sulfide and mercaptan molar feed rates of 2.5–3.5 and 4.8–6.7 mmol/d, respectively. H_2S concentrations in the outlet gas averaged 0–5 ppmv and MeSH concentrations averaged 10–30 ppmv. At the end of this period, the MeSH concentration in the outlet gas peaked at 50 ppmv, signaling a potential upset condition. For this reason, the medium in the system (fermentor vessel and fluidized-bed bioreactor) was removed and replaced with fresh medium. The mixed liquor suspended solids (MLSS) in the medium removed was 490 mg/L. No effort was made to recover this biomass; therefore, this step represented a selection in the system for biomass that had become associated with the beads or bioreactor walls. Some sulfate (57 mg/L) accumulated during this first period of fed-batch operation. SEM analysis of BIO-SEP beads taken from the system at 520 h showed very few bacterial cells on the interior of the beads. Therefore, it was determined that fed-batch operation would continue until the BIO-SEP beads were more heavily populated.

During the second phase of immobilization and fed-batch operation (additional 920 h), the caustic feed rate averaged 20.2 mL/d, corresponding to 7.1 mmol/d of sulfide and 13.3 mmol/d of mercaptan. System performance during this period was improved over the first period of operation in several respects owing to the accumulation and acclimation of biomass in the system. First, H_2S and MeSH concentrations in the off gas were much

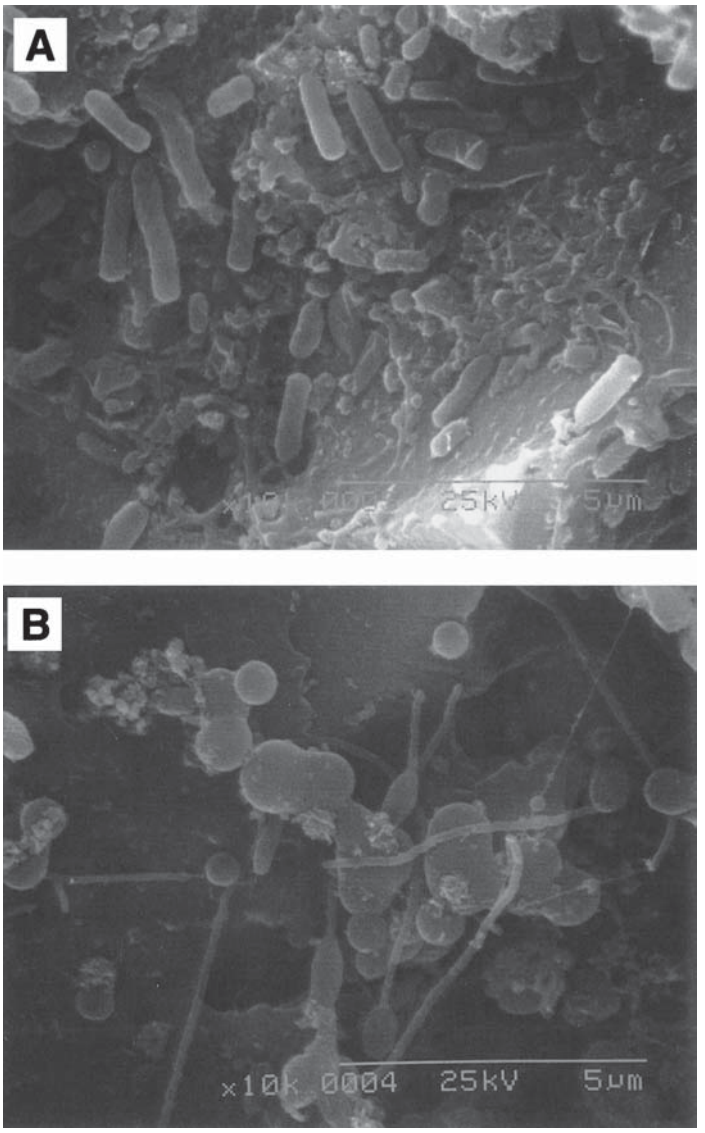

Fig. 2. Scanning electron micrographs of the interior of BIO-SEP beads at the end of the immobilization phase of operation of the refinery spent-sulfidic caustic biotreatment system. **(A)** Near surface; **(B)** intermediate; **(C)** center. ×10,000 magnification.

