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Scientific Note

Immobilization of a Sulfide-Oxidizing Bacterium in a Novel Adsorbent Biocatalyst Support

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Index Entries: Sulfidic caustic; *Thiobacillus denitrificans;* activated carbon; packed-bed reactor; volumetric productivity.

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 >13.0, 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)*.

We have previously demonstrated the biotreatment of refinery spent sulfidic caustic in stirred-tank reactors 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 that at least partially neutralized the caustic.

In the biological treatment of waste waters, advantage can be made of the immobilization of microbial cells. In a continuous process, immobilization of cells can result in an increase in volumetric productivity from (1) the decoupling of the hydraulic and biomass residence times, and (2) an increase in cell density relative to suspended cultures.

The benefits of the association of microorganisms with an adsorptive surface, such as activated carbon, in wastewater treatment applications are also well established *(4)*. Two common applications are the use of granular activated carbon with an associated biofilm in a fluidized-bed reactor and powdered activated carbon addition to activated sludge treatment systems. Activated carbon in these

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systems serves as a sink for adsorbable inhibitory substances from wastewater streams, which results in a reduced concentration of these species in the bulk liquid phase and a damping of inhibitory effects (*4*). Biological waste treatment systems supplemented with activated carbon are, therefore, more resistant to shock loads and exhibit greater stability.

T. denitrificans does not readily form a biofilm unless cocultured with heterotrophic organisms, which produce the necessary extracellular biopolymers to promote biofilm formation. In the previous work described above, *T. denitrificans* was flocculated by coculture with floc-forming heterotrophs from a refinery-activated sludge treatment system to allow for gravity sedimentation of the biomass for retention and recycle. The use of such commensal communities to promote flocculation or biofilm formation dilutes the sulfide-oxidizing organism limiting the effectiveness of immobilization in increasing cell densities over that obtained in suspended cultures.

In the work presented here, we have investigated a different approach to immobilization of *T. denitrificans*, which does not rely on biofilm formation, namely the entrapment of *T. denitrificans* in DuPont BIO-SEP™ beads. These beads are a composite of polymer and powdered activated carbon with a high porosity. The powdered activated carbon will potentially prevent excertion of toxic effects of inhibitory organic components, which may be present in a refinery spent sulfidic caustic. Heterotrophs needed to degrade these organics may also become immobilized in the beads resulting in simultaneous oxidation of sulfides and organic constituents of the caustic (*2*). The presence of the adsorptive surface will dampen or prevent a loading shock to the system. Immobilization of *T. denitrificans* within the beads may also result in higher cell densities and greater volumetric productivities in terms of sulfides oxidized per unit time per unit volume. In this article, we describe the immobilization of *T. denitrificans* strain F in DuPont BIO-SEP beads and present preliminary results on the treatment of refinery caustic in a packed-bed reactor.

MATERIALS AND METHODS

Organism and Stock 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 (*5*). Stock cultures were grown anoxically in 10-mL culture tubes at 30°C in thiosulfate mineral salts medium described previously (*6*). 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.

BIO-SEP Beads

The biocatalyst support used in this study consisted of 3- to 4-mm diameter spherical pellets (beads) manufactured by DuPont using a composite consisting of 25 wt% polymer and 75 wt% powdered activated carbon. The bulk density of the beads was approx 0.16 g/cc. 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 1.9 μ. Large macropores (>20 μ) exist inside the beads.

