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COUPLED ANAEROBIC/AEROBIC TREATMENT OF HIGH-SULFATE WASTEWATER WITH SULFATE REDUCTION AND BIOLOGICAL SULFIDE OXIDATION

Peter Fox and Venkatraghavan Venkatasubbiah

Department of Civil and Environmental Engineering, Arizona State University, Mail Code 5306, Tempe, AZ 85287-5306, USA

ABSTRACT

A pharmaceutical wastewater with a chemical oxygen demand (COD) concentration of 40,000 mg/l and a sulfate concentration of 5,000 mg/l was treated in a anaerobic baffled reactor. Treatment of the wastewater at 10% dilution was effective but at higher influent concentrations sulfide inhibition reduced efficacy of both COD conversion and sulfate conversion. A recycle line with an attached-film biological reactor was inserted into the anaerobic baffled reactor to facilitate biological sulfide oxidation. Recycling anaerobic effluent through a sulfide oxidizing biological system reduced inhibition in the anaerobic reactor by both reducing inhibitory sulfide concentrations within the reactor and by diluting the influent. The major product of the biological oxidation of sulfide by a *Thiobacillus* species appeared to be elemental sulfur. At an influent wastewater concentration of 40% and a HRT of 1 day, COD removal efficiencies were greater than 50% and the conversion of influent sulfate was greater than 95% with effluent sulfide concentrations of less than 20 mg/l. The major product observed from degradation of isopropyl acetate was acetic acid. Coupled anaerobic/aerobic provided removal of sulfur from the wastewater stream and helped to stabilize the pH in the reactor system.

KEY WORDS

Anaerobic, Inhibition, Isopropyl Acetate, Methanogenesis, Sulfate, Sulfide

INTRODUCTION

Wastewaters from oil refineries, gas manufacturing works, textile mills, pulp and paper mills, and pharmaceuticals may have high concentrations of sulfates. Anaerobic treatment of high strength wastewaters with high sulfate concentrations poses several unique problems. Anaerobic treatment has the potential advantages of high rate treatment, no oxygen addition requirements, low sludge production rates, and methane production. The production of sulfide during anaerobic treatment of sulfate containing wastewaters can reduce the efficiency of anaerobic treatment. Sulfate reducing bacteria (SRB) successfully compete with methanogens for substrates and produce sulfide as a product (Hilton and Oleszkiewicz, 1988). High sulfide concentrations can inhibit methanogens and can precipitate nutrients essential to methanogenesis. Unionized hydrogen sulfide is most toxic and sulfide toxicity can be alleviated at elevated pH (Anderson et al., 1982). Problems with sulfide can be overcome by removing sulfide during the anaerobic treatment process, inhibiting sulfate reduction, or by the addition of chelating agents to prevent the precipitation of nutrients (Parkin et al., 1991).

Biological sulfide oxidation has been used to remove sulfide in anaerobic effluents but it has not been used as a process to control sulfide inhibition in anaerobic systems (Buismann and Lettinga) (1990). Sulfide has been removed to control inhibition by

stripping biogas and converting the H_2S to elemental sulfur with a catalyst (Sarner et al., 1988). Biological oxidation of sulfide to elemental sulfur has several advantages over other physicochemical processes (Buismann, 1991): a.) saves money on oxidants and catalysts, b.) sulfur can be reused and no chemical sludge is produced, c.) low energy consumption, and d.) reduction in the sulfate or thiosulfate discharge. This study demonstrates the use of biological sulfide oxidation for control of sulfide and enhanced removal of sulfate in an anaerobic reactor system.

Biological sulfide oxidation in effluents from anaerobic reactors has been done by colorless sulfur bacteria from the genera of *Thiothrix* or *Thiobacilli* (Buismann et al., 1990). *Thiobacilli* species tend to deposit sulfur extracellularly which enhances the ability to recover sulfur as a product. The oxidation of sulfide has to be controlled to produce sulfur instead of thiosulfate and sulfate. A high sulfide to oxygen ratio is necessary for sulfur production (Chen and Morris, 1972) negligible sulfate production has been observed with sulfide concentrations greater than 20 mg/L. Buismann et al. (1989) found optimal pH and temperature for a *Thiobacilli* culture to be in the range of 8.0-8.5 and 25-35°C, respectively. Attached film reactors have been demonstrated to produce less sulfate than suspended-growth systems and rotating biological contactors have converted sulfide concentrations as high as 150 mg/L to elemental sulfur (Buismann et al., 1991).