reduced. H_2S was below detection limits and MeSH concentrations were, for the most part, below 10 ppmv even at higher caustic feed rates during this period. The system was also able to tolerate a greater cumulative caustic feed (610 mL) in this period compared to the first operating period (180 mL) without excessive breakthrough of MeSH. At the end of this second period of fed-batch operation, the MLSS was 230 mg/L and the accumulated sulfate was 480 mg/L. BIO-SEP beads taken from the system at the end of this operating period were examined by SEM at ×10,000 at

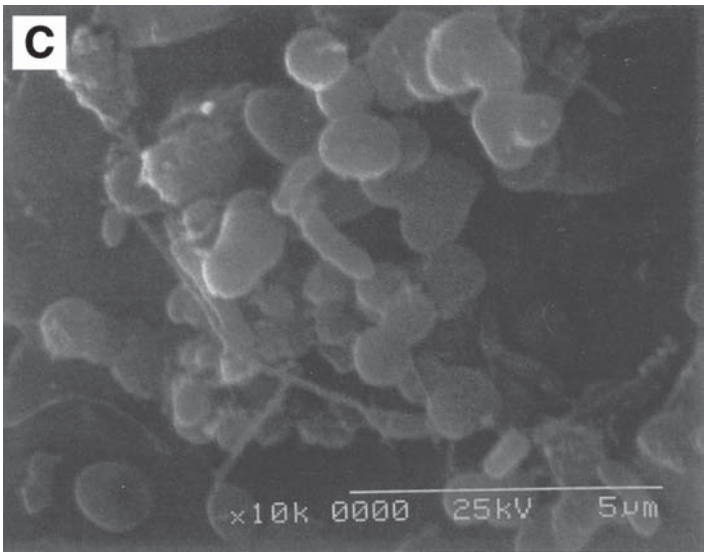

Fig. 2. *(continued)*.

three locations along the radial direction: near the center of the bead, very close to the surface of the bead and intermediate between the center and surface. Although extremely porous internally, BIO-SEP beads have an outer skin with a limited number of pores to provide access to the interior. However, once inside the bead, cells are virtually trapped and replicate to produce potentially high cell densities All beads examined showed large numbers of microorganisms in the internal void spaces. Interestingly, three different morphological types were seen: rod (possibly *Thiobacilli*), coccoidal, and oval with a threadlike structure attached. Rod-shaped bacteria were more abundant near the bead surface, and the oval-shaped bacteria were mainly observed near the center of the beads. Generally, bacteria were more numerous near the surface of the bead, with lower numbers observed near the center (Fig. 2).

To prepare for continuous operation, the system was once again drained of medium and replaced with fresh mineral salts medium.

Continuous Operation of Caustic Biotreatment System

The caustic biotreatment system was operated in a continuous mode with two types of aqueous feed: pure caustic and caustic plus mineral salts medium. Figure 3 summarizes operating conditions and outlet gas concentrations of H_2S and MeSH in the system during operation with pure caustic feed. As shown in Fig. 3, the highest caustic feed rate achieved was 49 mL/d. However, this feed rate also corresponded to the highest concentrations of MeSH in the system off gas. Subsequent reduction of the caustic feed rate resulted in a reduction in the outlet MeSH concentrations; however, MeSH concentrations in the off gas still ranged from 5 to 12 ppmv. Although H_2S concentrations in the outlet gas remained below detection limits (<1 ppmv)

