TOXICITY OF PHENOL TOWARDS ANAEROBIC BIOGRANULES

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Abstract—Toxity of phenol towards upflow anaerobic sludge blanket (UASB) biogranules treating wastewater containing different substrates was investigated. Under shock-loading conditions, the specific methanogenic activity (SMA) of the biogranules decreased with the increase of phenol concentration. The $C_{I,50}$ (i.e. the concentration at which bioactivity was 50% of the control) was 1750, 1000 and 1700 mg-phenol litre^{-1} for acetate-, propionate- and benzoate-degrading biogranules, respectively. Under continuous-loading conditions, however, phenol toxicity was not progressive. Instead, phenol had a threshold toxicity level for each type of biogranules: 1050–1600, >850 and 1100–1700 mg litre^{-1} for benzoate-, propionate- and acetate-degrading biogranules, respectively. Below these threshold levels, phenol was not inhibitive to the activity of biogranules; but above them, the inhibition was nearly 100%. Phenol toxicity was neither cumulative nor permanent. Once the phenol concentration in wastewater was lowered to levels below the threshold, biogranules were able to gradually regain 100% of their activity. Lowering substrate concentration expedited the recovering process. © 1997 Elsevier Science Ltd

Key words—anaerobic, biogranule, inhibition, methanogenic activity, phenol, shock, SMA, toxicity, UASB

INTRODUCTION

Anaerobic treatment of wastewater has a number of intrinsic advantages over the more conventional aerobic processes. It saves the energy needed for aeration, converts pollutants into methane, a readily usable fuel, and produces less sludge. The technology in recent years has been applied to the treatment of many high-strength industrial wastewaters. But its application is still limited mostly to the treatment of wastewater from agricultural and food/beverage industries for which pollutants are mostly readily biodegradable. This is partly due to a common misconception that anaerobic microorganisms are vulnerable to the toxicity of chemicals in wastewater (Speece, 1983). Thus, it is of great practical interest to investigate the feasibility of applying the anaerobic technology to the treatment of wastewater contaminated by toxic pollutants.

Phenol, a simple aromatic chemical, is toxic to most microorganisms and is commonly used as a general disinfectant. It is also used widely for the commercial production of a wide variety of resins, including phenolic resins as construction materials for automobiles and appliances, epoxy resins as adhesives, polycarbonate for soft-drink containers and polyamide for various applications. Wastewater containing residual phenol from resin production cannot be effectively treated by conventional biological processes. Most of the phenolic toxicity data in the literature is based on single-batch

experiments (Blum and Speece, 1991; Sierra-Alvarez and Lettinga, 1991; Nirmalakhandan *et al.*, 1994). Fang *et al.* (1996) studied the feasibility of anaerobic treatment of wastewater containing phenol as the sole substrate. They found that wastewater containing up to 1260 mg litre^{-1} of phenol could be treated anaerobically; however, the process required a lengthy start-up and was easily disturbed by the changes of temperature and phenol concentration.

Among the high-rate processes developed in recent years, the upflow anaerobic sludge blanket (UASB) reactor (Lettinga *et al.*,1980; Lettinga and Hulshoff Pol, 1991; Fang and Chui, 1993) is probably most successful commercially. It became popular first in Europe and more recently in Asia. In a UASB reactor, microorganisms agglutinate to form biogranules which have high bioactivity and superb settleability. The microstructure of a biogranule and its population dynamics are dependent on the nature of pollutants in wastewater (Fang *et al.*, 1994, 1995a). It is of practical interest to investigate the effect of phenol on biogranules of different microstructures and bacterial population dynamics. As a result, three types of biogranules were selected for this study: they were obtained from UASB reactors degrading acetate, propionate and benzoate, individually, as the sole substrate.

Acetate is one of the most important intermediates for the anaerobic degradation of complex organic substances. Conversion of acetate to methane is a one-step process. However, degradation of propi-

onate is a two-step process (Boone and Bryant, 1980). Propionate is first degraded to acetate and hydrogen by bacteria such as *Syntrophobacter wollinii* (Boone and Bryant, 1980; Chui, 1994). The two intermediates are then converted to methane by acetotrophic methanogens, such as *Methanothrix* (Zehnder et al., 1980) and hydrogenotrophic methanogens, such as *Methanospirillum hungatei* (Southam et al., 1990). Benzoate, on the other hand, is a key intermediate for the degradation of many aromatic compounds, including phenol and chlorophenol (Knoll and Winter, 1989; Kobayashi et al., 1989). Although its degradation follows a multi-step pathway, benzoate is directly converted to acetate and hydrogen by bacteria such as *Syntrophus buswellii* (Tarvin and Buswell, 1934; Mountfort et al., 1995). Furthermore, in addition to their differences in bacterial composition, the three selected biogranules also have different microstructures. Acetate-degrading biogranules exhibit a uniform microstructure comprising mostly *Methanothrix*-like bacteria. Benzoate-degrading biogranules exhibit a layered microstructure, in which the *Methanothrix*-like bacteria are in abundance in the centre core, shielded by a dense skin layer of *S. buswellii*-like bacteria (Li et al., 1995). Propionate-degrading biogranules, on the other hand, do not show any patterned microstructure (Fang et al., 1995b).

The objective of this study was to compare the effect of phenol on the methanogenic activity of these three types of UASB biogranules, which have different bacterial composition and microstructure, under shock-loading conditions in batch tests, as well as under continuous-loading conditions in upflow reactors.

MATERIALS AND METHODS

Cultivation of biogranules

Biogranules were cultivated at 37°C in six 2.8-litre UASB reactors (Fang et al., 1995c) for three wastewaters, containing acetate, propionate and benzoate as individual substrate—two reactors for each wastewater. Each reactor was seeded with 1 litre of UASB biogranules obtained from previous studies treating individual substrates (Chui, 1994; Li et al., 1995), plus 0.5 litres of digester sludge from a local municipal wastewater treatment plant. Each type of biogranules were cultivated for over 6 months by feeding with wastewater containing the individual substrate at the concentration equivalent to 10000 mg litre^{-1} of chemical oxygen demand (COD), plus buffering chemical, trace

metals and balanced nutrient (Fang and Chui, 1993). All reactors consistently removed over 98% of COD with a 24-h hydraulic retention time (HRT), which corresponded to a constant daily loading of 10 g-COD litre^{-1}. Biogranules were then taken after 6 months of cultivation from these reactors for the shock- and continuous-loading tests on phenol toxicity.

