EFFECT OF SULFATE ON ANAEROBIC DEGRADATION OF BENZOATE IN UASB REACTORS

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ABSTRACT: Wastewaters containing benzoate and sulfate were treated in two upflow anaerobic sludge blanket (UASB) reactors at 34–37°C for 320 d. The sulfate concentration was increased stepwise in Reactor-A up to 7,500 mg/L, and was kept mostly constant at 3,000 mg/L in Reactor-B. Both reactors removed over 98% of organic chemical-oxygen demand (COD) for sulfate up to 6,000 mg/L, despite the fact that the mixed liquor contained up to 769 mg S/L of total sulfides and up to 234 mg S/L of dissolved H_2S. Sulfate-reducing efficiency decreased with the increase in sulfate concentration, but increased with time at each sulfate concentration. Reactor-B consistently reduced 89% of sulfate. However, both organic COD removal and sulfate-reducing efficiencies of Reactor-A dropped drastically at 7,500 mg SO_4^{-2}/L, and showed no sign of recovery after 50 d. The system failure was likely due to the increased sulfate, instead of sulfide, toxicity. From the COD balance, 93.4% of COD removed was converted to methane instead of sulfides, with a net sludge yield of 0.047 g volatile suspended solids (VSS)/g COD. The sulfur balance was over 97%.

INTRODUCTION

Anaerobic processes have been widely used for the treatment of various high-strength industrial wastewaters. However, application has been limited for the treatment of sulfate-rich industrial wastewaters, such as those from pharmaceutical, pulp/paper, fermentation, petrochemical, and mining industries. It has been widely reported (Kroiss and Wabnegg 1983; Winfrey and Ward 1983; Rinzema and Lettinga 1988; Parkin et al. 1990) that sulfate at high concentrations could be detrimental to the anaerobic treatment process. Under anaerobic conditions, sulfate is converted by bacteria into hydrogen sulfide (H_2S), which is not only a malodorous and corrosive chemical when emitted as gas, but it is also a strong inhibitor of methanogenesis. Furthermore, sulfate-reducing bacteria (SRB) compete with methane-producing bacteria (MPB) and syntrophic acetogenic bacteria (SAB) for electron donors, including hydrogen, formate (Isa et al. 1986; Gupta et al. 1994), acetate (Isa et al. 1986; Yoda et al. 1987; Parkin et al. 1990; Gupta et al. 1994), propionate (Parkin et al. 1990; Visser et al. 1993a), butyrate (Visser et al. 1993a; Mizuno et al. 1994), and benzoate (Li et al. 1996).

There are many physiological similarities between SRB and MPB (Holt et al. 1994). Most of these two groups of bacteria are strictly anaerobic, chemoheterotrophic, and of similar optimum temperature and pH. Therefore, they are found coexisting in many anaerobic ecosystems. Since SRB have higher affinity to substrates (such as hydrogen, formate, and acetate) than MPB (Kristjansson et al. 1982; Widdel 1988), it is commonly believed that under sulfate-rich conditions, SRB can out-compete MPB for electron donors. Yoda et al. (1987) showed that SRB out-competed MPB in a biofilm reactor at low acetate concentration. However, Isa et al. (1986) found that, in high-rate anaerobic reactors, SRB could not compete with MPB, scavenging only 10–20% of the total electron donors. Harada et al. (1994) found that 53.3% of starch and sugar in wastewater was converted to methane and only 20.4% was used by SRB for sulfate reduction. Some recent studies suggested that, instead of sulfate concentration, the ratio of chemical-oxygen demand (COD) and sulfur (S) as sulfate in wastewater is the controlling parameter of electron flow. Uberoi and Bhattacharya (1995) reported that in a continuous chemostat reactor treating propionate-rich wastewater, SRB increased the degradation of acetate as the COD/S ratio dropped below 2.0. Mizuno et al. (1994) in a similar study treating butyrate-rich wastewater reported that methanogenesis was the predominant reaction for the COD/S ratio at 6.0 or higher, whereas sulfate reduction became the predominant reaction when the COD/S ratio was reduced to 1.5 or lower.

Many reported that the final product of sulfate reduction, H_2S, is inhibitory not only to methanogenesis (Mountfort and Asher 1979; Boone and Bryant 1980; Speece 1983; Koster et al. 1986; Karhadkar et al. 1987; Rinzema and Lettinga 1988; Parkin et al. 1990; Maillacheruvu et al. 1993; Visser et al. 1993b), but also to sulfate reduction itself (Okabe et al. 1995; Uberoi and Bhattacharya 1995). Parkin et al. (1990) reported that 50–80 mg/L of H_2S in chemostat reactors inhibited the degradation of acetate and propionate. Similarly, Maillacheruvu et al. (1993) reported that concentrated H_2S (over 60–75 mg/L) and total soluble sulfide (over 150–200 mg/L) could cause system failure in degrading acetate and propionate. Koster et al. (1986) reported that the methane production of biogranules of an upflow anaerobic sludge blanket (UASB) reactor treating wastewater from a potato factory was reduced by 50% when the feed solution contained 841 mg S/L of total sulfide or 252 mg S/L of H_2S. On the other hand, Okabe et al. (1995) found that sulfide inhibited the bioactivity of *Desulfovibrio desulfuricans*, and Uberoi and Bhattacharya (1995) reported that sulfide could be more toxic to SRB than to acetogens and methanogens.

The UASB process (Lettinga et al. 1980; Fang and Chui 1993) has been broadly used in industrial wastewater treatment in Europe and more recently in Asia as well. In a UASB reactor, biomass agglutinates into granules which have high bioactivity and superb settleability. It is estimated that about 600 full-scale UASB reactors were in operation worldwide in 1995, doubling the number estimated in 1991 (Lettinga and Hulshoff Pol 1992). Although it is mostly used for the treatment of high-strength carbohydrate-rich wastewaters, the UASB process has been found effective for wastewater containing aromatic pollutants, such as phenol (Fang et al. 1996) and benzoate (Li et al. 1995). Benzoate is an intermediate for the degradation of many aromatic chemicals, such as phenol (Kobayashi et al. 1989) and cresol (Londry and Fedorak

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1993). However, UASB treatment of wastewater containing both benzoate and sulfate has not yet been reported. This study was conducted to investigate the effect of concentrated sulfate on the degradation of benzoate in UASB reactors, including the degrees of COD and sulfate reduction, COD and sulfate balances, toxicity of sulfate/sulfide, methanogenic activity, and bacterial populations.

MATERIALS AND METHODS

Experimental Conditions

The experiments were conducted for 320 d in two UASB reactors treating wastewater containing benzoate as the sole organic substrate. One was a 8.5-1 reactor (Reactor-A), which was used in a previous study (Fang and Chui 1993), and the other was a 0.15-1 reactor (Reactor-B), as illustrated in Fig. 1. Both reactors were installed inside a temperature-controlled chamber, the temperature of which was kept at 34–37°C. Throughout the experiment, the benzoate concentration in wastewater for both reactors was kept at a constant level of 2,520 mg/L equivalent to about 5,000 mg/L of COD. With an average hydraulic retention time of 11–13 h, both reactors were operated at an average loading rate of 10 g COD/(L·d). Reactor-A was used to examine the effect of increased sulfate concentration on the anaerobic degradation of benzoate, whereas Reactor-B was used to examine the effect of sulfate at a constant level of 3,000 mg/L over an extended period of nine months. Reactor-A was seeded with granulated sludge obtained from a UASB reactor that had treated wastewater containing 2,520 mg/L of benzoate (Li et al. 1995) for over 12 months. The reactor had a sludge bed height averaging 750 mm, and a superficial velocity of 83 mm/h. The sulfate concentration in the wastewater was increased stepwise from the initial 100 mg/L to 7,500 mg/L, corresponding to a COD/S ratio of 2.0. Each level of sulfate was run for at least 16 d before increasing to the next level.

