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ANAEROBIC DEGRADATION OF BENZOATE AND CRESOL ISOMERS IN SULFATE-RICH WASTEWATER

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ABSTRACT

Results of a continuous wastewater experiment conducted in an upflow anaerobic sludge blanket (UASB) reactor at 37°C showed that m- and o-cresols (225 $mg \cdot l^{-1}$ each) could be partially degraded, and their presence did not adversely affect methanogenesis of benzoate (1000 $mg \cdot l^{-1}$) and sulfidogenesis of sulfate (1800-5600 $mg \cdot l^{-1}$). With 12 hours of hydraulic retention, the reactor on average was able to remove over 99.5% of benzoate, 11.0% of m- cresol, 8.3% of o-cresol and reduce up to 48% of sulfate. Sulfate was reduced at a constant rate of 1630 $mg \cdot (l \cdot d)^{-1}$, independent of sulfate concentration. Results of batch tests showed that biogranules were able to remove 46.2% of m-cresol and 37.4% of o-cresol in 100 hours. Furthermore, biogranules treating solutions containing 1250 $mg \cdot l^{-1}$ of sulfate degraded benzoate at an average rate 125% faster than those treating sulfate-free solutions; 74.4% of the electron flow was used for methanogenesis and only 25.6% for sulfidogenesis. Results suggest that most sulfidogens in the biogranule were acetogenic, producing acetate, most of which was subsequently converted by methanogens into methane. © 1997 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Anaerobic; benzoate; m-cresol; o-cresol; sulfate; methanogenesis; MPB; sulfidogenesis; SRB; UASB.

INTRODUCTION

Anaerobic technology has become popular in recent years for the treatment of high-strength industrial wastewaters. However, its application is still mostly limited to the treatment of food/beverage wastewater because anaerobes are commonly perceived as sensitive to the toxicity of many chemical pollutants. However, recent studies showed that the anaerobic process has great potential for the treatment of wastewater from chemical industry. The upflow anaerobic sludge blanket (UASB) process (Lettinga *et al.*, 1980; Fang and Chui, 1993) was found effective for the removal of simple aromatic chemicals, such as benzoate (Li *et al.*, 1995) and phenol (Fang *et al.*, 1996). Cresol, an aromatic pollutant with a slightly more complex structure, is mainly produced in coal liquefaction, oil refineries and some petrochemical plants. The isomers of cresol are recalcitrant to biodegradation and are believed to be toxic to bacteria. However, recent batch studies showed that cresol could be degraded under anaerobic conditions, particularly under a sulfate-rich environment (Ramanand and Suflita, 1991; Smolenski and Suflita, 1987; Suflita *et al.*, 1989).

Sulfate is commonly found in wastewaters of pharmaceutical, pulp/paper, fermentation, petrochemical and mining industries. In the anaerobic treatment of sulfate-rich wastewater, sulfate-reducing bacteria (SRB) compete with methane-producing bacteria (MPB) and syntrophic acetogenic bacteria (SAB) for electron donors, such as hydrogen and organic substrate, resulting in the production of hydrogen sulfide (H_2S). Many reported (Kroiss and Wabnegg, 1983; Parkin *et al.*, 1990; Rinzema and Lettinga, 1988; Winfrey and Ward, 1983) that sulfate at high concentration was detrimental to the anaerobic process. This was likely due to the H_2S production, which is not only malodorous and corrosive, but also believed to be toxic to both methanogens and sulfidogens. Competition between SRB and MPB was reported to be dependent upon a number of process variables, including ratio of COD (chemical oxygen demand) and sulfate (Li *et al.*, 1996; Mizuno *et al.*, 1994; Uberoi and Bhattacharya, 1995), substrate concentration (Isa *et al.*, 1986), organic loading rate (Yoda *et al.*, 1987) and even reactor type (Isa *et al.*, 1986). However, there is no consensus so far on the effect of these variables.

The study was thus conducted using both continuous and batch experiments to investigate: (1) the degrees of cresol degradation in wastewaters containing both sulfate and benzoate, (2) the rate of sulfate reduction, and (3) the interactions among MPB and SRB in the degradation of benzoate and cresols.