Shock-loading tests

At each shock-loading test, the specific methanogenic activities (SMA) (Owen et al., 1979) of the biogranules were measured for over 7 days in 157-ml serum vials. The substrate-dependent SMA represents the maximum methane production rate by the biogranules treating a given substrate. In each test, biogranules were added to the serum vial along with 100 ml of feed solution containing substrate, nutrient, vitamins and trace metals, plus phenol at various concentrations; the pH was buffered at 7.2–7.6 by bicarbonate. The exact amount of volatile suspended solids (VSS) in each vial was later measured after the SMA test was completed. As soon as biogranules and feed solution were added, each vial was flushed with a gas mixture of N_2/CO_2 (3:1) and then sealed by a rubber septum and aluminium cap. The vial was then placed in a 37°C shaking water-bath. The volume of biogas production was measured by a gas syringe, and the biogas composition was analysed using a gas chromatograph (GC, Hewlett Packard, model 5890A), as described by Fang et al. (1995c). Both the volume and composition of the biogas were monitored at regular intervals for 7 days: by then the biogas production was nearly exhausted.

The toxicities of phenol at various conditions were indicated by the decrease of SMA relative to the control treating a phenol-free solution. The SMA was measured at least in duplicate for each type of biogranule degrading a specific substrate at each phenol concentration. In all the SMA tests, unless specified otherwise, 100 mg of biogranules directly sampled from the cultivating reactors were tested using the same substrate as in the cultivating reactor at concentrations equivalent to 2500 mg litre^{-1} of COD.

Three series of shock-loading tests were conducted to examine the effects of (1) biomass quantities, (2) the use of intermediate acetate as substrate and (3) the microstructure of biogranules on the SMA measurements. Table 1 summarizes the conditions of

Table 1. Testing conditions of UASB biogranules under shock-loading conditions

Series[a]	Biomass (mg VSS)	Substrate (mg litre^{-1})	Remarks
1	50	1250	a
	100	2500	
	200	5000	
2	100	2500	Using acetate as substrate for propionate-degrading and benzoate-degrading biogranules
3	100	2500	Using disintegrated biogranules

[a]Unless specified otherwise, tests were conducted for three different types of biogranule obtained directly from cultivating reactors using the same individual substrates as in the cultivating reactors.

Table 2. Testing conditions of UASB reactors under continuous-loading conditions

Reactor no.	Substrate in wastewater	Inhibition-phase COD loading (g litre^{-1} per day)	Inhibition-phase phenol concentration (mg litre^{-1})	Recovery-phase COD loading (g litre^{-1} per day)
1	Benzoate	10	1700	10
2	Benzoate	10	1050; 1600	10; 5; 10
3	Propionate	10	850	10; 5; 10
4	Acetate	10	1700	5
5	Acetate	10	1100; 1700	5[a]

[a]Phenol concentration was kept at 1100 mg litre^{-1} during the recovery phase.

the three series of tests. In series 1, the SMA reduction was investigated for acetate-, propionate- and benzoate-degrading biogranules, using three different quantities of biomass (50, 100 and 200 mg VSS) for each type of biogranule; the respective substrate concentrations were equivalent to 1250, 2500 and 5000 mg litre^{-1} of COD so that the COD/VSS ratio was kept at 2.5. In the series 2, propionate- and benzoate-degrading biogranules were tested using the intermediate acetate as substrate at concentration equivalent to 2500 mg-COD litre^{-1}. In series 3, all three types of biogranules were disintegrated first by a Warring blender and then by a ultrasonic homogenizer (Cole-parmer 4710 Series) prior to the test.

At the end of series 1, two additional tests were conducted. One was to investigate the adsorption of phenol by biogranules, if any, during the SMA tests, and the other was to see if the biogranules activity could be recovered after being submerged in phenol-laden feed solution for over 7 days in the serum vials. For the first test, phenol concentration in the supernatant was immediately measured after each SMA test and compared with the original concentration in the feed solution. For the second test, the supernatant after each SMA test was replaced with a fresh phenol-free feed solution, and the SMA of this batch was compared to the control.

A second GC (Hewlett Packard, model 5890 Series 2) was used to analyse the concentrations of phenol, benzoate, acetate, propionate and other fatty acids (C$_4$-C$_7$), using the detector, column and operating conditions as described in a previous study (Fang et al., 1997). Parameters such as VSS and COD were measured according to the Standard Methods (ALPHA, 1985).

Continuous-loading tests

After three series of shock-loading tests, continuous-loading tests were conducted in five small upflow reactors with 150 ml of working volume (Fang et al., 1997). Two small reactors were used to treat benzoate-containing wastewater, two others for acetate- and one for propionate-containing wastewaters. Loading rates and HRTs in this study were calculated according to the reactor working volume, excluding the volume of the gas–liquid–solid separator. Each reactor was packed with 3 g of anaerobic biogranules (as VSS) obtained from the biogranule-cultivating reactor treating the same kind of wastewater. The tests were conducted in three phases. In the preliminary phase, which lasted 5–22 days, only phenol-free solution was fed to each reactor. In the inhibition phase, the duration of which varied

from 33 to 210 days, phenol at a selected concentration was added to the feed solution. Finally, in the recovery phase, phenol was again removed from the feed solution. In all reactors, the wastewater flowrate was mostly kept at a constant 200 ml per day, corresponding to a 18-h HRT and a COD loading rate of 10 g per day. Table 2 summarizes the key operating parameters for the continuous loading tests.

All reactors were kept at 34°C. Volume of biogas produced was monitored daily, and its composition including methane, carbon dioxide and nitrogen content was analysed by GC weekly. Parameters such as pH, COD, total suspended solids (TSS) and VSS of both influent and effluent were measured at least once a week, following the Standard Methods (ALPHA, 1985). Concentrations of phenol, benzoate, acetate, propionate and other fatty acids (C$_4$-C$_7$) were also analysed at least weekly using GC.