The goal of this research was to study the treatment of high sulfate pharmaceutical wastewater with an anaerobic baffled reactor (Boopathy et al., 1988) coupled with biological sulfide oxidation. A three-phase research approach was used. The first-phase of the research was acclimation of the anaerobic reactor to the wastewater and isolation of sulfur oxidizing bacteria from municipal sewage. During the second phase of this research, the influent wastewater concentration was increased until inhibition was observed. The second phase of this research also included a series of anaerobic batch tests with the wastewater and individual components of the wastewater including isopropanol, sulfate, and glucose. The final phase of this research was to implement biological sulfide oxidation and continue to increase the influent wastewater concentration.

MATERIALS AND METHODS

Anaerobic Baffled Reactor. The anaerobic baffled reactor (Figure 1) was constructed primarily of 1.27 cm and 0.63 cm plexiglas. The active reactor volume was 10 liters and the volume was divided by baffles into five equal 2 L chambers by baffles having an angular section (154°) at the bottom. Each compartment was equipped with ports for the sampling of liquid, gas, and biological solids. The top of the reactor was sealed with a butyl rubber o-ring. All experiments were done in a temperature control room with a temperature of 35±1°C. Gas production was monitored with a wet tip gas meter. Two peristaltic pumps were used to pump influent to the reactor. One pump was used for the wastewater while the second pump was used for a nutrient solution. The total flow to the reactors was maintained at 10 L/d throughout the study so the average hydraulic retention time was approximately one day. The nutrient solution was similar to that presented in Table 2 except that no thiosulfate was added and a 10% dilution was used.

Wastewater. The wastewater studied was from a pharmaceutical process that used isopropyl acetate and sulfuric acid to extract products following fermentation. Therefore, the wastewater contained isopropyl acetate, sulfates, and cellular products. Since the cells were filtered from the wastewater during processing, isopropyl acetate comprised the majority of chemical oxygen demand (COD) in the wastewater.

Start-up of the reactor was done with a mixture of wastewater (1-6.6% dilution), glucose, and isopropanol. The concentrations of individual components was gradually increased over the first 100 days until the influent COD was 20,000 mg/L and the influent wastewater concentration was 6.6%. Hydrolysis of the ester isopropyl acetate yields isopropanol and acetate. Therefore, acclimation to isopropanol was considered essential. Glucose was used to rapidly develop a complete anaerobic consortium. During days 92-95, the influent wastewater was erroneously increased to 20%.