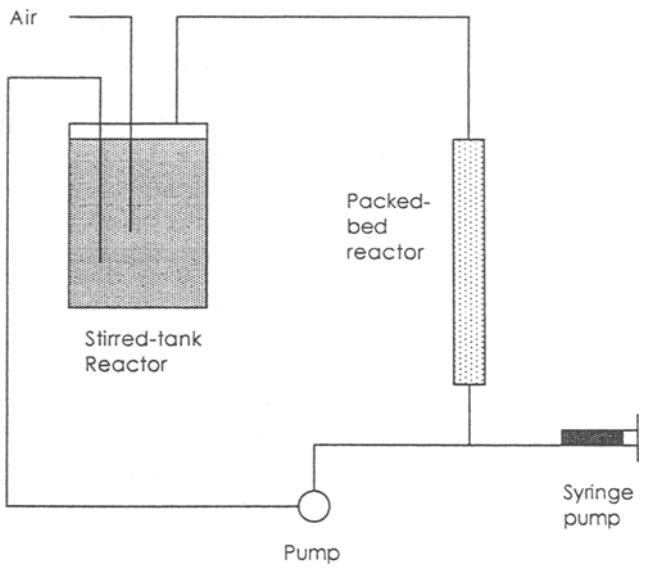

Fig. 1. Schematic diagram of equipment used for immobilizing *T. denitrificans* strain F and treating refinery spent sulfidic caustic.

Immobilization of *T. denitrifcans* in BIO-SEP Beads

T. denitrifcans strain F was immobilized in DuPont BIO-SEP beads as follows. *T. denitrificans* strain F was grown in thiosulfate mineral salts medium with nitrate as the terminal electron acceptor in a B. Braun Biostat M (1.5 L) at pH 7.0 and 30°C as previously described *(6)*. The culture received a gas feed of 30 mL/min of 5% CO_2 in nitrogen. When the OD (460 nm) reached 0.9 corresponding to about 10^9 cells/mL, the culture was circulated through a glass column packed with BIO-SEP beads held in place with glass wool plugs.

The packed bed of BIO-SEP beads was 40.6 cm long with an inside diameter of 1.4 cm. Mixed liquor from the fermenter was pumped to the bottom of the packed bed by a Masterflex (Cole-Parmer) peristaltic pump at 10 mL/min. The effluent from the top of the bed was returned to the fermenter.

The thiosulfate concentration in the fermenter was monitored with time. Whenever thiosulfate was depleted (every 24–48 h), half of the contents of the fermenter was removed and replaced with fresh thiosulfate medium. At 15, 30, 45, and 60 d, BIO-SEP beads were removed from the column, cut in half with a razor blade, fixed and coated, and examined at 15,000× by scanning electron microscopy. Figure 1 shows a schematic diagram of the equipment used for immobilizing *T. denitrificans* in BIO-SEP beads. (The syringe pump was not used during immobilization.)

Treatment of Refinery Caustic by Immobilized *T. denitrificans*

When scanning electron microscopy confirmed that *T. denitrificans* was immobilized in the BIO-SEP beads in the column, the beads were removed and washed with phosphate buffer (pH 7.0) to remove cells and elemental sulfur, which had

Table 1
Results of Analysis of Refinery Spent Sulfide Caustic

pH	13.0
COD	26,700 mg/L
Sulfide	180 m*M*
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	2.11*N*
MDEA/MEA	<1000 mg/L
TOC	4080 mg/L
TDS	130,000 mg/L
Total phenolics	2753 mg/L

COD = chemical oxygen demand.
TOC = total organic carbon.
TDS = total dissolved solids.
MDEA/MEA = methyl diethanolamine/methyl ethanolamine.

become entrapped in the interstitial spaces between beads. The column was then repacked with the clean beads, which showed no visible biofilm on the outside of the beads.

The *T. denitrificans* culture was removed from the fermenter and replaced with fresh medium without thiosulfate. This medium was circulated to the column for 24 h to allow any residual thiosulfate to be oxidized to sulfate. During this time, and hereafter, the medium was sparged with 300 mL/min of air through a glass-fritted sparger. After 24 h, the medium in the fermenter was again changed for fresh medium.

A spent sulfidic caustic sample was obtained from a major refinery and characterized as described previously *(7)*. The results of this characterization are given in Table 1. After the medium in the fermenter was replenished, undiluted caustic was fed continuously to the reactor system using a Harvard apparatus syringe pump just ahead of the packed-bed reactor as shown in Fig. 1.