Reactor-B was seeded with granulated sludge obtained from Reactor-A when it had been treating wastewater containing 2,000 mg SO_4^{-2}/L for one month. Reactor-B had a sludge bed height averaging 150 mm, and a superficial velocity of 24 mm/h. Reactor-B was fed initially with wastewater containing 2,000 mg SO_4^{-2}/L; after 60 d, the sulfate concentration was raised to 3,000 mg/L and was kept at that level until the end. In addition to benzoate and sulfate, each liter of synthetic wastewater for both reactors was dosed with balanced nutrient, trace elements, and buffering chemical, using the following formula: $NaHCO_3$, 5,000 mg; NH_4Cl, 1,300 mg; $MgSO_4 \cdot 7H_2O$, 213 mg; K_2HPO_4, 125 mg; KH_2PO_4, 50 mg; $CaCl_2$, 58 mg; sodium citrate, 113 mg; $NiSO_4 \cdot 7H_2O$, 21 mg; $FeCl_3 \cdot 6H_2O$, 17 mg; $MnCl_2 \cdot 4H_2O$, 7 mg; $ZnCl_2$, 4 mg; $CoCl_2 \cdot H_2O$, 4 mg; $(NH_4)_2MoO_4 \cdot 4H_2O$, 2.7 mg; $CuCl_2 \cdot 2H_2O$, 2 mg; and $NaBO_2 \cdot 10H_2O$, 1 mg. Both reactors were operated without recycling of effluent. The influent pH averaged 8.5 for Reactor-A and 8.3 for Reactor-B.

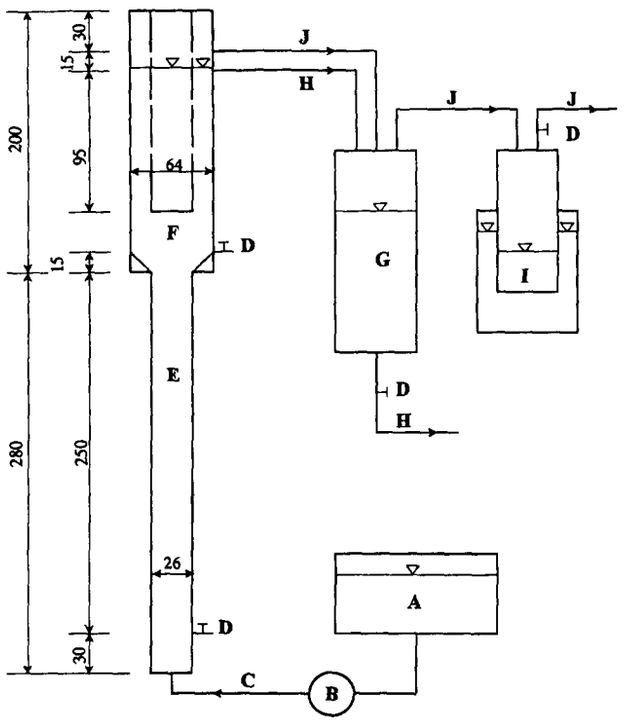

FIG. 1. Experimental Setup for the 0.15-1 UASB Reactor (All Dimensions in mm): A. Substrate Feed Tank; B. Pump; C. Influent; D. Valve; E. Reactor; F. Separator; G. Equilibrium Tank; H. Effluent; I. Water Displacement; J. Gas

Analytical Methods

Gas Composition

The amount of biogas produced in each reactor was recorded daily using a water replacement method. The contents of methane, carbon dioxide, nitrogen, and H_2S in the biogas were analyzed by a gas chromatograph (GC) (Hewlett-Packard, Model 5890 Series II) equipped with a thermal conductivity detector and a 10 m stainless steel column packed with HayeSepQ (80/100 mesh). Helium was used as the carrier gas at a flow rate of 22 ml/min. The column was operated at a temperature program of 90°C for 1.2 min, followed by 2 min at 110°C. The temperatures of the injector and detector were 130°C and 200°C, respectively.

Sulfate and Sulfide

Sulfate concentration in the effluent was analyzed by an ion chromatograph (Shimadzu HPLC 10A) equipped with a CDD-6A conductivity detector and a Shim-Pack IC-A3 column. A solution containing 8.0 mM of 4-hydroxybenzoic acid and 3.2 mM of bis[2-hydroxyethyl]iminotris-[hydroxymethyl]methane was used as the mobile phase. The flow rate of the mobile phase was 1.0 ml/min, oven temperature was 40°C, and detector temperature was 43°C. Sulfide concentration in the effluent was analyzed using the iodometric method (*Standard* 1989).

Benzoate and Volatile Fatty Acids

The concentration of benzoate and volatile fatty acids (VFA) (from acetic to heptanoic acids) were determined by a second gas chromatograph equipped with a flame ionization detector and a 10 m × 0.53 mm HP-FFAP fused-silica capillary column. Samples were filtered through a 0.2 μm filter, acidified by phosphoric acid, and measured for free acids. The initial temperature of the column was 70°C for 4 min and then 140°C for 3 min, and finally 170°C for 4 min. The temperatures of injector and detector were both 200°C. Helium was used as the carrier gas at a flow rate of 25 ml/min. The column used in this study was unable to detect formic acid.

Organic COD, Total Suspended Solids, Volatile Suspended Solids, and pH

The effluent comprised residual benzoate, VFA intermediates, and reduced sulfides. The total COD of the effluent is the sum of organic COD and sulfide COD. The organic COD in this study was calculated from the concentrations of benzoate and individual VFA in the effluent as measured by GC. The content of total suspended solids (TSS) and volatile suspended

solids (VSS) were measured according to methods in *Standard* (1989) and the pH was measured using a pH/ISE meter with a Ross sure-flow pH electrode.

Specific Methanogenic Activity and Most-Probable Number

The bioactivity of the biogranules was measured in duplicate using the specific methanogenic activity (SMA) method developed by Owen et al. (1979) and Dolfing and Mulder (1985). The SMA for a given type of biogranule using a specific substrate was measured in serum vials using concentrated substrate, so that the availability of the substrate was not a limiting factor. The SMA of two types of benzoate-degrading biogranules was measured in this study: one was sampled from Reactor-A when it was treating wastewater containing 2,000 mg/L of sulfate, and the other was sampled from another UASB reactor for a parallel study treating sulfate-free wastewater containing 5,000 mg/L of benzoate. The COD loading for both reactors was 10 g/(L·d). Benzoate, acetate, and formate were the three substrates selected for the SMA measurement. The COD equivalent of the former two substrates were 2,500 mg/L and that of formate was 1,500 mg/L. The sulfate concentration was kept at 2,000 mg/L in all the feed solutions.