RESULTS AND DISCUSSION

Shock loading studies

Phenolic toxicity and effect of biomass quantity. The average SMA for the control of acetate-degrading biogranules was 1.36 g-methane-COD (VSS)$^{-1}$ per day^{-1}, whereas those for propionate- and benzoate-degrading biogranules were 0.84 and 1.02 g-methane-COD (VSS)$^{-1}$ per day, respectively. There was no noticeable difference between the biogranules sampled from the two separate reactors treating wastewater of the same substrate. Figure 1 illustrates the reduction of SMA of the three biogranules relative to the controls using the same substrate as in the biogranule-cultivating reactors. For each type of biogranule, three different dosages of biogranules (50, 100 and 200 mg VSS) and substrates concentration (1250, 2500 and 5000 mg-COD litre^{-1}) were tested. Figure 1 illustrates that, in general, the higher the phenol dosage, the greater the SMA reduction and, thus, the higher the inhibition towards the overall methanogenic activity. However, varying the biomass quantity used for each test had little effect on SMA measurements.

The toxicity of phenol for each type of biogranules can be represented by $C_{1,50}$, the concentration at which the bioactivity of the biogranules was reduced to 50% of the control. Table 3 shows that the average value of $C_{1,50}$ for acetate-, propionate- and benzoate-degrading biogranules were 1750, 1000 and 1700 mg litre^{-1}, respectively. Judging from the $C_{1,50}$ values, the propionate-degrading biogranules were more vulnerable to the phenol toxicity than those degrading acetate or benzoate, as also illustrated in Fig. 1.

Recovery of phenol-inhibited biogranules. After SMA measurements, the phenol-rich supernatant in vials testing benzoate-degrading biogranules were replaced by a fresh phenol-free feed solution. The quantities and rates of methane production of these phenol-inhibited biogranules were then re-examined and compared with the control. Figure 1(c) illustrates that the methanogenic activity was totally inhibited for biogranules in contact with 2000 mg-phenol litre^{-1} feed solution. However, Fig. 2 illustrates that the rate and quantity of methane production of those biogranules which had been in contact with solution containing 2000 mg-phenol litre^{-1} was same as those of the control, indicating a full recovery of bioactivity. This shows that the toxic effect of phenol to biogranules was not permanent. But, biogranules which had in contact with 3000 mg-phenol litre^{-1}, solution required a lengthy period to regain their
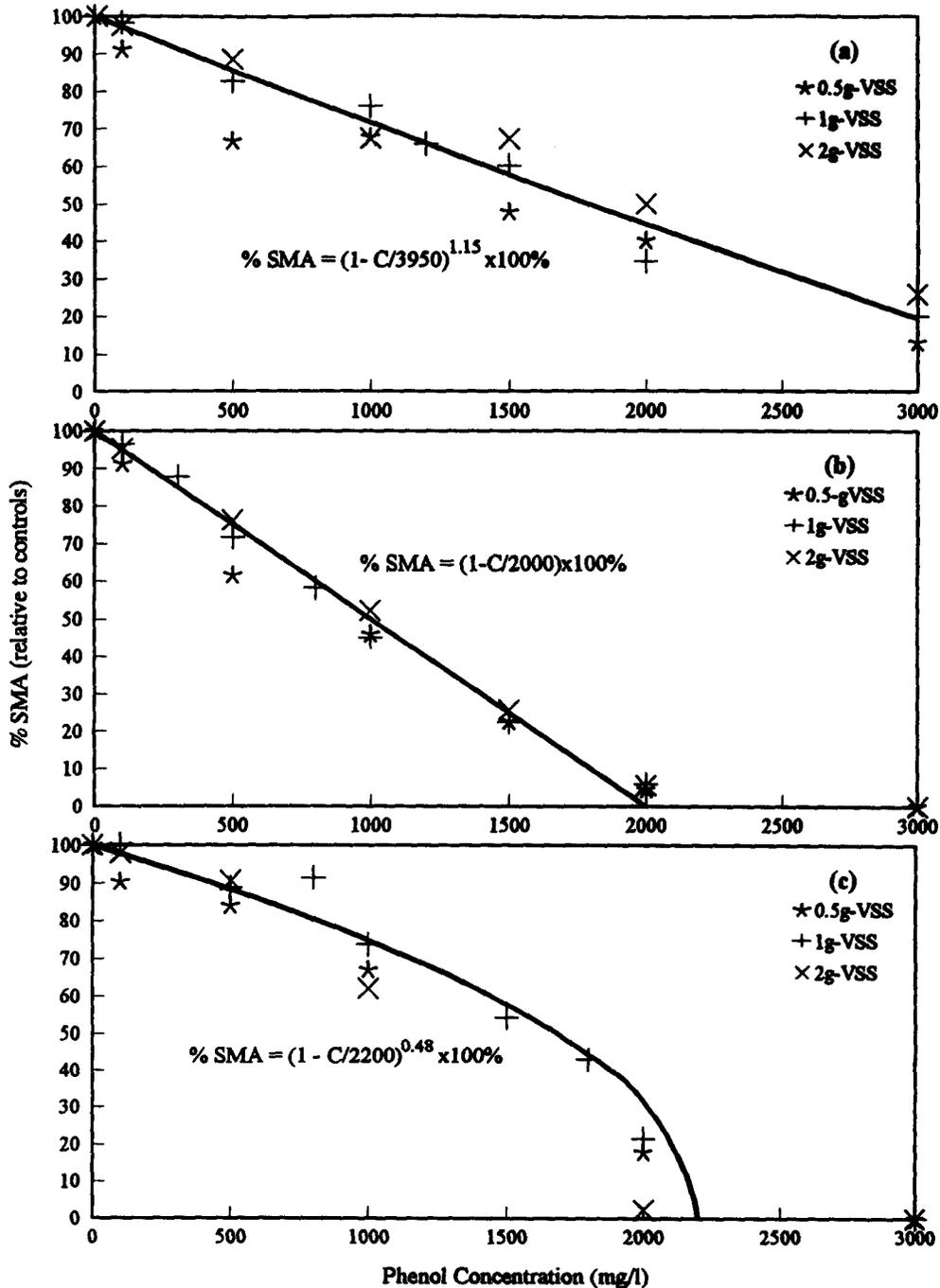

Fig. 1. Reduction of methanogenic activity of UASB biogranules at various phenol concentrations: (a) acetate-degrading biogranules, (b) propionate-degrading biogranules, (c) benzoate-degrading biogranules.