MATERIALS AND METHODS

The continuous experiment was carried out in a 2.8-l UASB reactor (Fang *et al.*, 1996) at 37°C over 391 days. Digester sludge from a local municipal wastewater treatment plant was used to seed the reactor. Balanced nutrients, trace elements and buffer chemicals were added to the synthetic wastewater throughout this study using the formula established previously (Fang and Chui, 1993). The experiment comprised three phases. The organic constituents in the wastewater had a COD-equivalent of 3100 $mg \cdot l^{-1}$ in all phases. The acclimation phase lasted 148 days, at the beginning of which sucrose was used as the sole substrate but it was gradually replaced by benzoate, m- and o-cresols at increased concentrations. At the last stage (Days 84-148) of the acclimation phase, the reactor was treating wastewater containing 500 $mg \cdot l^{-1}$ of sucrose, 800 $mg \cdot l^{-1}$ of benzoate, 200 $mg \cdot l^{-1}$ each of m- and o- cresol, and 1800 $mg \cdot l^{-1}$ of sulfate. In the second phase, which started on Day 149, sucrose was completely replaced by benzoate and cresols. The second phase lasted for 148 days, during which the reactor treated a wastewater containing 1000 $mg \cdot l^{-1}$ of benzoate, 225 $mg \cdot l^{-1}$ each of m- and o-cresols plus 1800 $mg \cdot l^{-1}$ of sulfate. In the last phase, sulfate concentration was gradually increased reaching 5600 $mg \cdot l^{-1}$ at the end of the experiment, while benzoate and cresol isomers remained at the same levels as in the previous phase.

The biogas composition was 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 30 $ml \cdot min^{-1}$. The column was operated at a temperature program of 90°C for 1 minute and then 110°C for 2 minutes. The temperatures of injection port and detector were 200°C. Concentrations of benzoate, cresol isomers, and volatile fatty acids were determined by a second gas chromatograph of the same model equipped with a flame ionization detector and a 10m (length) x 0.53mm (ID) capillary column. The column was operated at a temperature program of 70°C for 4 minutes and then 140°C for 3 minutes, and finally 170°C for 3 minutes. The temperatures of injection port and detector were the same at 200°C. Helium was used as the carrier gas at a flow rate of 40 $ml \cdot min^{-1}$. Sulfate was analyzed by an ion chromatograph (Shimadzu, Model LC-10) with the Shim-pack IC-A3 column. Other parameters, such as VSS (volatile suspended solids) and COD were measured according to the *Standard Methods* (APHA, 1985).

Batch tests were also conducted on the activities of biogranules sampled from the UASB reactor near the end of phase 2 on Days 271 and 293. All batch tests were conducted in duplicate in 157-ml serum vials. About 100 mg of biogranules (as VSS) were added to each serum vial along with 100 ml of feed solution containing individual substrate plus nutrient, vitamins, trace metals, and bicarbonate to buffer the pH at 7.2~7.6. The exact amount of VSS in each vial was later measured after the test was completed. The vials were placed in a 37°C shaking water bath throughout the test. The production of biogas was monitored at regular intervals, and its methane content was measured by GC.

Methanogenic and sulfidogenic activities of biogranules sampled on Day 271 were measured using feed solutions containing 1250 $mg \cdot l^{-1}$ of sulfate and 2500 mg-$COD \cdot l^{-1}$ equivalent of individual substrates. The four individual substrates and their respective concentrations used in the tests were: acetate (2300 $mg \cdot l^{-1}$), propionate (1630 $mg \cdot l^{-1}$), butyrate (1360 $mg \cdot l^{-1}$) and benzoate (1260 $mg \cdot l^{-1}$). Biogas was monitored at regular intervals for methane production. The test was terminated when the biogas production was exhausted. Residual sulfate and individual substrate in the mixed liquor were then measured. In order to evaluate the effect of sulfate, the methanogenic activity of biogranules was also measured in another batch treating a sulfate-free solution containing benzoate as the sole substrate. Lastly, another batch test was conducted on biogranules sampled on Day 293 treating a feed solution containing benzoate, m- and o-cresol and sulfate, all at the same concentrations as those in the influent of the continuous experiment in phase 2. In this test, the concentrations of benzoate, cresols and sulfate in the mixed liquor were regularly measured over 260 hours.