The most-probable number (MPN) method was used for the enumeration of MPB, SRB, and benzoate-degrading SAB in biogranules sampled on day 150 from Reactor-A using the established technique (Balch et al. 1979; *Official* 1980; Chartrain and Zeikus 1986; Jain et al. 1991). For the MPN tests of MPB and SAB, each liter of basal medium contained: KH_2PO_4, 0.4 g; K_2HPO_4, 0.4 g; NH_4Cl, 1.0 g; $MgCl_2 \cdot 6H_2O$, 0.21 g; $NaHCO_3$, 6.0 g; yeast extract, 0.2 g; $Na_2S \cdot 9H_2O$, 0.25 g; L-(+)-cysteine·HCl·H_2O, 0.5 g; mineral solution 10 ml (Li and Noike 1989) and vitamin solution, 10 ml (Balch et al. 1979), plus a selected organic substrate. The organic substrates in the basal medium were sodium formate (5.0 g/L) for MPN test of formate-consuming MPB, sodium acetate (3.0 g/L) for acetate-consuming MPB, and sodium benzoate (3.0 g/L) for benzoate-consuming SAB, respectively. The detailed handling procedures followed those recommended by Jain et al. (1991). All glassware and utensils were sterilized in an autoclave, and the medium was dispensed into each tube inside an ultraviolet-sterilized laminar flow workstation (HLF-120/75, Gelman Science, Australia) under the continuous purge of N_2 to ensure the anaerobic condition. In most tests, the head space was filled with a gas mixture of N_2/CO_2 (80/20), except for the MPN test for the hydrogen-consuming MPB; in that case, it was filled with a H_2/CO_2 (80/20) mixture at 2 atm of pressure. All the gases (supplied by Hong Kong Oxygen Company) used for the MPN tests flowed through a heated tubing (370°C) packed with copper pellets to remove any trace amount of oxygen. All MPN tests were conducted in triplicate. All tubes were incubated at 35°C for one month, as specified by Jain et al. (1991). The growth of MPB was interpreted on the basis of the presence of methane in gas phase of the tube.

For the MPN tests of SRB, each liter of basal medium contained: Na_2SO_4, 1.0 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 2.0 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; Na_2SO_3, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.5 g; $NaHCO_3$, 6.0 g; sodium thioglycolate, 0.1 g; L(+)-ascorbic acid, 0.1 g; biotin, 0.1 mg; and p-aminobenzoic acid, 0.05 mg. The concentration of organic substrate was same as the corresponding tests for MPB. The growth of SRB was interpreted by the black appearance of the medium, indicating the presence of FeS.

RESULTS AND ANALYSIS

Both reactors were operated continuously for 320 d. Throughout the experiment, the effluent pH of both reactors was steady, ranging from 7.3 to 8.4 in Reactor-A and from 7.3 to 7.9 in Reactor-B. Fig. 2 illustrates the key results for Reactor-A throughout the experiment: (a) sulfate concentration in wastewater, (b) organic COD removal efficiency, (c) sulfate-reducing efficiency, (d) soluble sulfide concentration in the effluent, and (e) methance production rate. Figs. 3(a)–3(e) illustrate the corresponding results for Reactor-B. The organic COD removal and sulfate reducing efficiencies in this study are defined as follows:

organic COD removal efficiency

$$= (COD_{infl} - \text{soluble organic } COD_{effl})/COD_{infl} \times 100\%$$

sulfate-reducing efficiency

$$= (SO_{4infl}^{-2} - \text{soluble } SO_{4effl}^{-2})/SO_{4infl}^{-2} \times 100\%$$

Both reactors did not experience sludge washout, and their effluent contained only small amount of VSS, averaging 166 mg/L in Reactor-A and 85 mg/L in Reactor-B. The small amount of COD in the washed-out solids was neglected in calculating the organic COD removal efficiency.

Organic-COD Removal

Throughout this study, the wastewater COD was kept at a constant level of 5,000 mg/L for both reactors. Fig. 2(a) illustrates that the sulfate concentration in the wastewater treated in Reactor-A was increased stepwise from the initial 100 mg/L to 7,500 mg/L. Fig. 2(b) illustrates that the organic COD removal efficiency was consistently 98–100% throughout the experiment for wastewater sulfate up to 6,000 mg/L, corresponding to a COD/S ratio of 2.5. As the sulfate was increased to 7,500 mg/L (COD/S = 2.0) on day 270, the organic COD removal efficiency dropped drastically to below 32%, and did not show any sign of recovery after 50 d. The study was terminated on day 320.

Fig. 3(b) illustrates that the organic COD removal efficiency averaged 99.3% in Reactor-B for wastewater containing 3,000 mg/L of sulfate over an extended period of 260 d. The content of residual benzoate in the effluent averaged less than 13 mg/L throughout the experiment in both reactors and never exceeded 57 mg/L, indicating that over 99% of benzoate was transformed. The effluent contained only small amount of acetate and little propionate and butyrate. In Reactor-A, the average VFA and acetate concentrations throughout the experiment were less than 1 mg/L, even when only 32% of benzoate was degraded while treating wastewater containing 7,500 mg/L of sulfate.

Sulfate Reduction

Fig. 2(c) illustrates that in Reactor-A, the sulfate-reducing efficiency in general decreased with the increase of sulfate concentration in the wastewater. At the end, the efficiency was nearly diminished after treating 7,500 mg SO_4^{-2}/L for 50 d. For wastewater containing 4,000 mg/L or less of sulfate, the sulfate-reducing efficiency was improved slightly over time as the biomass was acclimated to the new level of concentrated sulfate and more SRB were produced. When the wastewater sulfate was increased from 1,000 mg/L to 2,000 mg/L on day 62, the sulfate-reducing efficiency decreased from 76 to 40% immediately, but it gradually recovered, reaching 56% by day 137. When the sulfate was further increased to 3,000 mg/L, the efficiency was again dropped followed by gradual recovery. A similar phenomenon was observed in Reactor-B. Fig. 3(c) illustrates that when treating wastewater containing 2,000 mg SO_4^{-2}/L, the sulfate-reducing efficiency increased from 40% on day 1 to 60% by day 59. The efficiency was then reduced to 49% after the sulfate in wastewater was increased to 3,000 mg/L on day 60. But the efficiency gradually in-