Table 3. Parameters of phenol toxicity for various types of biogranule and substrate

Biogranules from Reactors treating wastewater containing	Form of biogranules	Substrate tested	$C_{1,50}$ (mg l^{-1})	$C_{1,100}$ (mg l^{-1})	n	Source of data
Acetate	As-is	Acetate	1750	3950	1.15	Present study
Propionate	As-is	Propionate	1000	2000	1.00	Present study
Benzoate	As-is	Benzoate	1700	2200	0.48	Present study
Propionate	As-is	Acetate	1750	2950	0.75	Present study
Benzoate	As-is	Acetate	1650	2000	0.40	Present study
Acetate	Disintegrated	Acetate	1500	2500	0.75	Present study
Propionate	Disintegrated	Propionate	850	1600	0.90	Present study
Benzoate	Disintegrated	Benzoate	1000	1200	0.35	Present study
Distillery	As-is	Acetate	1100	NA	NA	Sierra-Alvarez *et al.* (1991)
Acetate (sludge)	As-is	Acetate	< 1500	NA	NA	Hanaki *et al.* (1994)
Acetate (immobilized sludge)	As-is	Acetate	> 1500	NA	NA	Hanaki *et al.* (1994)
Starch	As-is	Starch	1370	NA	NA	Fang *et al.* (1994b)

activity. Methane production did not begin until 100 h after switching to phenol-free feed solution, and only about 10% of methane was produced after 230 h. Figure 2 illustrates only the results for those tests using 100 mg-VSS of biogranules; similar results were also observed for those used 50 and 200 mg-VSS of biogranules.

Acetate as substrate. Unlike acetate-degrading biogranules, which were predominantly composed of acetotrophic methanogens, propionate- and benzoate-degrading biogranules had more complex bacterial population dynamics. The latter two biogranules are composed of acetogens, plus both hydrogenotrophic and acetotrophic methanogens. The toxicity of phenol towards the acetotrophic methanogens in these two biogranules was evaluated by using acetate as the substrate in SMA tests. Figure 3(a) illustrates that propionate-degrading biogranules had considerably higher tolerance towards phenol using acetate as substrate than using propionate as substrate, as illustrated in Fig. 1(b). Figure 3(b), on the other hand, illustrates that benzoate-degrading biogranules had similar tolerance towards phenol using benzoate and acetate as the substrate. The $C_{1,50}$ using acetate as substrate were 1750 mg-phenol litre^{-1}

for the propionate-degrading biogranules and 1650 mg-phenol litre^{-1} for the benzoate-degrading biogranules. Both are comparable to the corresponding $C_{1,50}$ of 1750 mg-phenol litre^{-1} for the acetate-degrading biogranules. This indicates that all acetotrophic methanogens in the three biogranules had the same degrees of tolerance towards phenol.

Both propionate- and benzoate-degrading biogranules are composed of hydrogenotrophic and acetotrophic methanogens. The main difference of their population dynamics is in their acetogens. Comparison of Figs 1 and 3 shows that propionate-degrading biogranules using propionate as substrate had the least tolerance towards phenol toxicity. This implies that the propionate-utilizing acetogens were probably more vulnerable to phenol than the benzoate-utilizing acetogens and the two groups of methanogens.

Disintegrated biogranules. Figure 4 illustrates the SMA for the three biogranules after having been disintegrated by a Waring blender and a ultrasonic homogenizer. It illustrates that the SMA of all the disintegrated biogranules decreased with the phenol concentration, following the same patterns as the as-is biogranules illustrated in Fig. 1. The $C_{1,50}$ for the disintegrated acetate- and propionate-biogranules
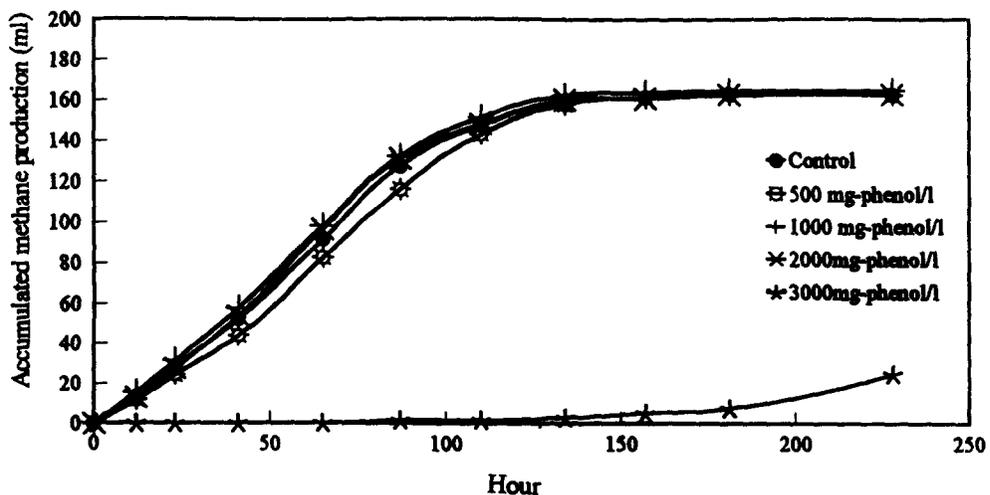

Fig. 2. Methane production of bezoate-degrading biogranules after having contact with phenol.