RESULTS AND DISCUSSIONS

Continuous operation studies

Figure 1 illustrates the major operational conditions and results of the continuous experiment, including (a) influent benzoate concentration, (b) influent sulfate concentration, (c) benzoate removal efficiency, (d) sulfate-reducing efficiency, and (e) methane production rate. Data of the acclimation phase were plotted starting on Day 70; data prior to that date were incomplete.

At the last stage of the acclimation period, while treating wastewater containing 500 $mg \cdot l^{-1}$ of sucrose, 730 $mg \cdot l^{-1}$ of benzoate, 200 $mg \cdot l^{-1}$ each of m- and o-cresol , and 1800 $mg \cdot l^{-1}$ of sulfate, the reactor was able to remove 7% each of m- and o-cresol, and over 99.5% of benzoate, and to reduce 39.6% of sulfate. A distinct granulated sludge bed was developed at the end of this phase; the biogranules were about 1 mm in diameter.

During phase 2 the reactor treated the wastewater containing 1000 $mg \cdot l^{-1}$ of benzoate, 225 $mg \cdot l^{-1}$ each of m- and o-cresols plus 1800 $mg \cdot l^{-1}$ of sulfate for 148 days to see if biogranules were able to improve the degradability of cresole isomers. However, it was found that after such an extended period degradation of cresol isomers was improved only slightly. At the end of phase 2, only 11.0% of m-cresol and 8.3% of o-cresole were degraded, even though the reactor was able to consistently degrade over 99.5% of benzoate throughout this period. The fraction of sulfate being reduced increased from 36%, initially, to 48% at the end of phase 2. The increase of sulfate- reducing efficiency over time was likely due to the increase of the SRB population as the experiment progressed. The poor cresol removal could be due to the relatively short HRT (hydraulic retention time) of 12 hours (and/or the high concentrations of the isomers in this experiment). The average degradation rate of m-cresol in this study was 45.8 $mg \cdot (l \cdot d)^{-1}$, considerably higher than the value reported in literature. Ramanand and Suflita (1991) reported that over 85% of m-cresol (initial concentration of 32.4 $mg \cdot l^{-1}$) was consumed in less than 6 days in an anoxic aquifer slurry kept under sulfate-reducing conditions; the average degradation rate was 4.6 mg-$cresol \cdot (l \cdot d)^{-1}$.

On the other hand, results of the experiment showed that the presence of m- and o-cresols at concentrations up to 225 $mg \cdot l^{-1}$ each did not adversely affect benzoate degradation over the long period of operation. It has been recently reported (Li *et al.*, 1995; Fang *et al.*, 1995) that benzoate-degrading biogranules exhibited a layered structure, in which the more vulnerable methanogens and syntrophic acetogens were populated in the interior of the granule and were shielded by a dense layer of presumably benzoate-degrading acidogens. It is thus likely that, due to the presence of such a protection layer, biogranules have a higher degree of tolerance to chemical pollutants, such as cresol isomers in this study.

Phase 3 started on day 297, during which influent sulfate concentration was increased stepwise from 1800 $mg \cdot l^{-1}$ to 5600 $mg \cdot l^{-1}$ while the organic constituents remained unchanged. The increase of sulfate did not affect the removal efficiencies of cresol isomers and benzoate, as illustrated in Figure 1(c); but, it resulted in

a decrease in sulfate-reducing efficiency, as illustrated in Figure 1(d). The daily methane production rate in phases 2 and 3 averaged 1.2 l-methane•(l•d)$^{-1}$, as illustrated in Figure 1(e).