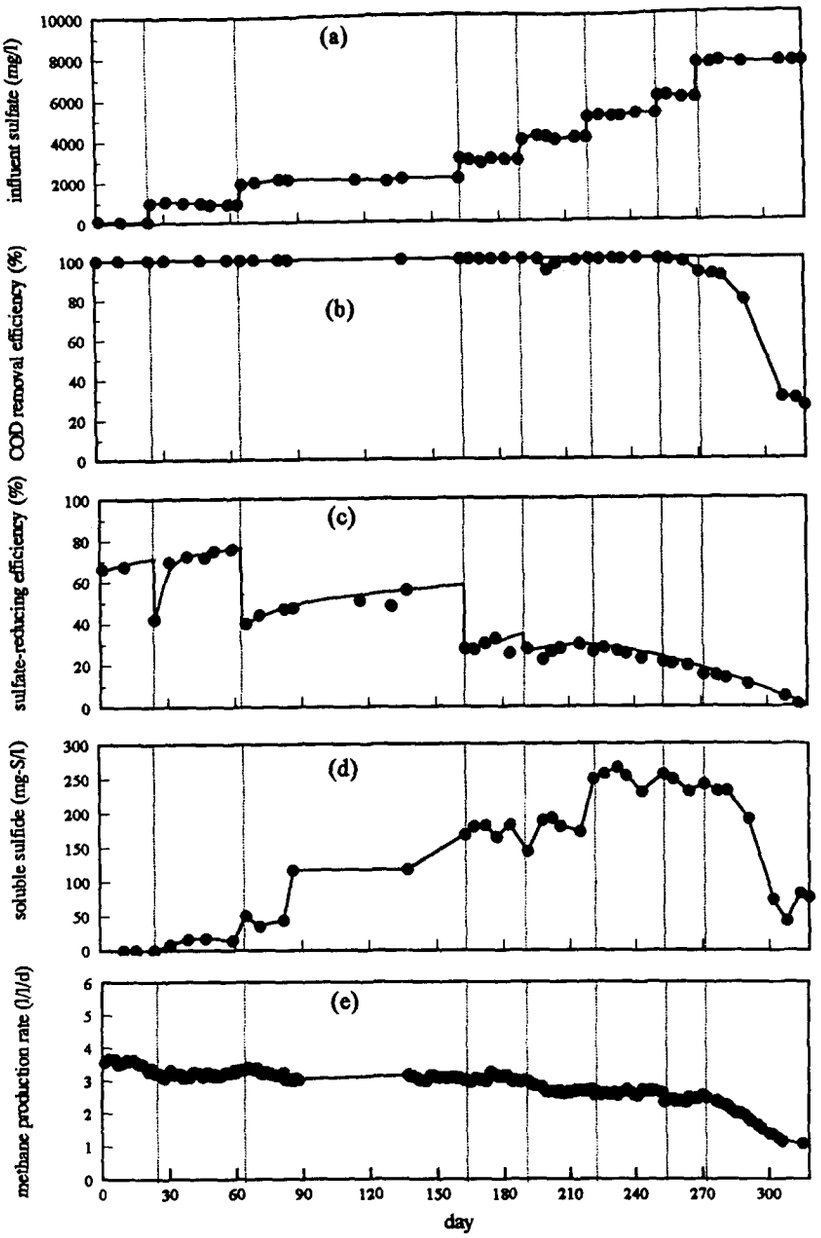

FIG. 2. Results for Reactor-A Treating Wastewater Containing 5,000 mg COD/L at 10 g COD/(L · d): (a) Sulfate Concentration in Wastewater; (b) Organic COD Removal Efficiency; (c) Sulfate-Reducing Efficiency; (d) Soluble Sulfide Concentration in Effluent; (e) Methane Production Rate

creased over time, reaching 80% by day 167, and 95% by day 214, and leveled off to about 89% from day 214 to day 320 [Fig. 3(c)]. Reactor-B had better sulfate-reducing efficiency than Reactor-A in treating wastewater containing 3,000 mg SO_4^{-2}/L, because the former was treating that wastewater for 260 d so that bacteria were well acclimated to that level of sulfate and more SRB may have grown; the latter was treating that wastewater for only 28 d. This agrees with similar observations reported by others that sulfate-reducing efficiency improved with time (Harada et al. 1994; Visser et al. 1993a), but decreased with increased sulfate concentration (Maillacheruvu et al. 1993; Mizuno et al. 1994; Nedwell and Reynolds 1996; Visser et al. 1993a).

Under anaerobic conditions, sulfate is converted by SRB into various forms of sulfide, including soluble sulfide, precipitated metal sulfide, and gaseous H_2S. The soluble sulfide can be present as H_2S, HS^-, or S^{-2}, depending strictly on the pH. Fig. 2(d) illustrates that the soluble sulfide concentration in the effluent of Reactor-A increased with the sulfate concentration in wastewater.

Methane Production

Anaerobic degradation of benzoate in the presence of sulfate is a complex process, involving a number of bacteria and steps, as summarized in Table 1. Benzoate is first either converted by SAB into acetate and hydrogen (reaction 1), or converted by SRB either into acetate (reaction 2) or converted by SRB directly into bicarbonate (reaction 3). In the subsequent degradation process, MPB and SRB compete for acetate and hydrogen, producing methane by the former (reactions 4 and 6) and sulfides by the latter (reactions 5 and 7). Electron donors (in the form of COD) in the wastewater could be converted ultimately into either methane or various forms of sulfide. The methane production in Reactor-A was gradually reduced [Fig. 2(e)] as the reactor produced more sulfide because of increased sulfate treatment of the wastewater. But the

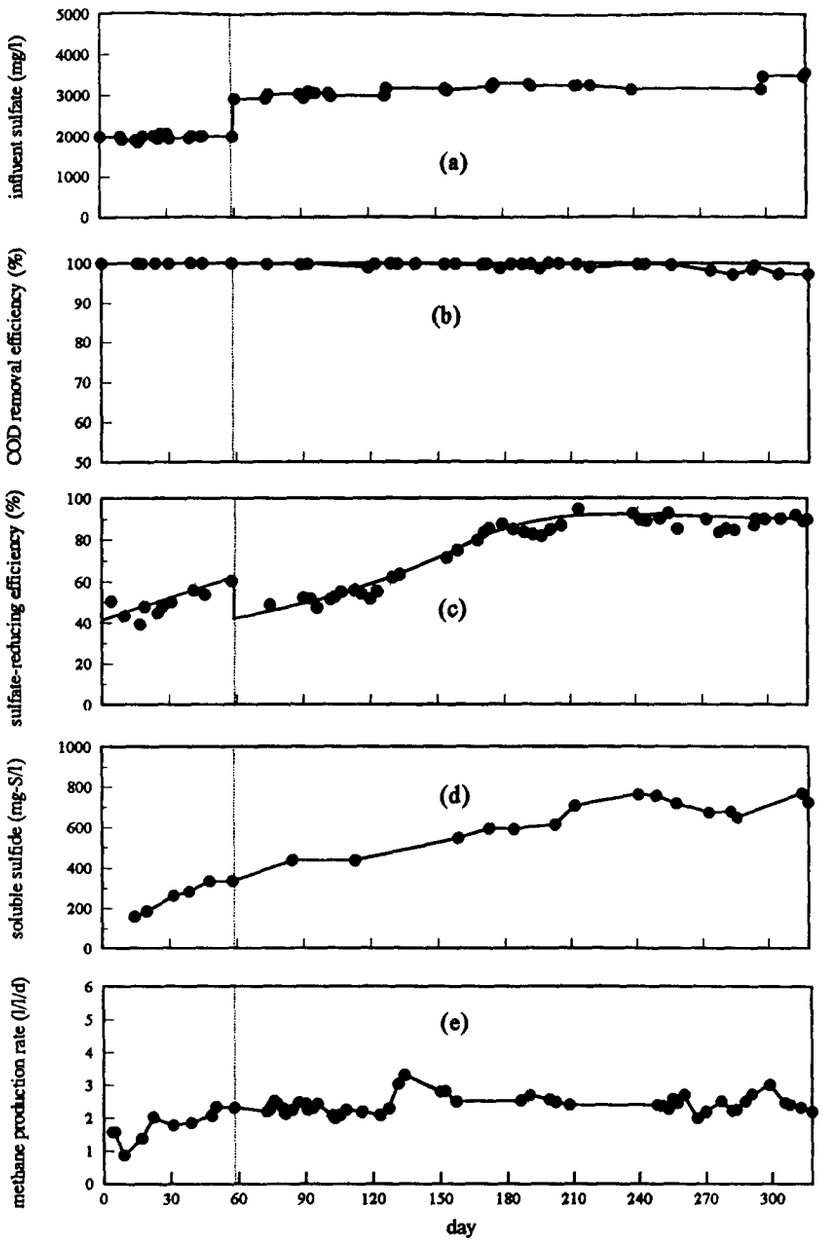

FIG. 3. Results for Reactor-B Treating Wastewater Containing 5,000 mg COD/L at 10 g COD/(L · d): (a) Sulfate Concentration in Wastewater; (b) Organic COD Removal Efficiency; (c) Sulfate-Reducing Efficiency; (d) Soluble Sulfide Concentration in Effluent; (e) Methane Production Rate

methane production in Reactor-B remained steady [Fig. 3(e)], despite the increase of sulfide production, because the COD loading was slightly increased during the period. In general, methane production in both reactors was not suppressed for sulfate concentrations of up to 6,000 mg/L (COD/S = 2.5).