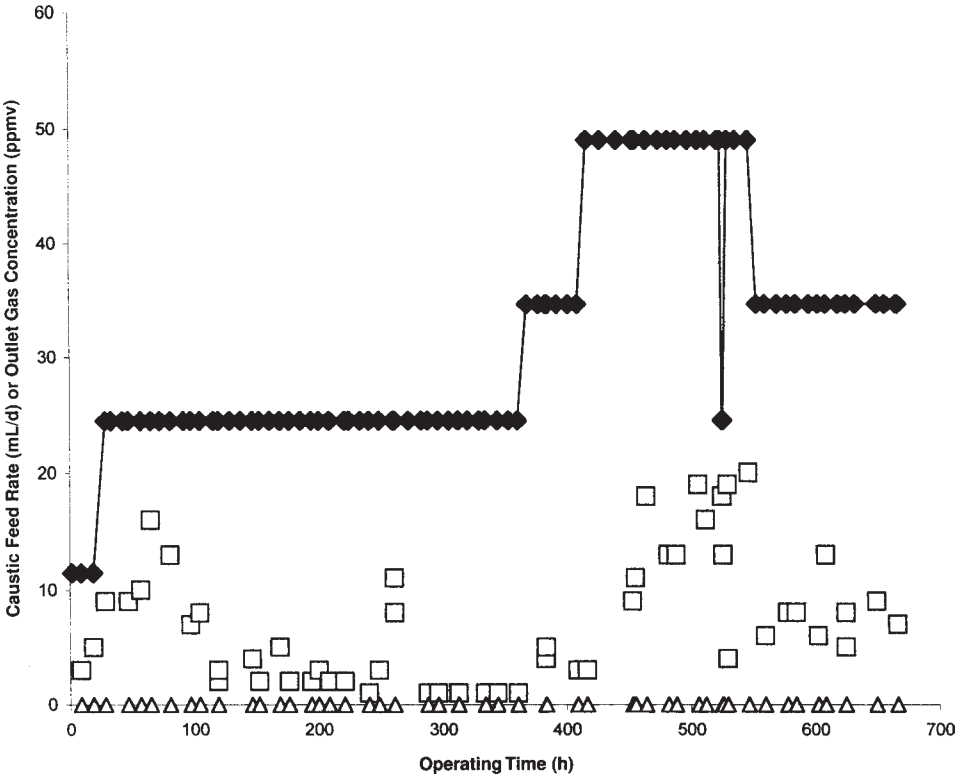

Fig. 3. Caustic feed rate and outlet gas composition of the caustic biotreatment system operating continuously with a pure caustic feed. (—◆—), Caustic feed rate; □, methylmercaptan; △, hydrogen sulfide.

throughout this period of operation, MeSH breakthrough was, of course, undesirable. Sulfate concentrations in the system medium during the period of higher MeSH breakthrough were 10–15 g/L or 104–156 m*M*. Pure cultures of *T. denitrificans* have been shown to be inhibited by sulfate concentrations exceeding 250 m*M*. At this point it was hypothesized that mercaptan-oxidizing organisms in the culture were possibly inhibited by accumulating sulfate. This led to a second period of continuous operation in which a diluent of mineral salts medium was fed to the system along with caustic to reduce the sulfate concentrations in the system.

After 680 h of continuous operation with a pure caustic feed, the system medium was once again removed and replaced with fresh mineral salts medium. Caustic feed (33 mL/d) and mineral salts diluent was then initiated at a 1:1 ratio. The amount of diluent relative to caustic and the caustic feed rate were then both increased in an effort to maximize the caustic feed rate while maintaining sulfate concentrations in the system that would not cause significant MeSH concentrations in the outlet gas.