$$\% \text{SMA} = (1 - C/2950)^{0.75} \times 100\%$$

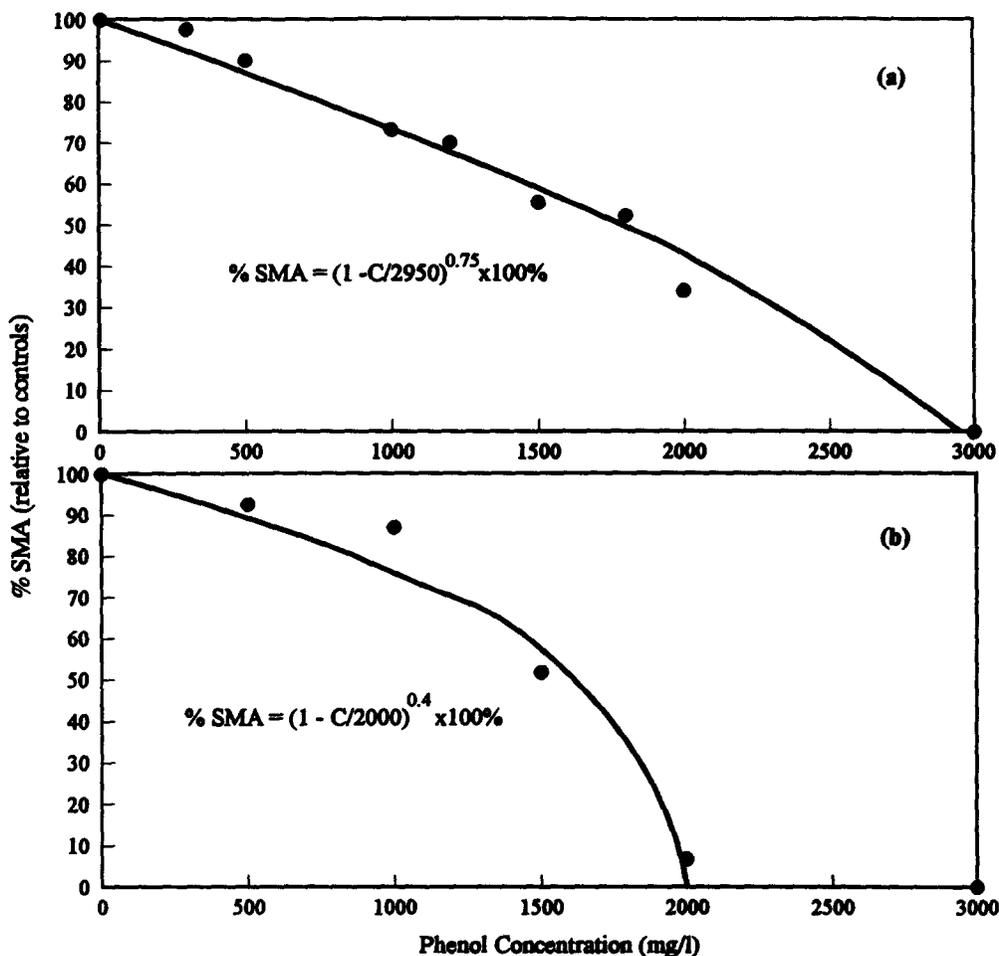$$\% \text{SMA} = (1 - C/2000)^{0.4} \times 100\%$$
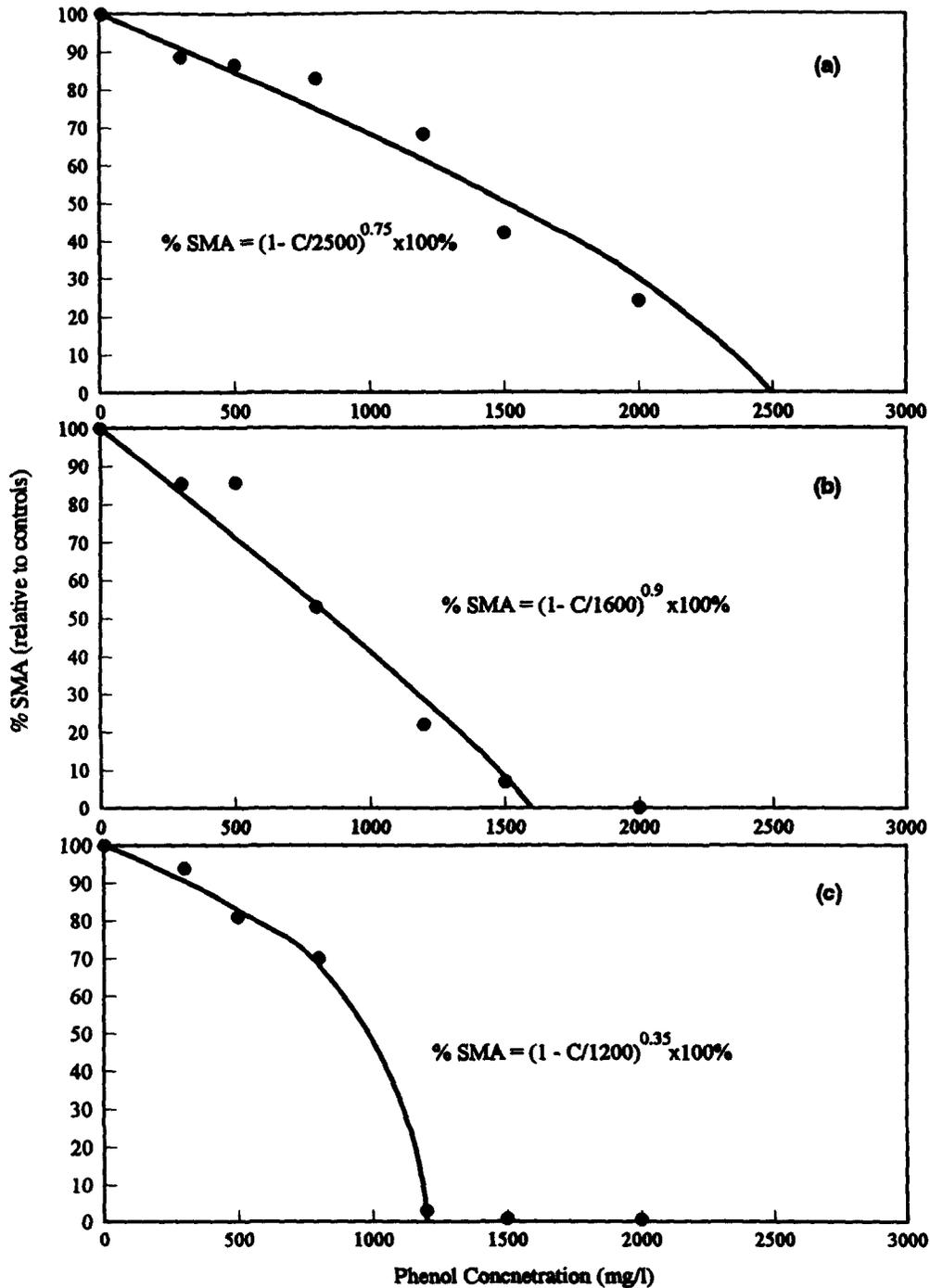
Fig. 3. Reduction of methanogenic activity of UASB biogranules at various phenol concentrations: (a) propionate-degrading biogranules, (b) benzoate-degrading biogranules.

were 1500 and 850 mg-phenol litre^{-1} only about 15% lower than the corresponding values for the as-is biogranules, as shown in Table 3. This indicates that the granular formation for these two types of biomass did not significantly affect their degrees of tolerance towards phenol toxicity. This may be due to their uniform microstructure (Fang et al., 1995b). On the other hand, the $C_{I,50}$ for the disintegrated benzoate-degrading biogranules was 1000 mg-phenol litre^{-1}, considerably lower than the 1700 mg-phenol litre^{-1} for the as-is biogranules. The as-is biogranules had superior tolerance to phenol, probably due to their layered microstructure (Li et al., 1995), in which a dense skin layer shielded the bacteria inside from being exposed to phenol. Such a protection was destroyed when biogranules were disintegrated.