Sulfur and COD Balances

By mass balance, the amount of sulfur (S) as sulfate in the wastewater should equal to the sum of those in (1) residual sulfate in effluent; (2) total soluble sulfide in effluent; (3) H_2S in the vapor phase; (4) sulfide precipitated by metals; and (5) total S in the accumulated biomass. Among them, the first three were measured routinely, and the fourth could be estimated assuming all the metals in the feedwater were precipitated as metal sulfides. The quantity of the last parameter, however, could not be estimated because data on biomass accumulation were unavailable.

The COD balance is more complex. Standard COD measurement is a reduction/oxidation reaction, in which pollutants with reducing capacity are oxidized by a selected strong oxidant, e.g., dichromate. Electrons flow from reducing pollutants to oxygenated products, such as CO_2, H_2O, and SO_4^{-2}. In wastewater treatment, engineers are often only interested in the organic fraction of COD. Thus, conventionally, COD is meant to be the organic COD alone; the sulfide COD, if there is any, has to be subtracted from actual COD measurements. However, strictly speaking, COD by definition is a measurement of reducing capacity, namely, electrons available for chemical oxidation of wastewater. It comes from the organic as well as the sulfidous pollutants. In an anaerobic treatment process, the principle of conservation of available electrons, and thus COD, applies. The COD contents originally in the pollutants are transformed into those of the reduced products, such as methane and H_2S. A balance of COD is, therefore, a balance of the available electrons for chemical oxidation.

The amount of wastewater COD, which was wholly contributed by benzoate, could be converted into either organic

TABLE 1. Reactions Involved in Converting Benzoate into Methane

Reaction number (1)	Electron donor (2)	Bacteria (3)	Reaction (4)
1	Benzoate	SAB	$C_6H_5COO^- + 7H_2O \rightarrow 3CH_3COO^- + HCO_3^- + 3H_2 + 3H^+$
2	Benzoate	SRB(i)	$C_6H_5COO^- + 0.75SO_4^{-2} + 4H_2O \rightarrow 3CH_3COO^- + 0.75HS^- + HCO_3^- + 2.25H^+$
3	Benzoate	SRB(c)	$C_6H_5COO^- + 3.75SO_4^{-2} + 4H_2O \rightarrow 7HCO_3^- + 3.75HS^- + 2.25H^+$
4	Acetate	MPB-A	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$
5	Acetate	SRB(c)	$CH_3COO^- + SO_4^{-2} \rightarrow 2HCO_3^- + HS^-$
6	Hydrogen	MPB-H	$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$
7	Hydrogen	SRB(c)	$SO_4^{-2} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O$

Note: SAB = syntrophic acetogenic bacteria; SRB(i) = the sulfate-reducing bacteria conducting incompletely degradation; SRB(c) = the sulfate-reducing bacteria conducting completely degradation; MPB-A = the methane-producing bacteria consuming acetate; and MPB-H = methane-producing bacteria consuming hydrogen.

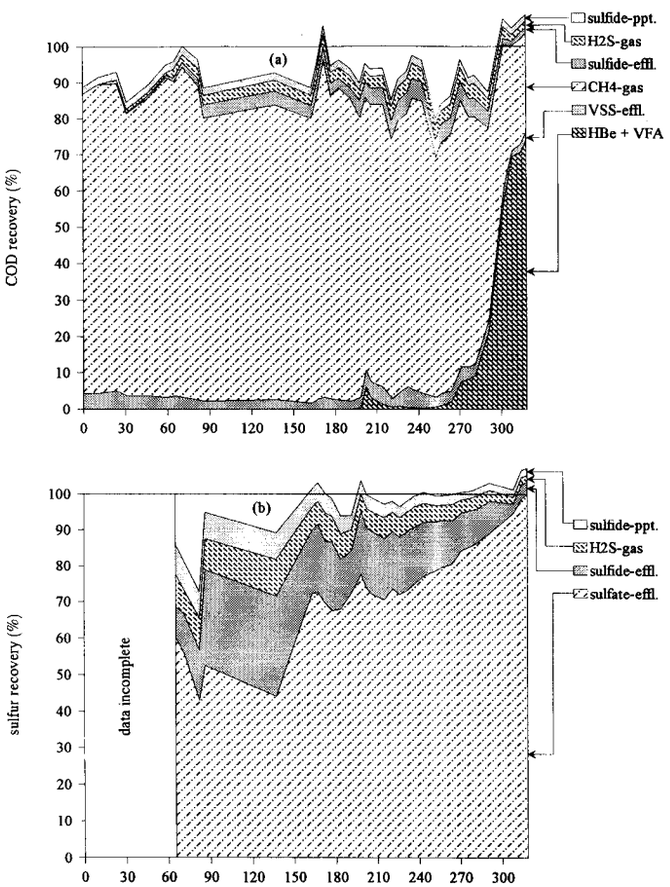

FIG. 4. Reactor-A: (a) COD Balance; (b) Sulfur Balance

COD or sulfide COD, plus those in the accumulated biomass. Although the amount of biomass inside the reactor could not be accurately measured, the first two fractions could be accurately measured. The organic COD is the sum of (1) soluble organic COD in effluent; (2) suspended-solids COD in the effluent; and (3) methane COD in biogas. In comparison, the sulfide COD is the sum of (1) COD of total soluble sulfide in effluent; (2) COD of H_2S in biogas; and (3) COD of sulfides precipitated by metal. The individual quantities of these parameters throughout this study could be estimated based on routinely gathered data. According to the stoichiometry of the two following reactions, each gram of methane and S are equivalent to 4 g and 2 of COD, respectively.

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

$$H_2S + 2O_2 \rightarrow 2H^+ + SO_4^{-2}$$

Thorough examination of the COD and S balances elucidates the fates of electron donors and S in the wastewater. Fig. 4(a) illustrates the COD balance for Reactor-A throughout the experiment. Similar plots for S balance are illustrated in Fig. 4(b). All the accountable COD and S are expressed as a percentage of those in the incoming wastewater. Although data for sulfides were incomplete prior to day 65, the fraction of sulfide COD during this period was insignificant because of their low concentrations. Similarly, Fig. 5(a) illustrates the corresponding COD balance for Reactor-B from day 25 to day 320, and Fig. 5(b) illustrates the S balance throughout the experiment. Overall, the fates of 93% of COD and 98% of S in the wastewater of Reactor-A can be identified throughout this 320-d study, as illustrated in Fig. 4. For Reactor-B, the corresponding figures are 90% and 97%, as illustrated in Fig. 5.

Figs. 4(a) and 5(a) also illustrate that, except near the end of the experiment, benzoate was mostly converted to methane in both reactors. GC analysis of effluent showed that there was little residual benzoate and intermediate VFAs in the effluent, as discussed in the previous section.