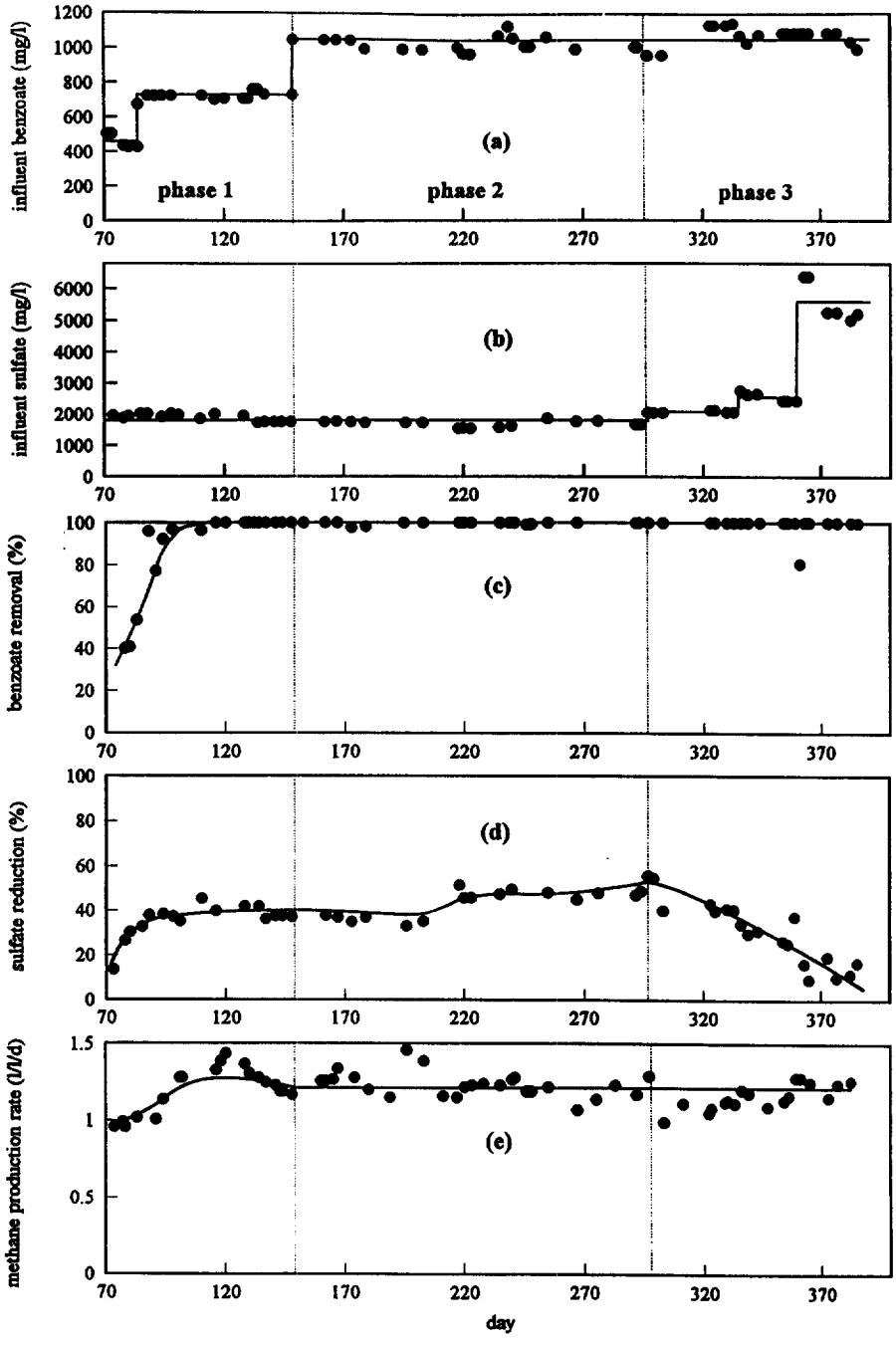

Figure 1. (a) Influent benzoate concentration, (b) influent sulfate concentration, (c) benzoate removal efficiency, (d) sulfate reducing efficiency, and (e) methane production rate.

Figure 2(a) illustrates that the sulfate-reducing rate was near constant, averaging 1630 mg-sulfate•(l•d)$^{-1}$, even the sulfate concentration in wastewater increased from 1800 to 5600 mg•l^{-1}; correspondingly, the sulfate-reducing efficiency was lowered from 48% to 14% , as illustrated in Figure 2(b). This was because SRB were unable to reduce the increased amount of sulfate without an increase of substrate for extra carbon source. Thus, sulfate concentration in the wastewater is probably not the determining parameter for the efficiency of sulfate reduction, as illustrated in Figure 2(a). Inside the reactor, SRB and MPB competed for electrons supplied by the benzoate and cresols. The rates of methane production (Figure 1(e))and sulfate reduction (Figure 2(a)) were both near constant.