Table 3 shows the $C_{I,50}$ of phenol for other anaerobic biogranules or sludges for comparison, including those treating distillery wastewater (Sierra-Alvarez and Lettinga, 1991), colloidal starch (Fang et al., 1994b) and acetate (Hanaki et al., 1994).

Inhibition model. In many cases, the concentration of residual phenol in the supernatant was measured by GC after the SMA tests. Results of 12 measurements show that, for all three types of biogranules, on average 99.5% of phenol in the feed solution remained in the supernatant after 7 days of SMA test. This indicates that only a very small quantity of phenol was accumulated, probably by surface adsortption if any, in the biogranules. This is probably due to the low octane-water partition coefficient of molecular phenol (Tsezos and Bell, 1989; Kennedy and Mohn, 1992), which is more predominant than ionized phenol at near-neutral pH. The interaction between phenol and microorganisms in the biogranules is very complex. Phenolic toxicity is believed to be non-reactive (Blum and Speece 1991) and, thus, is likely to result from the phenol adsorbed by the biogranules. As a result, the toxicity is dependent on the phenol concentration in the solution, as observed in the present study.

The relationship between toxicity and phenol concentration under shock-loading conditions can be

expressed by the following non-linear equation (Han and Levenspiel, 1988), as illustrated in Figs 1, 3 and 4:

$$\%SMA = (1 - C/C_{1,100})^n \times 100\%$$

where %SMA is the SMA of biogranules relative to those in phenol-free solution, C is the concentration of phenol in solution, $C_{1,100}$ is the concen-

tration of phenol causing 100% inhibition of bioactivity and n is the empirical coefficient.

The $C_{1,100}$ and the n values for all the biogranules and substrates tested in the present study are also summarized in Table 3. The inhibition effect of a toxicant can be classified simply as competitive or not competitive. Since the %SMA decreased with the

Fig. 4. Reduction of methanogenic activity of disintegrated UASB biogranules at various phenol concentrations: (a) acetate-degrading biogranules, (b) propionate-degrading biogranules, (c) benzoate-degrading biogranules.
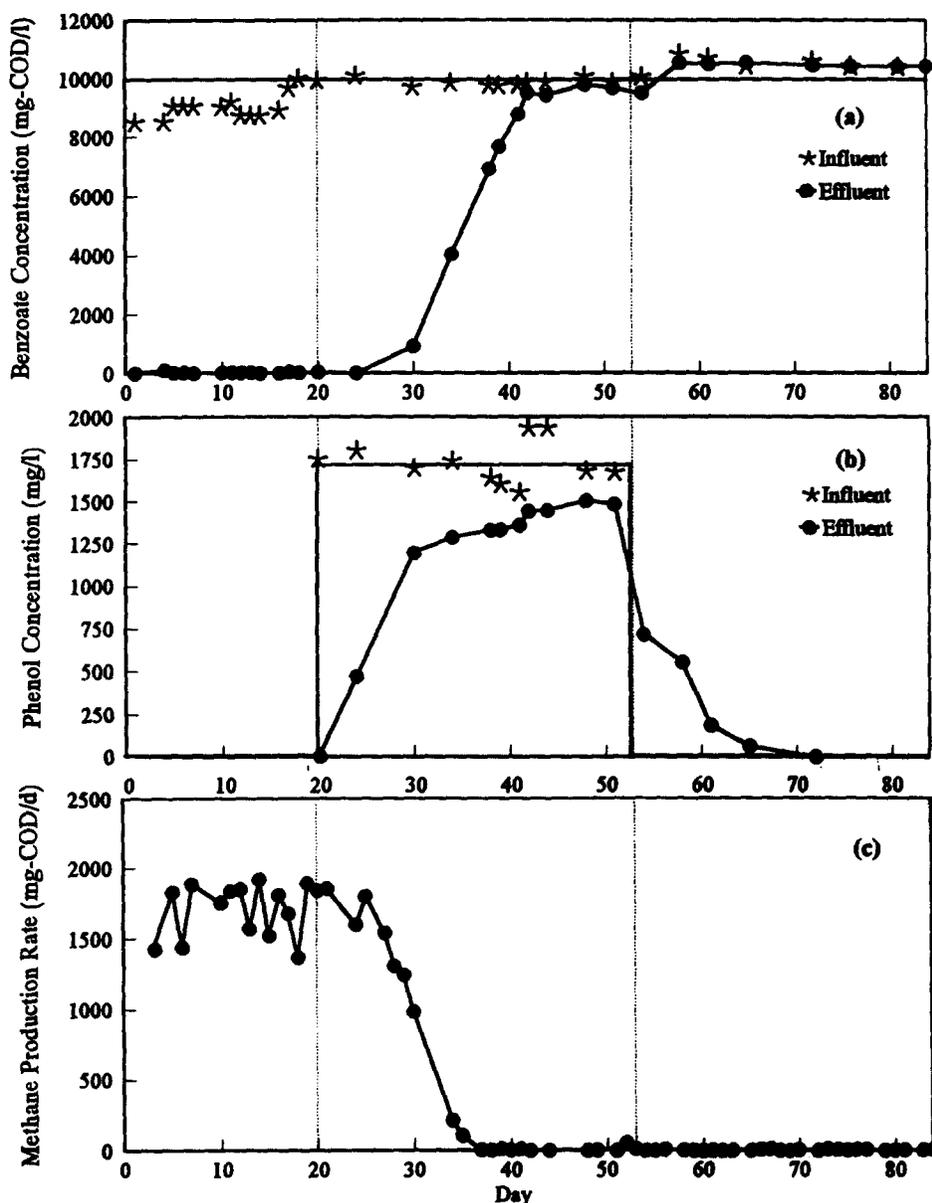
Fig. 5. Operational parameters of reactor 1 treating benzoate-containing wastewater: (a) benzoate concentrations, (b) phenol concentrations, (c) methane production rate.

increase of C in all tests, the toxicity of phenol is classified as not competitive according to the analysis of Kim et al. (1994).