The best operating regime in our study was a caustic feed rate of 49 mL/d with 490 mL/d of mineral salts diluent. This caustic feed rate

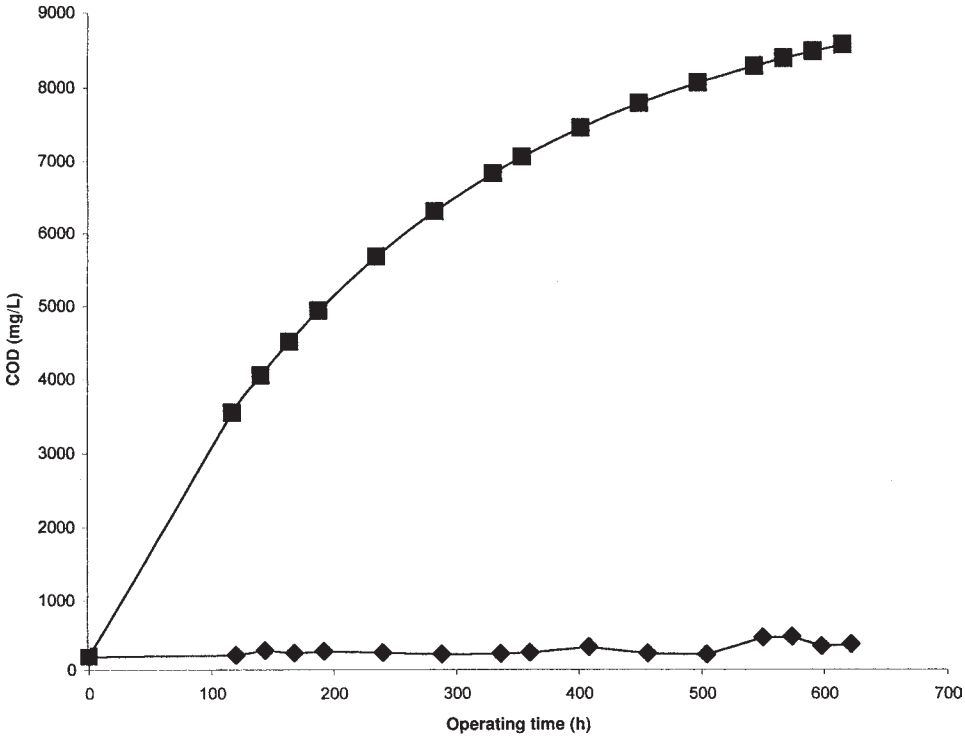

Fig. 4. sCOD concentration in the caustic biotreatment system operating continuously with a feed of caustic (49 mL/d) and mineral salts diluent (490 mL/d). Also shown is the sCOD concentration to be expected without biooxidation of caustic components. (—◆—), Actual; (—■—), dilution alone.

corresponds to molar feed rates of 17.2 mmol/d of sulfide and 32.3 mmol/d of mercaptan. The volumetric productivity for caustic treatment based on the bead bed volume was 33.7 mmol/(L·d) for sulfide oxidation and 63.4 mmol/(L·d) for mercaptan oxidation. This is a fivefold increase over that observed with this enrichment culture in suspended culture *(7)*.

After an initial start-up period, the MeSH in the outlet gas was ≤2 ppmv and H_2S was undetectable during the 900 h of operation under these conditions. Figure 4 displays the sCOD concentration in the system during this operating period. Also shown is the sCOD concentration that would be expected based on dilution alone of the caustic feed in the system (no biooxidation). Significant sCOD reduction through biological oxidation of caustic components is clearly indicated. As seen in Fig. 5, the sulfate concentration in the system reached a steady state of about 97 m*M* after 600–700 h of operation. Based on the caustic analysis in Table 1, the predicted steady-state concentration was 92 m*M*. Therefore, complete oxidation of both inorganic and MeSH sulfur to sulfate is indicated.