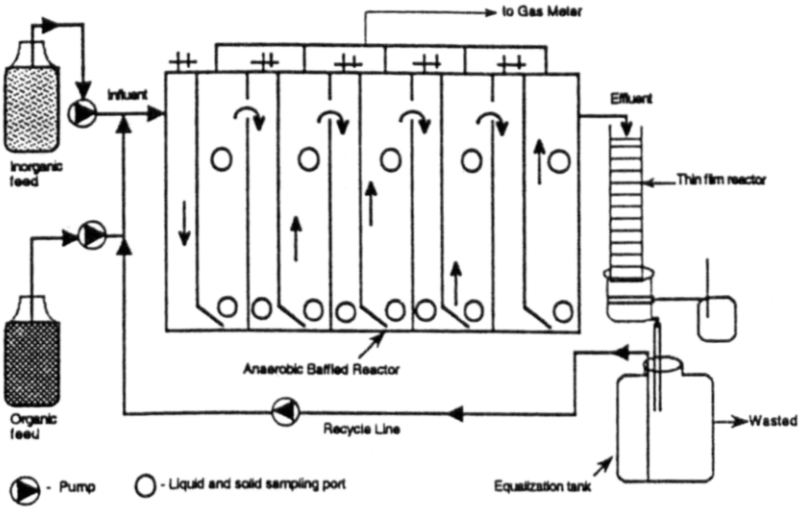

Figure 1. Schematic of Anaerobic Baffled Reactor

An operating schedule is presented in Table 1. By day 70 the nominal influent COD concentration was 20,000 mg/L and from days 99-155, the wastewater concentration was maintained at 6.6% until stable operation was observed. From days 155-176, the wastewater concentration was increased to 20% while the glucose and isopropanol concentrations were reduced to maintain a nominal influent COD concentration of 20,000 mg/L. From days 176-316, only wastewater was fed to the reactor with step-wise increases in concentration from 5 to 50%. On day 260, recycle at a recycle ratio of 10:1 was added through a thin-film sulfide-oxidizing bioreactor and recycle was maintained throughout the remainder of the experiment. Without the presence of the thin-film sulfide-oxidizing bioreactor, recycle was periodically turned on during Phases 1 and 2 and no impact on anaerobic baffled reactor performance was observed.

Sulfur Oxidizing Bacteria: Sulfur oxidizing bacteria were cultured from domestic sewage in a 1 L fill and draw aerobic reactor. A thiosulfate medium (Table 2) used by Vishniac and Santer (1957) to isolate *Thiobacillus Thioparus* was fed to the reactor on a daily basis. Initially, the reactor was fed 20 mL/d of medium. After efficient conversion of elemental sulfur was achieved and a monoculture was observed microscopically, addition of growth medium was increased in increments of 10 mL/d until 100 mL/d was added. The total suspended solids concentration was maintained in the range of 350 to 450 mg/L.

A microorganism similar in morphology and chemolithotrophic activity as *Thiobacillus* sp. was isolated. The microorganism excreted elemental sulfur and sulfur granules were easily separated by gravity. Approximately 10% of the reactor biomass was harvested on a daily basis for 60 days and a stock of the *Thiobacillus* sp. was stored at 4°C for use in the thin-film sulfide-oxidizing reactor.

Thin-Film Bioreactor. A thin-film bioreactor, resembling a ladder structure, was constructed of plexiglas with dimensions of 20.5 cm by 3.8 cm. There were twelve 2.54 cm 'steps' on the ladder and wire mesh was placed between steps to enhance biofilm attachment. The bioreactor was place at a 45° angle and reactor effluent flowed evenly over the reactor. Oxygen mass transfer was limited by surface area and flowrate. Sulfur deposits were collected in an equalization chamber located below the reactor.

Table 1. FEED COMPOSITION TO REACTOR SYSTEM

COD of Wastewater = 40,000 mg/L
Sulfate Concentration of Wastewater = 5,000 mg/L
pH of Wastewater = 5.6
Suspended Solids in Wastewater = Negligible
Color of Wastewater = Dark Brown

Phase	Days	% Volume Wastewater	Glucose, mg/L	Isopropanol, mg/L
ONE	0-99	1-6.6	1,000-9,375	0-5,300
	99-155	6.6	9,375	5,300
	155-176	20	7,375	4,150
TWO	176-211	5	0	0
	211-232	10	0	0
	232-246	15	0	0
	246-260	20	0	0
THREE	260-274	20	0	0
	274-316	25-50	0	0

TABLE 2 - GROWTH MEDIUM/NUTRIENT SOLUTION

Constituent	Mass or Volume per L
$Na_2SO_3(H_2O)_5$	10.0 g
KH_2PO_4	4.0 g
K_2HPO_4	4.0 g
$MgSO_4(H_2O)_7$	0.8 g
NH_4Cl	0.4 g
Trace Metal Solution	15 mL
TRACE METAL SOLUTION	
Ethylenediamine Tetracetic Acid	50.0 g
$ZnSO_4(H_2O)_7$	22.0 g
$CaCl_2$	5.54 g
$MnCl_2(H_2O)_4$	5.06 g
$FeSO_4(H_2O)_7$	5.0 g
$(NH_4)_6Mo_7O_{24}(H_2O)_4$	5.0 g
$CoCl_2(H_2O)_6$	1.61 g
$CuSO_4(H_2O)_5$	1.57 g

Sampling Methodology. Daily measurements included pH, flowrates, temperature, and gas production. Liquid and gas samples from each of the five chambers, the anaerobic baffled reactor effluent, and the thin-film bioreactor effluent were taken once a week. Gas samples were analyzed for carbon dioxide and methane while liquid samples were analyzed for sulfide and suspended solids immediately. Liquid samples were filtered, acidified, and stored at 4°C. Stored liquid samples were analyzed for COD, volatile acids, and sulfate.