The caustic feed rate was varied from 10.6 to 28.8 mL/d. The reactor system was operated intermittently (during working hours—8 h/d) for a total of 42.4 h to obtain preliminary data. During this time the outlet gas was monitored for hydrogen sulfide (H_2S), and the medium in the fermenter was monitored for sulfate, the product of sulfide oxidation. The pH in the reactor system was monitored and controlled in the fermenter by addition of 5*N* HNO_3 as needed *(2)*.

Analytical

Thiosulfate was determined by titration with a standard iodine solution with a starch indicator *(8)*. Sulfate was determined turbidometrically following precipitation with $BaCl_2$ *(8)*. Hydrogen sulfide in the outlet gas was determined using chromophoric GasTech Analyzer tube (Yokohama, Japan), 0.25–60 ppm range.

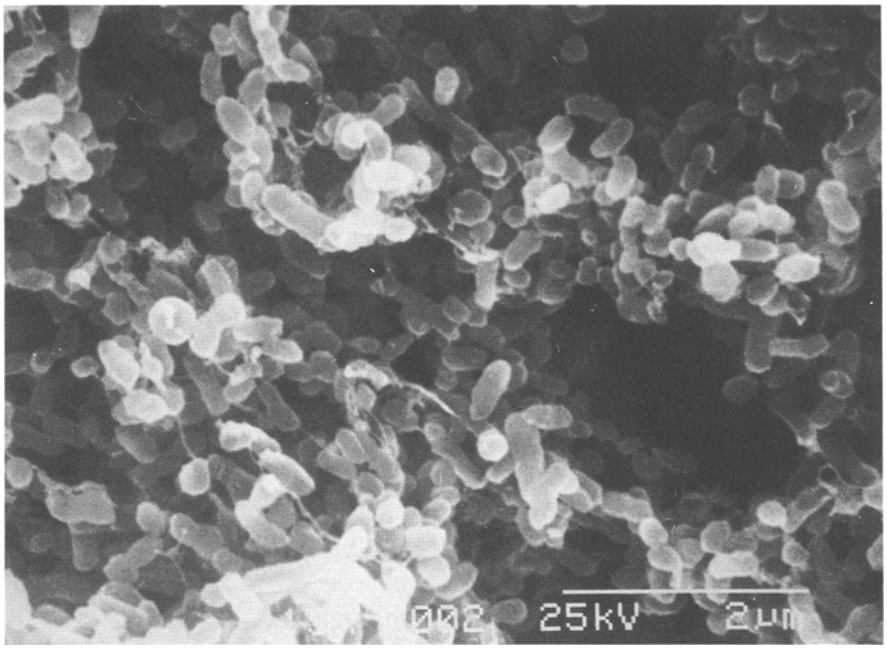

Fig. 2. Scanning electron microraph of interior of BIO-SEP bead showing immobilized cells of *T. denitrificans* at 1500×.

RESULTS AND DISCUSSION

Immobilization of *T. denitrificans*

Although extremely porous internally the DuPont BIO-SEP beads have an outer skin with a limited number of pores to provide access to the interior of the bead. However, once inside the bead cells are virtually trapped and replicate to produce potentially high cell densities. Copious amounts of *T. denitrificans* cells were not observed in BIO-SEP beads until some 60 d following initiation of the immobilization procedure. At this time very large numbers of cells could be found in 10 beads removed from various points in the column (Fig. 2).

Treatment of Refinery Caustic by Immobilized *T. denitrificans*

As the packed-bed reactor received a continuous feed of refinery spent caustic, sulfate accumulated in the medium of the fermenter (Fig. 3). Hydrogen sulfide (1 ppm) was detected in the outlet gas of the fermenter after about 3 h of operation and persisted at this level for another 3 h. The H_2S concentration in the outlet gas then decreased to undetectable levels (<0.25 ppm) for the remainder of the experiment, except for one measurement of 1 ppm at 32.4 h of operating time.