These results clearly suggest that the performance of this reactor system, in terms of volumetric productivity for caustic treatment, is limited by

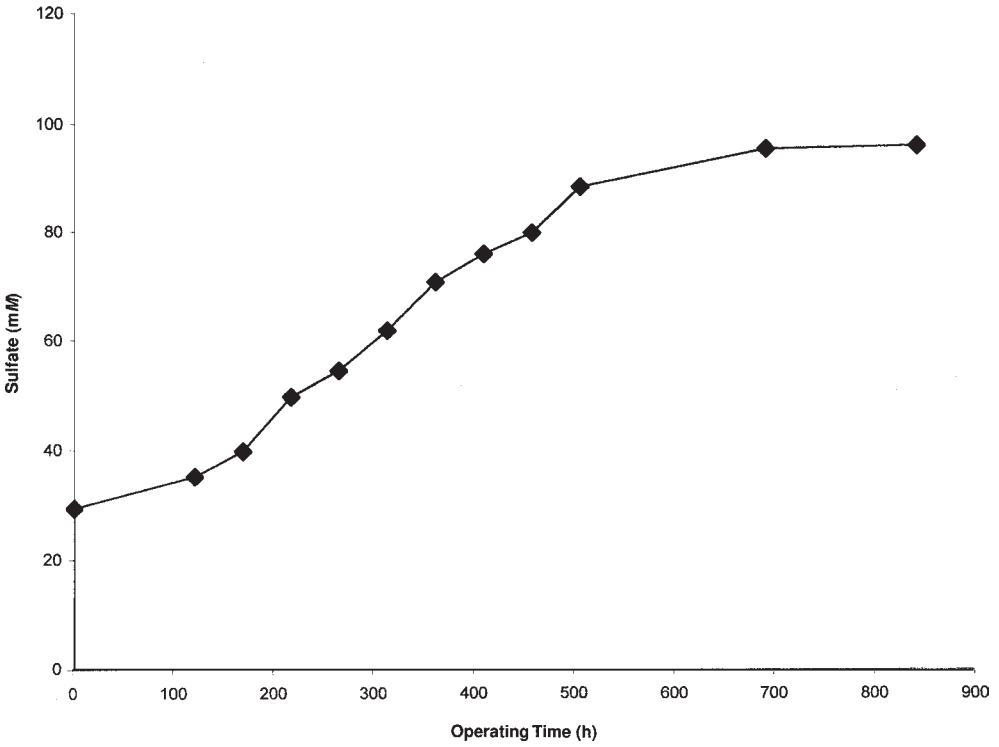

Fig. 5. Sulfate concentration in the caustic biotreatment system operating continuously with a feed of caustic (49 mL/d) and mineral salts diluent (490 mL/d).

the steady-state sulfate concentration. In other words, further improvement in volumetric productivity of a bioreactor system based on this enrichment culture will depend on maintenance of sulfate concentrations below inhibitory levels.

Microbial Analysis of Process Culture

Biomass existed in the caustic treatment system in basically two forms: that immobilized within BIO-SEP beads and that adhering to the walls of the fluidized-bed column bioreactor. Each of these reservoirs of biomass was examined by PLFA and DGGE of extracted 16S rDNA to ascertain any differences in the two populations and to identify as many species as possible in each locale.

PLFA analysis of BIO-SEP and wall growth suggested that the community structure of the two populations was similar. However, the BIO-SEP growth was enriched in Gram-negative bacteria compared to the wall growth. DGGE analysis showed that the bead and wall growths were distinctly different populations of bacteria. The only known sulfur-metabolizing organism identified was *T. thioparus*, which was found predominantly in the beads.

Conclusion

A refinery caustic containing both inorganic sulfides and organic sulfur in the form of mercaptans has been successfully biotreated by an enrichment culture immobilized in BIO-SEP beads in a fluidized-bed column bioreactor. Both inorganic and organic sulfur were oxidized to sulfate. DGGE analysis of the enrichment culture and sequencing of 16S rDNA suggests that *T. thioparus* may be involved with the oxidation of both types of sulfur. The volumetric productivity of the biotreatment system was fivefold higher than that observed previously in suspended culture but was limited by sulfate inhibition. Further significant improvement in performance of this system will require a means of reducing the steady-state sulfate concentration without uneconomical high hydraulic throughput.

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