Continuous-loading studies

Five small upflow reactors were used for the study of toxic effect of phenol under continuous loading conditions for up to 285 days. Reactors 1 and 2 were for the treatment of benzoate-containing wastewater, reactor 3 for propionate- and reactors 4 and 5 for acetate-containing wastewaters. Results of reactor 1 are illustrated in Fig. 5, including (a) benzoate concentration, (b) phenol concentration, plus (c) methane production rate. Figures 6–9

illustrate the corresponding plots for reactors 2–5, respectively.

Benzoate as substrate. Figure 5(a) illustrates that the wastewater treated in reactor 1 contained an average of 9900 mg-COD litre^{-1} of benzoate throughout the experiment. Prior to day 20, the reactor performed steadily treating phenol-free wastewater. The effluent contained less than 200 mg-COD litre^{-1} of residual benzoate, equivalent to a removal efficiency of over 98%, and less than 20 mg litre^{-1} of other volatile fatty acids (VFAs). Methane production was equivalent to 1800 mg-COD per day, corresponding to over 90% of the total COD conversion. The pH of the effluent average 7.4.

From days 20 to 53, phenol was added to the wastewater at about 1700 mg litre^{-1}, which was chosen based on the $C_{1,50}$ of phenol for the benzoate-degrading biogranules determined from the shock-loading tests. By day 25, benzoate in effluent started to increase, and within a few days the reactor completely lost its benzoate-removing capability (Fig. 5a), as also reflected by the ceasing of methane production (Fig. 5c). Phenol addition was then stopped on day 54, and the experiment continued until day 85. During this last period, the reactor could not recover its benzoate-degrading capability, even though the phenol had been gradually elutriated from the reactor. Throughout the experiment, the effluent had less than 100 mg litre^{-1} of acetate and less than

40 mg litre^{-1} of other VFAs. The effluent pH was slightly increased, after the addition of phenol, to pH 8.3 but levelled off to pH 8.0 after day 54. The addition of phenol did not cause sludge washout (the effluent VSS was under 150 mg litre^{-1}) nor cause any disintegration of biogranules.

Reactor 2 was operated under similar conditions as reactor 1, except started from a lower phenol concentration. Figure 6(a) illustrates that the wastewater treated in reactor 2 contained 9900–10100 mg litre^{-1} of benzoate from day 1 to 76. Phenol was dosed to the wastewater, averaging 1050 mg litre^{-1} from days 10 to 40, during which the reactor continued to remove over 98% of benzoate (Fig. 6a), maintained a steady effluent pH of 7.4–7.6
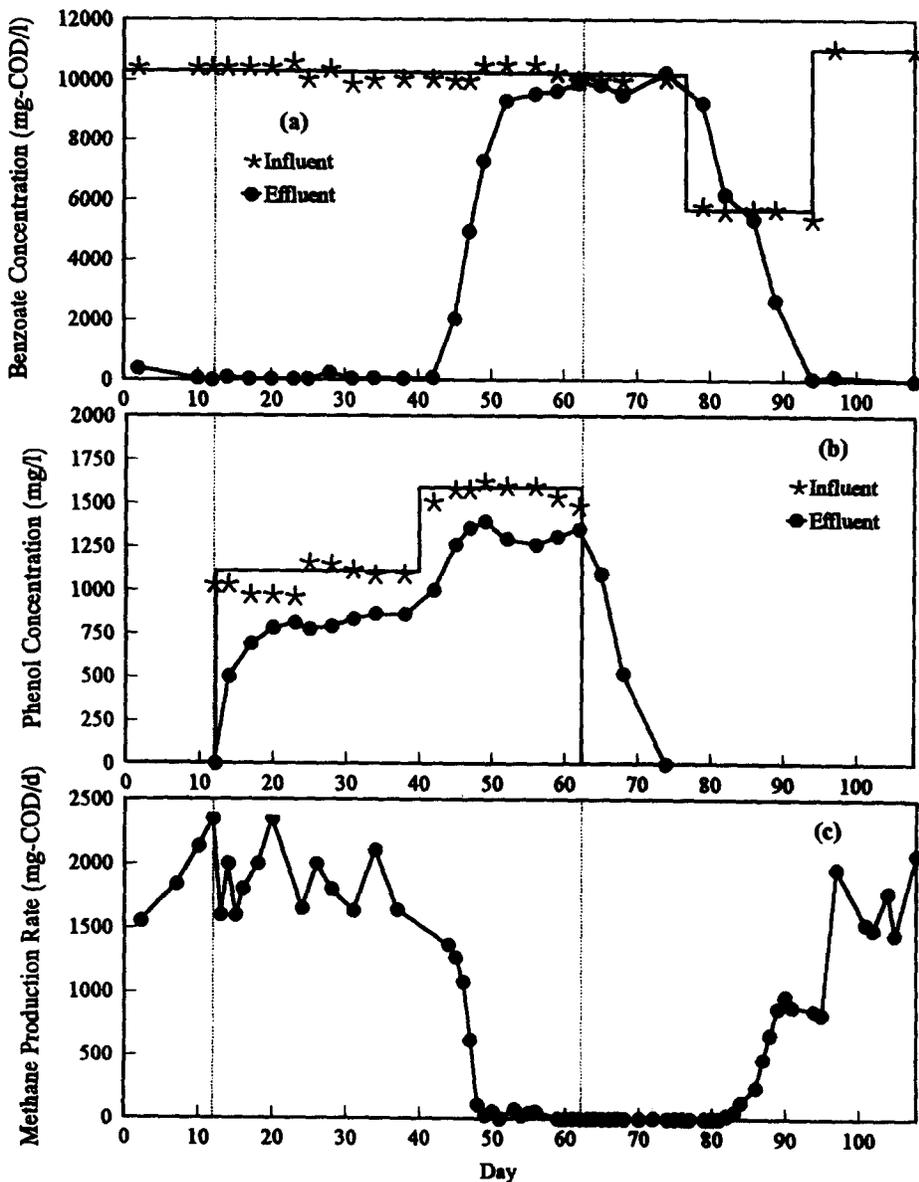