A sulfur balance showed that during this time, more sulfate accumulated in the fermenter than could be accounted for purely from sulfide oxidation. The ratio of mol sulfate produced to mol of sulfide fed to the packed-bed reactor was 1.8. This has been attributed to the oxidation of elemental sulfur, which accumulated in the beads while thiosulfate was circulated through the bed during the immobilization procedure. Subsequent experiments showed that sulfate accumulated in the

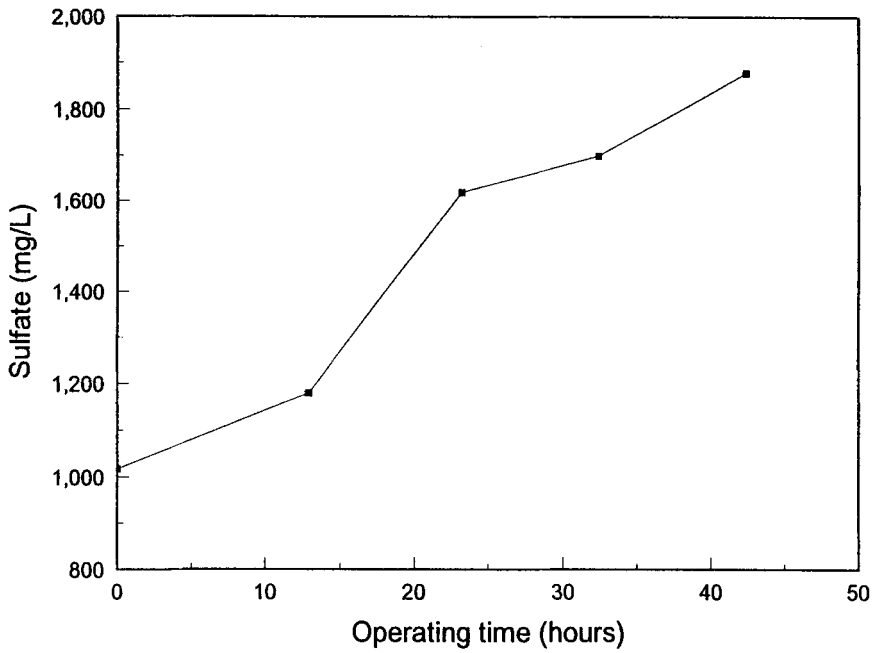

Fig. 3. Accumulation of sulfate in medium circulated in the treatment system for refinery spent sulfidic caustic.

medium of the fermenter initially when mineral salts medium was circulated through the packed-bed reactor containing freshly immobilized *T. denitrificans* in the absence of a caustic feed.

The highest volumetric productivity observed in this experiment in terms of the rate of caustic treatment per unit volume of the packed-bed reactor was 1.4 mL/d/cm^3. This is not necessarily the maximum volumetric productivity of the packed-bed system. In contrast, this same caustic has been used as feed to a stirred-tank reactor containing a suspended culture of *T. denitrificans (7)*. The maximum volumetric productivity observed in these experiments with this caustic was 0.2 mL/d/cm^3 at a mixed liquor suspended solids concentration of 1500 mg/L. Clearly, the packed-bed reactor offers advantages in this regard over suspended cultures, suggesting that the packed-bed reactor contains significantly higher cell densities of the sulfide-oxidizing bacterium.

Finally, the presence of heterotrophs did not lead to biofouling of the beads. However, longer periods of operation are required before a definitive conclusion can be reached about the fouling potential.

CONCLUSION

Preliminary experiments have shown that *T. denitrificans* strain F can be immobilized in DuPont BIO-SEP beads and used to treat refinery spent sulfidic caustic in a packed-bed reactor. Sulfides were oxidized to sulfate with very little off-gassing of sulfide as H_2S. The volumetric productivity of the packed-bed reactor was shown to be almost an order of magnitude greater than the maximum observed in a stirred-

tank reactor treating the same caustic and using the same organism. This is especially significant since the packed-bed reactor had not been optimized and was not operating at its maximum rate.

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