Table 2 summarizes the relative amounts of electron donors in five identifiable fates in Reactor-A. It shows that the large majority of reducing capacity ended up as methane. But, the relative amount of methane steadily decreased with the in-

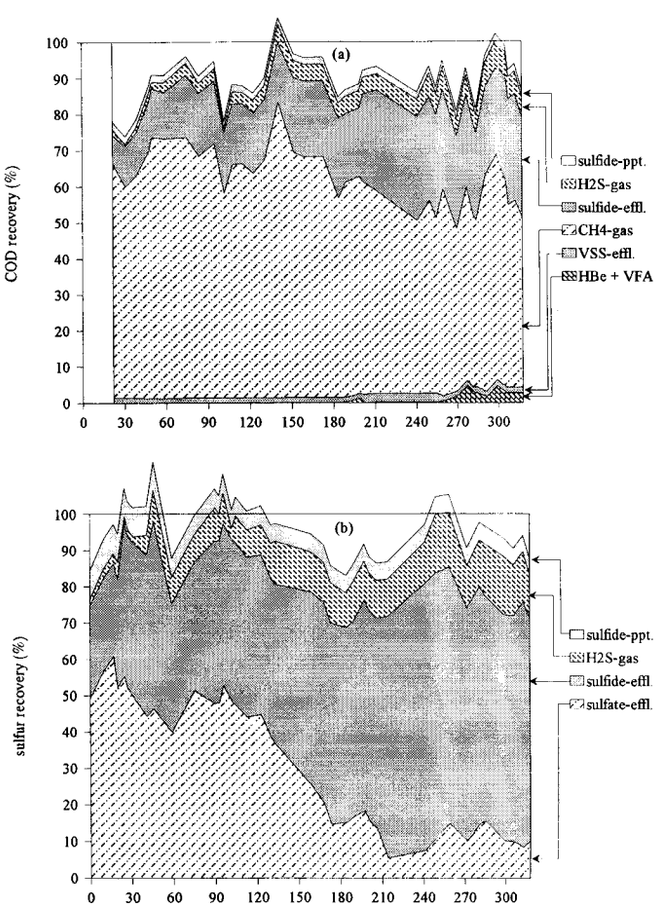

FIG. 5. Reactor-B: (a) COD Balance; (b) Sulfur Balance

TABLE 2. Fates of Electron Donors in Reactor-A as Percent of Those in Wastewater

Sulfate in wastewater ($mg \cdot l^{-1}$) (1)	VSS in effluent (%) (2)	Methane in biogas (%) (3)	Sulfide in effluent (%) (4)	H_2S in biogas (%) (5)	Sulfide ppt (%) (6)
0	7	93	0	0	0
1,000	6	92	1	1	0
2,000	4	87	5	2	2
3,000	3	85	7	3	2
4,000	5	81	9	3	2
5,000	6	77	11	4	2
6,000	4	78	12	4	2
7,500	7	76	10	4	3

TABLE 3. Fates of Sulfur in Reactor-A as Percent of That in Wastewater

Sulfate in wastewater ($mg \cdot l^{-1}$) (1)	Sulfide in effluent (%) (2)	H_2S in biogas (%) (3)	Sulfide ppt (%) (4)
2,000	52	26	22
3,000	57	26	17
4,000	64	22	14
5,000	64	24	12
6,000	63	24	13
7,500	54	25	21

crease of wastewater sulfate concentration from 92% at 1,000 mg SO_4^{-2}/L to 78% at 6,000 mg SO_4^{-2}/L. As the sulfate concentration increased, more SRB were produced and, thus, more reducing capacity was converted to soluble sulfide and gaseous H_2S; thus, less was converted to methane. Among the fates of the remaining electron donors in Reactor-A, soluble sulfide increased from less than 1 to over 10%, and gaseous H_2S increased from 1.0 to 4.5%, whereas those in the effluent VSS and in metal precipitates were more stable. Results of Reactor-B treating 3,000 mg SO_4^{-2}/L over 260 days also showed that most of the electron donors ended up as methane, and the relative amount of methane steadily decreased from 75 to 56%. The fraction of reducing capacity that became soluble sulfide increased from 18.2 to 32.7%, and the fraction of reducing capacity that became gaseous H_2S increased from 3.3 to 6.8%, whereas those in the effluent VSS and in metal precipitates were near constant.

Table 3 shows that in treating wastewaters containing higher than 2,000 mg SO_4^{-2}/L in Reactor-A, 52–64% of reduced S became soluble sulfide in the effluent, 21–26% became H_2S in the biogas, and 12–22% became sulfide precipitates. The average corresponding figures for Reactor-B are 78, 14, and 8%.

Sulfide and Sulfate Toxicity

Soluble sulfide can be present as H_2S, HS^-, or S^{-2}, depending strictly on the pH. Because the effluent of both reactors was slightly alkaline (pH ranging from 7.3 to 8.4 in Reactor-A and 7.3 to 7.9 in Reactor-B) and the dissociation constant for H_2S was $10^{-7.0}$, the predominant species of sulfide in both reactors was HS^-. The maximum effluent sulfide concentration in this study was 769 mg S/L, as illustrated in Fig. 3(d). Whereas the maximum dissolved H_2S concentration was 234 mg S/L when the effluent contained 707 mg S/L at pH 7.3 in Reactor-B. In both cases, the reactor performed steadily, indicating that there was no toxic effect to both MPB and SRB by either sulfide (up to 769 mg S/L) or H_2S (up to 234 mg S/L). However, methane production and sulfate reduction were both inhibited in Reactor-A as the sulfate concentration was increased to 7,500 mg/L (COD/S = 2.0) despite that, during that 50-day period, the maximum total sulfide was only 242 mg S/L and maximum dissolved H_2S was 10 mg S/L. The inhibition, therefore, probably resulted from the concentrated sulfate, instead of total sulfide or H_2S. On the other hand, Nedwell and Reynolds (1996) reported satisfactory sulfate reduction in treating landfill leachate containing 16,800 mg SO_4^{-2}/L (COD/S = 2.53) and 10,080 mg SO_4^{-2}/L (COD/S = 1.93). Reasons for such a discrepancy are not clear.

Maillacheruvu et al. (1993) reported that H_2S at 60–75 mg S/L and soluble sulfide at 150–200 mg S/L caused stress in chemostat reactors treating acetate and propionate; higher levels could result in system failure. A similar observation was reported by Li et al. (1996) for treating benzoate-rich wastewater in chemostat reactors. They found that H_2S at 50 mg S/L, soluble sulfide at 260 mg S/L inhibited bacterial activity. Biogranules from the UASB reactor in this study appeared to have superior resistance to sulfide/H_2S toxicity to the sludge in the chemostat reactors reported in these studies. This could be partly due to the patient acclimation process, and partly due to the layered microstructure of the UASB biogranules (Fang et al. 1994a).

Sludge Yield

The net sludge yield in a reactor is usually estimated by monitoring the organic COD removal as well as the VSS contents in both the reactor and the effluent. The amount of VSS accumulated inside the reactor plus those washed out divided by the total COD removed in the same period equals the net sludge yield. However, the accuracy of this conventional means of estimation is strongly dependent on the reliability of the VSS data. In reality, it is difficult to obtain accurate data of VSS in both reactor and effluent. However, the net sludge yield in an anaerobic reactor can be estimated by another means without any VSS measurements. During anaerobic degradation all the COD removed is converted to either methane, sulfides, carbon dioxide, or biomass. The COD equivalents are 4 g COD/g methane and 2 g COD/g S, but is nil for carbon dioxide. Thus, the amount of COD that converts to biomass equals the difference between the total COD removed and those in methane and all sulfides. The net sludge yield can be estimated from the COD balance. This method has been recently used to estimate the net yields of sludge treating wastewaters containing various fatty acids (Chui et al. 1994; Fang et al. 1995a,b), proteins (Fang et al. 1994b), and starch (Kwong and Fang 1996).