RESULTS AND DISCUSSION

BAFFLED ANAEROBIC REACTOR (PHASE ONE Days 0-176). The influent and effluent COD concentration profiles for days 0-176 are presented in Figures 2. The COD removal efficiency ranged from 68% to 36%. Following acclimation, COD removal efficiency was rather consistent until day 120 when a significant increase in the effluent COD concentration was observed. However, no decrease in methane production or increase in sulfide concentration was observed (Figure 3). During the time period of consistent COD removal, there was accumulation of microorganisms in the reactor as evidenced by the low effluent solids concentration which averaged less than 50 mg/L.

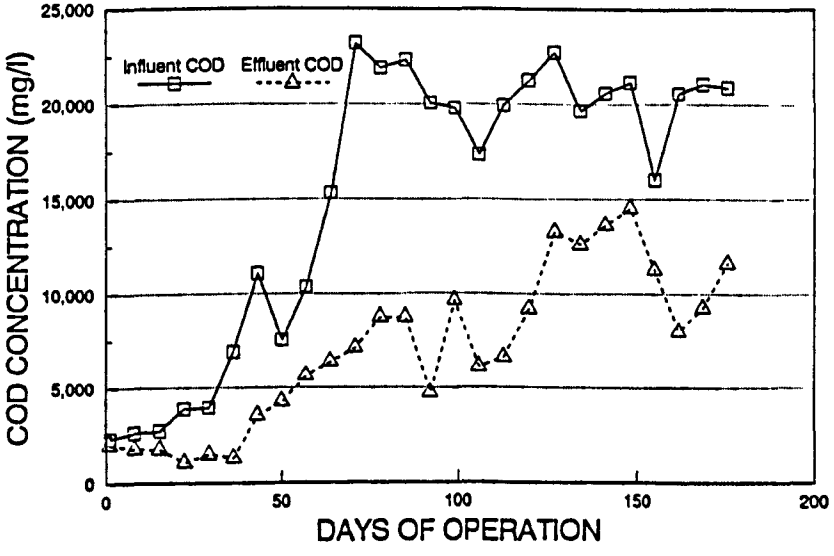

Figure 2. COD Concentration Profile for Days 0-176

Sulfate conversion to sulfide was greater than 95% during the first 100 days and the influent COD/SO_4 ratio was greater than 150 during the majority of this period. When the influent wastewater concentration was increased to 6.6% after day 100, the conversion efficiency of sulfate to sulfide decreased and the influent COD/SO_4 ratio was maintained at 70.0. The spike in effluent sulfide concentration near day 100 was a consequence of an error in the feed composition (Figure 3). At an influent wastewater concentration of 6.6% (Days 99-155), sulfate conversion efficiency ranged from 60 to 70% and when the influent wastewater concentration was increased to 20% (COD/SO_4 ratio = 23.7), the sulfate conversion efficiency was less than 50%. These data indicate that sulfate reduction was limited at higher sulfate concentrations when glucose and isopropanol were present in the influent along with the wastewater.