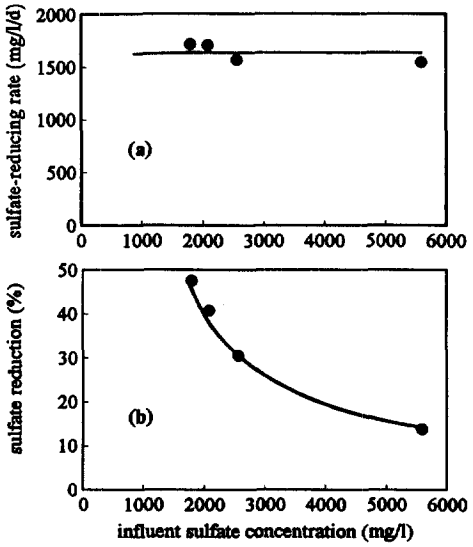

Figure 2. (a) Sulfate-reducing rate, and (b) sulfate-reducing efficiency at various sulfate concentrations.

Throughout the experiment, the pH of sludge bed in the reactor was steady, ranging 7.0-7.2. The concentrations of various forms of sulfide dissolved in mixed liquor depend upon the H_2S in the gas phase and the mixed liquor pH. The concentrations of total sulfide-S and dissolved H_2S-S in the effluent could be calculated, based on the amounts of sulfate reduced, the concentration of gaseous H_2S and the mixed liquor pH. Results showed that, in this study, the maximum concentration of total sulfides was 270 mg-S•l^{-1} and the maximum dissolved H_2S was 98 mg-S•l^{-1}; at these levels, both sulfides and dissolved H_2S did not appear to have noticeable toxic effect to methanogenesis and sulfidogenesis.

Batch tests

Electron flow. Reduction/oxidation is the kind of chemical reaction involving electron transfer. Electrons flow from reducing chemicals, such as organic substrates and hydrogen, to the oxidizing chemicals, such as oxygen and sulfate. Anaerobic degradation of benzoate in the presence of sulfate is a complex process. SRB can conduct either complete oxidation converting all organic carbon in benzoate into carbon dioxide, or partial oxidation converting most carbon into acetate. In the former case, SRB compete with SAB and MPB for substrate and electron, whereas in the latter case, SRB is syntrophically associated with MPB. The synergistic association among SAB, MPB and SRB has been reported for treating wastewater containing substrates such as propionate (Harada *et al.*, 1994), butyrate (Mizuno *et al.*, 1994), and benzoate (Li *et al.*,1995).

The distribution of electron flow between the reactions conducted by SRB and MPB could be calculated from the amounts of sulfate reduced and methane produced (Isa *et al.*, 1986). Results of the batch tests treating sulfate-rich solution showed that electron flow toward sulfate-reduction was dependent upon the

substrate. Using benzoate as the sole substrate, sulfate-reduction consumed 25.6% of electron flow, which was slightly lower than the 30.5% in the continuous experiment. The electron flows consumed by sulfate reduction using other substrates were: 33.3% for butyrate; 50.4% for propionate, but only 12.4% for acetate. The last figure indicates that 87.6% of acetate, the key intermediate product of benzoate degradation, was utilized by MPB, suggesting that most of the SRB in the biogranules conducted only the partial oxidation.

Effect of sulfate on methane production. Figure 3 illustrates the methane production in two batch tests treating identical feed solutions, both containing 1260 $mg \cdot l^{-1}$ of benzoate, except one contained 1250 $mg \cdot l^{-1}$ of sulfate and the other was sulfate free. In the sulfate-free solution, benzoate was first converted by SAB into acetate and hydrogen, and the latter two were subsequently converted by MPB to methane. While in the presence of sulfate, some of the benzoate was utilized by SRB, in competition with SAB, for partial oxidation and thus less amount of methane was produced. Figure 3 illustrates that reactor treating sulfate-free solution produce 100.4 ml of methane, 25% higher than the quantity produced by the reactor treating solution containing 1250 $mg \cdot l^{-1}$ sulfate. Figure 3 also illustrates that methane production was exhausted within 155 hours in the latter reactor and within 335 hours in the former. Analyses by GC showed that benzoate was depleted from solution in all batch tests when methane production was exhausted. The average benzoate-degradation rate was 9.11 $mg \cdot (l \cdot h)^{-1}$ in the sulfate-rich solution, about 125% higher than that in the sulfate-free solution.