Based on COD balance, on average 93.4% of COD removed from Reactor-A was accountable in the forms of methane COD and sulfide COD. The remaining 6.6% was assumed to be converted to biomass accumulated in the reactors. Because each gram of VSS in biomass has 1.41 g of COD, the net yield of the biomass in Reactor-A is estimated at 0.047 g VSS/g COD. The yield in Reactor-B was estimated, similarly, as 0.072 g VSS/g COD. However, the latter value was probably less reliable, because data for Reactor-B could have higher degrees of error due to the small reactor size.

SMA

Table 4 summarizes the SMA of two types of benzoate-degrading biogranules. One was sampled from Reactor-A when it was treating wastewater containing 2,000 mg/L of sulfate, and the other was sampled from another UASB reactor for a parallel study treating sulfate-free wastewater containing 5,000 mg/L of benzoate. For the benzoate-degrading biogranules, the SMA for treating sulfate-free solutions and for treating those solutions containing 2,000 mg SO_4^{-2}/L were comparable. This indicates that sulfate at 2,000 mg/L had no toxic

TABLE 4. Specific Methanogenic Activity of Biogranules

Substrate (1)	SMA (g CH_4 COD·g VSS^{-1}·d^{-1}) of Biogranules Treating: Benzoate (2)	Benzoate/sulfate (3)
Benzoate + sulfate	1.01	0.68
Acetate + sulfate	1.50	0.23
Formate + sulfate	1.23	1.01
Benzoate	1.05	
Acetate	1.32	
Formate	1.15	

TABLE 5. Bacterial Composition in UASB and Chemostat Reactors

(1)	UASB (2)	Chemostat[a] (3)
Condition		
SO_4^{-2} (mg·l^{-1})	2,000	5,000
COD (mg·l^{-1})	5,000	10,000
COD/S (g/g)	7.5	6
Bacteria	MPN·g VSS^{-1}	MPN·g VSS^{-1}
Benzoate-degrading SAB	5.2×10^9	1.0×10^9
Acetate-degrading MPB	5.2×10^9	4.7×10^8
Formate-degrading MPB	8.2×10^9	4.7×10^8
Hydrogen-consuming MPB	5.2×10^{10}	2.6×10^8
Benzoate-degrading SRB	2.5×10^{10}	1.0×10^{11}
Acetate-degrading SRB	8.2×10^9	1.0×10^{11}
Formate-degrading SRB	2.5×10^9	2.6×10^{10}
Hydrogen-consuming SRB	2.5×10^8	4.7×10^8

[a]Li et al. (1996).

effect on SAB and MPB. On the other hand, the biogranules from Reactor-A had lower SMA than the benzoate-degrading biogranules for all substrates. This may partly be due to the fact that a significant fraction of bacterial population in Reactor-A were SRB, as shown in the next section, that were unable to produce methane. Furthermore, the SRB consumed a significant fraction of acetate as substrate, and thus biogranules from Reactor-A had a very slow SMA of 0.23 g CH_4 COD/(g VSS·day) using acetate, as compared to the corresponding SMA of 1.50 g CH_4 COD/(g VSS·day) for the benzoate-degrading biogranules.

MPN

Table 5 summarizes the MPN for each individual trophic group of bacteria for sludge in Reactor-A. The sludge was sampled on day 150 when the reactor was treating wastewater containing 2,000 mg SO_4^{-2}/L and having 56% sulfate-reducing efficiency. The benzoate-degrading SAB, and the three trophic groups of MPB (utilizing acetate, formate, and hydrogen, individually, as substrate) were ranging from 10^9 to 10^{10} MPN/g VSS. The benzoate-, acetate-, and formate-utilizing SRB were found having the same orders of magnitude. However, the hydrogen-utilizing SRB was found having a slightly lower magnitude of 10^8 MPN/g VSS. These results imply that the relationship between SRB and SAB and MPB was both synergistic and competitive.

Table 5 also lists for comparison the MPN data reported in a previous study (Li et al. 1996), which was conducted in a chemostat reactor treating wastewater containing 10,000 mg/L of benzoate COD and 5,000 mg/L of sulfate. The MPN of MPB in this study were about one order of magnitude higher than those in the chemostat reactor, whereas the MPN of SRB were about one order of magnitude lower. This was probably due to the difference in the sulfate concentration of wastewater. More concentrated sulfate concentration in the chemostat study resulted in higher growth rates of SRB, which outcompete MPB for electron donors.

CONCLUSION

Both reactors removed over 98% of organic COD at various sulfate concentrations up to 6,000 mg/L, despite the mixed liquor concentration of up to 769 mg S/L of total sulfides and up to 234 mg S/L of dissolved H_2S. Sulfate-reducing efficiency decreased with the increase of sulfate concentration, but increased with time at each sulfate concentration. Reactor-B consistently reduced 89% of sulfate treating wastewater containing 3,000 mg SO_4^{-2}/L. But, both organic COD removal and sulfate-reducing efficiencies of Reactor-A dropped drastically at 7,500 mg SO_4^{-2}/L, and showed no sign of recovery after 50 d. The system failure was likely due to the increased sulfate, instead of sulfide, toxicity. From the COD balance in Reactor-A, 93.4% of COD removed was converted to methane, instead of sulfides, with net sludge yields of 0.047 g VSS/g COD. The sulfur balance was over 97%. Results from SMA analysis show that SRB consumed a significant fraction of acetate as substrate. All groups of SAB, MPB, and SRB had 10^9–10^{10} MPN/g VSS, except hydrogen-consuming SRB, which was one order of magnitude lower.

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APPENDIX. REFERENCES

Balch, W. E., Fox, G. E., Magrum, L. J., and Wolfe, R. S. (1979). "Methanogens: reevaluation of unique biological group." *Microbiol. Rev.*, 43, 260–296.

Boone, D. R., and Bryant, M. P. (1980). "Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems." *Appl. Envir. Microbiol.*, 40(3), 626–632.

Chartrain, M., and Zeikus, J. G. (1986). "Microbial ecophysiology of whey biomethanation: characterization of trophic populations and prevalent species composition in continuous cultures." *Appl. Envir. Microbiol.*, 51, 188–196.

Chui, H. K., Fang, H. H. P., and Li, Y. Y. (1994). "Removal of formate from wastewater by anaerobic process." *J. Envir. Engrg.*, ASCE, 120(5), 1308–1321.

Dolfing, J., and Mulder, J. W. (1985). "Comparison of methane production rate and coenzyme F_{420} content of methanogenic consortia in anaerobic granular sludge." *Appl. Envir. Microbiol.*, 49(5), 1142–1145.

Fang, H. H. P., and Chui, H. K. (1993). "Maximum COD loading capacity in UASB reactors at 37°C." *J. Envir. Engrg.*, ASCE, 119(1), 103–119.

Fang, H. H. P., Chui, H. K., and Li, Y. Y. (1994a). "Microbial structure and activity of UASB granules treating different wastewaters." *Water Sci. and Technol.*, 30(12), 87–96.

Fang, H. H. P., Chui, H. K., Li, Y. Y., and Chen, T. (1994b). "Performance and granule characteristics of UASB process treating wastewater with hydrolyzed proteins." *Water Sci. and Technol.*, 30(8), 55–63.

Fang, H. H. P., Li, Y. Y., and Chui, H. K. (1995a). "UASB treatment of wastewater with concentrated mixed VFA." *J. Envir. Engrg.*, ASCE, 121(2), 153–160.

Fang, H. H. P., Li, Y. Y., and Chui, H. K. (1995b). "Performance and sludge characteristics of UASB process treating propionate-rich wastewater." *Water Res.*, 29(3), 895–898.

Fang, H. H. P., Chen, T., Li, Y. Y., and Chui, H. K. (1996). "Degradation of phenol in an UASB Reactor." *Water Res.*, 30(6), 1353–1360.