For the most of samples taken, the sulfide concentration from the first baffled reactor was within 10% of the sulfide concentration in the final reactor effluent. Sulfate reduction occurred primarily in the first chamber and sulfide concentrations often

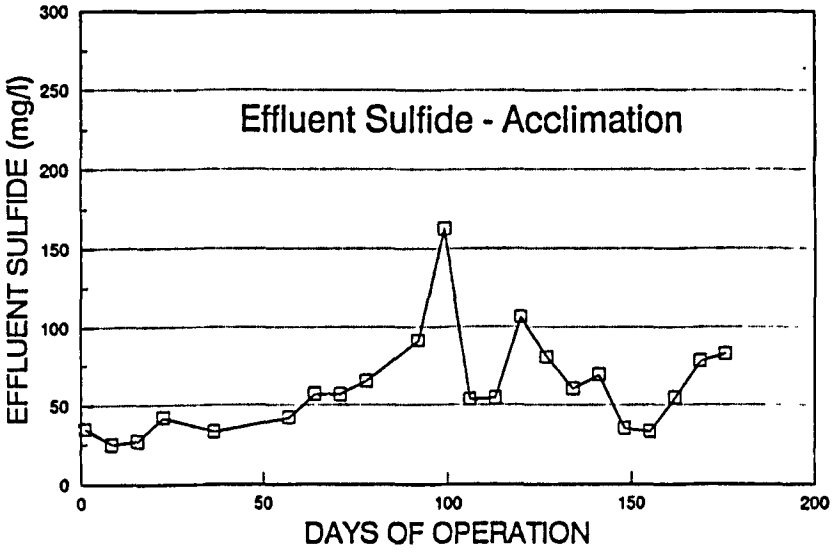

Figure 3. Effluent Sulfide Concentration Profile for Days 0-176

decreased in subsequent chambers. This is evidence that sulfate reduction was rapid in the first chamber and that some sulfide was removed in subsequent chambers by either precipitation or microbial uptake.

Reactor performance was very stable with respect to pH and percentage of methane in the gas. The pH ranges from 7.0 to 7.25 and the percentage of methane in the gas ranged from 60 to 70%.

Volatile fatty acids (VFAs) revealed that 2,000 to 4,500 mg/L of VFAs was produced. The VFAs represented 20 to 35% of the effluent COD and the majority of the VFAs were acetic and propionic acids with butyric acid concentrations less than 200 mg/L.

BAFFLED ANAEROBIC REACTOR (PHASE 2 Days 176-260). Figure 4 presents the COD and effluent sulfide concentration profiles for the anaerobic baffled reactor during Phases 2 and 3. The anaerobic baffled reactor was fed only wastewater at concentrations ranging from 5 to 20% (Table 1) and the COD/SO_4 ratio was 8 throughout Phase 2. From days 176-232 while the influent wastewater concentration was 5% or 10%, no inhibition was observed and greater than 50% removal of COD occurred after washout of residual COD from Phase 1. As the influent wastewater concentration was increased to 15% and 20%, the reactor performance began to deteriorate as the effluent sulfide concentration increased to inhibitory levels (> 200 mg/l) and the effluent COD concentration correspondingly increased while COD removal efficiency decreased to less than 20%.

Sulfate conversion to sulfide was greater than 90% from days 176-232. While the influent wastewater concentration was 15% or 20%, the sulfate conversion efficiency ranged from 70% to 80% from days 232-260.

In comparison to Phase 1, both VFA composition and concentration changed. Acetic acid was the only VFA detected after day 190 and the acetic acid concentration was less than 300 mg/L from days 176-232. The acetic acid concentration increased dramatically after day 232 from 655 mg/L to 3490 mg/L. Acetic acid represented over 65% of the COD in the effluent at the end of Phase 2. Since the primary organic in the feed was isopropyl