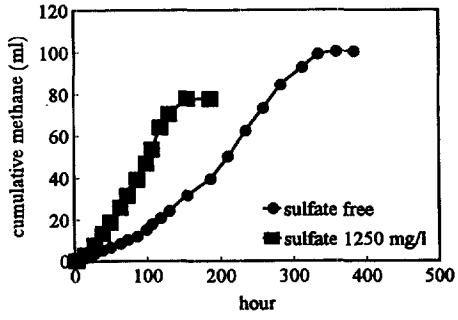

Figure 3. Conversion of benzoate to methane in the presence and absence of sulfate.

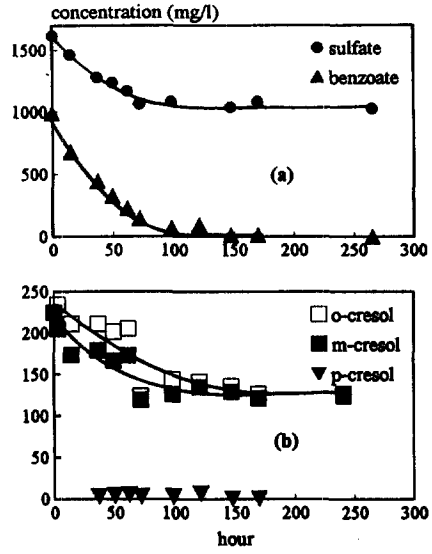

Figure 4. (a) Degradation of benzoate and reduction of sulfate, and (b) degradations of m- and o-cresols.

Degradation of m- and o-cresols. Figure 4 illustrates that concentrations of m- and o-cresols in the mixed liquor decreased along with decreases of sulfate and benzoate. Concentrations of m- and o-cresols were levelled off after about 100 hours to the levels of 125.8 $mg \cdot l^{-1}$ and 144.3 $mg \cdot l^{-1}$, respectively, corresponding to 46.2% and 37.4% of removal. The removal efficiencies of m- and o-cresols were considerably higher in the batch tests than those in the continuous experiment; this was because the former were conducted at a longer retention time (over 100 hours) than the latter (12 hours only). Figure 4 also illustrates that degradation of cresol isomers appeared to stop by the time when benzoate became depleted. This suggests that degradation of cresols required benzoate as a co-substrate. Figure 4 (b) also illustrates a trace amount of p-cresol was present in the mixed liquor after 40 hours, probably a transformation product from the other two isomers.

CONCLUSIONS

Results of a continuous experiment showed that m- and o-cresols at concentration of 225 $mg \cdot l^{-1}$ each could be partially degraded, and their presence did not adversely affect methanogenesis of benzoate (1000 $mg \cdot l^{-1}$) and sulfidogenesis of sulfate (1800-5600 $mg \cdot l^{-1}$). With 12 hours of hydraulic retention, the reactor on average was able to remove over 99.5% of benzoate, 11.0% of m-cresol, 8.3% of o-cresol and reduce up to 48% of sulfate. Sulfate was reduced at a constant rate of 1630 $mg \cdot (l \cdot d)^{-1}$, independent of sulfate concentration. Results of batch tests showed that biogranules were able to remove 46.2% of m-cresol and 37.4% of o-cresol in 100 hours. Furthermore, biogranules treating solutions containing 1250 $mg \cdot l^{-1}$ of sulfate degraded benzoate at an average rate 125% faster than those treating sulfate-free solutions; 74.4% of the electron flow was used for methanogenesis and only 25.6% for sulfidogenesis. Results suggest that most SRB in the biogranule were acetogenic, producing acetate which was subsequently converted by MPB into methane.

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