Gupta, A., Flora, J. R. V., Gupta, M., Sayles, G. D., and Suidan, M. T. (1994). "Methanogenesis and sulfate reduction in chemostats—I. kinetic studies and experiments." *Water Res.*, 28(4), 781–793.

Harada, H., Uemura, S., and Momonoi, K. (1994). "Interaction between sulfate-reducing bacteria and methane-producing bacteria in UASB reactors fed with low strength wastes containing different levels of sulfate." *Water Res.*, 28(2), 355–367.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., and Williams, S. T. (ed.) (1994). *Bergey's manual of determinative bacteriology*, 9th Ed., Williams and Wilkins, Baltimore, Md., 335–346, 719–737.

Isa, Z., Grusenneyer, S., and Verstraete, W. (1986). "Sulfate reduction

relative to methane production in high-rate anaerobic digestion: microbial aspects." *Appl. Envir. Microbiol.*, 51(3), 580–587.
Jain, M. K., Zeikus, J. G., and Bhatnagar, L. (1991). *Anaerobic microbiology—a practical approach.* P. N. Levett, ed., Oxford University Press, New York, N.Y., 223–246.
Karhadkar, P. P., Audic, J., Faup, G. M., and Khanna, P. (1987). "Sulfide and sulfate inhibition of methanogenesis." *Water Res.*, 21(9), 1061–1066.
Kobayashi, T., Hashinaga, T., Mikami, E., and Suzuki, T. (1989). "Methanogenic degradation of phenol and benzoate in acclimated sludges." *Water Sci. and Technol.*, 21, 55–65.
Koster, I. W., Rinzema, A., DeVegt, A. L., and Lettinga, G. (1986). "Sulfide inhibition of the methanogenic activity of granular sludge at various pH levels." *Water Res.*, 20(12), 1561–1567.
Kristjansson, J. K., Schonheit, P., and Thauer, R. K. (1982). "Different Ks values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate." *Archives of Microbiol.*, 131, 278–282.
Kroiss, H., and Wabnegg, F. P. (1983). "Sulfide toxicity with anaerobic wastewater treatment." *Proc., Eur. Symp. on Advanced Anaerobic Waste Treatment (AWWT)*, W. J. van der Brink, ed., 72–85.
Kwong, T. S., and Fang, H. H. P. (1996). "Anaerobic degradation of cornstarch in wastewater in two upflow reactors." *J. Envir. Engrg.*, ASCE, 122(1), 9–17.
Lettinga, G., van Velsen, A. F. M., Hobma, S. M., de Zeeuw, W., and Klapwijk, A. (1980). "Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment." *Biotechnol. Bioengrg.*, 22, 699–734.
Lettinga, G., and Hulshoff Pol, L. W. (1992). "UASB process design for various types of wastewaters, in design of anaerobic process for the treatment of industrial and municipal wastes." *Design of anaerobic process for the treatment of industrial and municipal wastewater*, J. F. Malina and F. G. Pohland, eds., Technomic Publishing Co., Lancaster, Pa., 119–145.
Li, Y. Y., Fang, H. H. P., Chen, T., and Chui, H. K. (1995). "UASB treatment of wastewater containing concentrated benzoate." *J. Envir. Engrg.*, ASCE, 121(10), 748–751.
Li, Y. Y., Lam, S., and Fang, H. H. P. (1996). "Effect of sulfate on anaerobic benzoate degradation." *Water Res.*, 30(7), 1555–1562.
Li, Y. Y., and Noike, T. (1989). "Characteristics of bacterial population and organic matter degradation in anaerobic sludge digestion: on methanogenic bacteria and homoacetogenic bacteria." *Japan J. Water Pollution Res.*, 12, 771–780.
Londry, K. L., and Fedorak, P. M. (1993). "Use of fluorinated compounds detect aromatic metabolities from m-cresol in a methanogenic consortium: evidence for a demethylation reaction." *Appl. Envir. Microbiol.*, 59(7), 2229–2238.
Maillacheruvu, K. Y., Parkin, G. F., Chen, Y. P., Kou, W. C., Oonge, Z. I., and Lebduschka, V. (1993). "Sulfide toxicity in anaerobic systems fed sulfate and various organics." *Water Envir. Res.*, 65(2), 100–108.
Mizuno, O., Li, Y. Y., and Noike, T. (1994). "Effects of sulfate concentration and sludge retention time on the interaction between methane production and sulfate reduction for butyrate." *Water Sci. and Technol.*, 30(8), 45–54.
Mountfort, D. O., and Asher, R. A. (1979). "Effect of inorganic sulfide in the growth and metabolism of *Methanosarcina barkeri* strain DM." *Appl. Envir. Microbiol.*, 37, 670–675.
Nedwell, D. B., and Reynolds, P. J. (1996). "Treatment of landfill leachate by methanogenic and sulfate-reducing digestion." *Water Res.*, 30(1), 21–28.
Official methods of analysis, 13th Ed. (1980). Association of Official Analytical Chemists, Washington, D.C., 825–851.
Okabe, S., Nielsen, P. H., Jones, W. L., and Characklis, W. G. (1995). "Sulfide product inhibition of *Desulfovibrio desulfuricans* in batch and continuous cultures." *Water Res.*, 29(2), 571–578.
Owen, W. F., Stuckey, D. C., Healy, J. B., Young, L. Y., and McCarty, P. L. (1979). "Bioassay for monitoring biochemical methane potential and anaerobic toxicity." *Water Res.*, 13(6), 485–492.
Parkin, G. F., Lynch, N. A., Kou, W. C., Vankeuren, E. L., and Bhattacharya, S. K. (1990). "Interaction between sulfate reducers and methanogens fed acetate and propionate." *J. Water Pollution Control Fed.*, 62(6), 780–789.
Rinzema, A., and Lettinga, G. (1988). "The effect of sulfide on the anaerobic degradation of propionate." *Envir. Technol. Letters*, 9, 83–88.
Speece, R. E. (1983). "Anaerobic biotechnology for industrial wastewater treatment." *Envir. Sci. Technol.*, 17, 416A.
Standard methods—for the examination of water and wastewater, 17th Ed. (1989). American Public Health Association, Washington, D.C.
Uberoi, V., and Bhattacharya, S. K. (1995). "Interactions among sulfate reducers, acetogens, and methanogens in anaerobic propionate systems." *Water Envir. Res.*, 67(3), 330–339.
Visser, A., Beeksma, I., vander Zee F., Stams, A. J. M., and Lettinga, G. (1993a). "Anaerobic degradation of volatile fatty acids at different sulfate concentrations." *Appl. Microbiol. and Biotechnol.*, 40, 549–556.
Visser, A., Nozhevnikova, A. N., and Lettinga, G. (1993b). "Sulfide inhibition of methanogenic activity at various pH levels at 55°C." *J. Chem. Tech. Biotechnol.*, 57, 9–13.
Widdel, F. (1988). "Microbiology and ecology of sulfate and sulfur-reducing bacteria." *Biology of anaerobic microorganisms*, A. J. B. Zehnder, ed., John Wiley & Sons, Inc., New York, N.Y., 469–585.
Winfrey, M. R., and Ward, D. M. (1983). "Substrates for sulfate reduction and methane production in intertidal sediments." *Appl. Envir. Microbiol.*, 45(1), 193–199.
Yoda, M., Kitagawa, M., and Miyaji, Y. (1987). "Long term competition between sulfate-reducing and methane-producing bacteria for acetate in anaerobic biofilm." *Water Res.*, 21(12), 1547–1556.