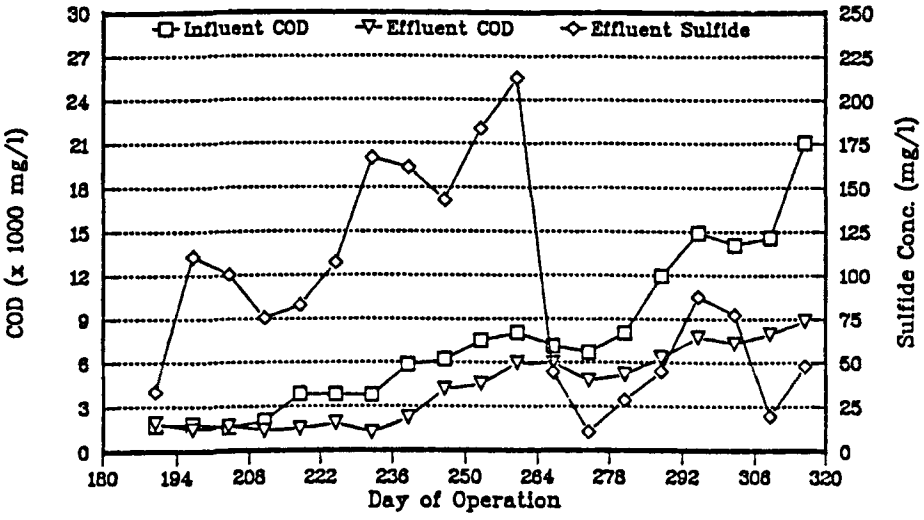

Figure 4. COD and Effluent Sulfide Concentration Profiles for Days 176-316

acetate, the presence of acetate as the primary organic in the effluent was logical. The high sulfide concentration appears to have inhibited both sulfate reduction and the conversion of acetate to methane by methanogens.

ANAEROBIC BAFFLED REACTOR AND THIN-FILM SULFIDE OXIDIZING REACTOR (Phase 3 Days 260-316). On day 260, the attached film biological sulfide oxidizing reactor was added to the anaerobic baffled reactor and recycling effluent through the sulfide-oxidizing reactor was initiated at a recycle ratio of 10:1. After seeding with sulfide-oxidizing culture harvested from the fill and draw reactor, the effluent sulfide concentration decreased to less than 10 mg/L and the COD removal efficiency increased from 20% to greater than 50% by day 274. Methane gas production rates correspondingly increased over 100% clearly indicating that decreasing the sulfide concentration and diluting the influent with recycled effluent alleviated inhibition of methanogenesis.

The influent wastewater was increased from 20% to 50% during days 274-316. Overall reactor performance was consistent with greater than 50% removal of influent COD and low effluent sulfide concentration less than 90 mg/l from the anaerobic baffled reactor. Overall sulfur removal efficiencies were greater than 95% and distinct sulfide and sulfate profiles were observed across the reactor. Sulfide concentrations steadily increased while sulfate concentration correspondingly decreased. Therefore, the addition of biological sulfide oxidation alleviated inhibition of both methanogenesis and sulfate reduction.

Although a complete sulfur balance was not completed on the reactor system, strong circumstantial evidence that sulfide was not being stripped or oxidized further exists. Visual observation indicated that the majority of sulfur was removed as elemental sulfur which accumulated in the equalization basin and on the thin film reactor in the form of white granules. Prior to addition of the sulfur oxidizing reactor, strong sulfide odors were observed in the temperature control room (Volume = 35 m^3) and in adjacent rooms. Immediately after the addition of the sulfide-oxidizing reactor, odors were almost eliminated. Finally, the pH within the anaerobic baffled reactor increased 0.5 units.

If sulfide was oxidized to sulfur compounds more oxidized than elemental sulfur such as thiosulfate or sulfate, strong acid would be produced and a pH reduction would have been observed (Buismann and Lettinga, 1990). The increase in pH was evidence of acid consumption by sulfate reduction and increased methanogenesis of acetate. Mass transfer of oxygen in the thin-film reactor was limited by the surface area and the liquid flow-rate. The mass transfer of oxygen was sufficient to provide sulfide oxidation to sulfur without increasing the dissolved oxygen concentration to greater than 0.1 mg/L.

SUMMARY AND CONCLUSIONS

Coupled anaerobic/aerobic treatment of high sulfate wastewater was effective at alleviating sulfide inhibition of both methanogenesis and sulfate reduction. Sulfide oxidizing organisms were cultured from municipal sewage and applied for the biological oxidation of sulfide in anaerobic reactor effluent. A thin-film sulfide-oxidizing reactor was effective at converting sulfide to elemental sulfur without adding excess oxygen which made recycle of the anaerobic effluent through the sulfide-oxidizing reactor feasible. Biological sulfide oxidation could provide an alternative method to removing sulfides produced during anaerobic treatment and alleviating sulfide inhibition by removing sulfur from the wastewater stream.

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