Fig. 6. Operational parameters of reactor 2 treating benzoate-containing wastewater: (a) benzoate concentrations, (b) phenol concentrations, (c) methane production rate.
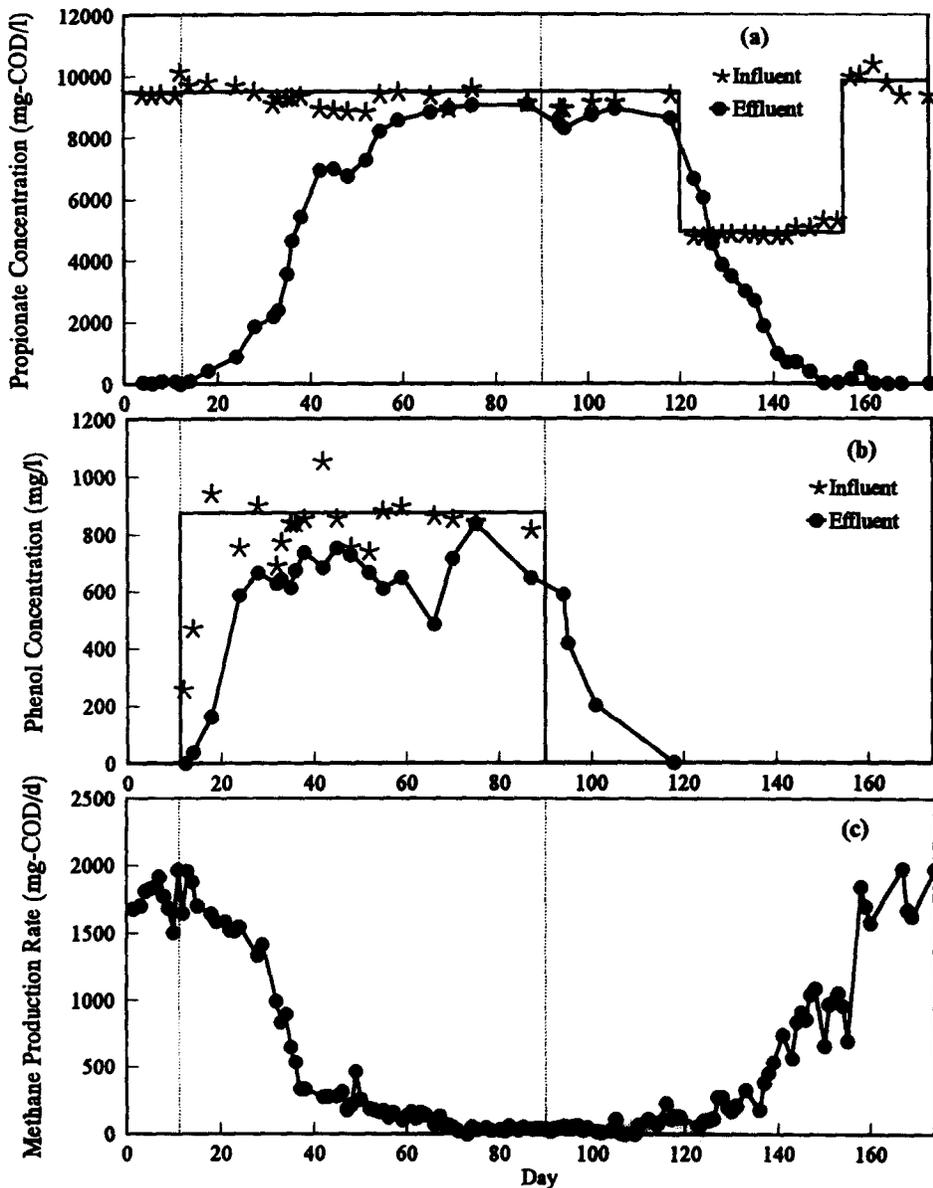
Fig. 7. Operational parameters of reactor 3 treating propionate-containing wastewater: (a) benzoate concentrations, (b) phenol concentrations, (c) methane production rate.

and converted over 1800 mg COD per day into methane (Fig. 6c). The effluent phenol concentration increased gradually after day 10 and reached the steady level of 850 mg litre^{-1} by day 20. About 20% of phenol in the wastewater was removed by the reactor, even though the biogranule had not been acclimated to phenol prior to this test. The concentration of phenol in wastewater was increased further from 1050 to 1600 mg litre^{-1} during days 41–62 (Fig. 6b). Within 2 days of concentration increase, the residual benzoate started to increase, readily reaching 9500 mg COD litre^{-1} within 12 days. The methane production was lowered correspondingly (Fig. 6c).

On day 63, the dosing of phenol was stopped. However, the reactor could not regain its benzoate-degrading capability in the next 14 days. A further effort was made on day 77 by lowering the benzoate concentration to 5500 mg COD litre^{-1}. Immediately, the residual benzoate concentration was lowered, reaching the level of less than 200 mg COD litre^{-1} by day 95. The benzoate-degrading capability was fully recovered in 18 days, and it remained unchanged even after the benzoate concentration was later raised to 11000 mg litre^{-1} on day 95. Throughout this test, there was no VFA accumulation in the effluent.

Propionate as substrate. Wastewater treated in reactor 3 contained 9500 mg litre^{-1} of propionate as

substrate. From days 12 to 92, phenol was dosed to the wastewater at 850 mg litre^{-1}, slightly less than the $C_{1,50}$ for the propionate-degrading biogranules observed in the shock-loading tests. Prior to day 12, the reactor removed over 98% of propionate. After the phenol addition, the effluent propionate was increased gradually from less than 230 to 8600 mg-COD litre^{-1} over 2 months. During this period, methane production was decreased to below 50mg-COD per day and the effluent pH was dropped from the normal 7.7 to 5.9. The effluent contained an average of 700 mg litre^{-1} of phenol, plus less than 100 mg litre^{-1} of acetate, less than 50 mg litre^{-1} of other VFAs (C$_3$–C$_7$) and about 100–400 mg litre^{-1} of benzoate, a key intermediate in the pathway of phenol degradation.

Phenol dosage to wastewater was ceased on day 92, resulting in a gradual increase of effluent pH, but the removal of propionate was still less than 5% in the next 30 days. The biogranules began to regain the activity only after the propionate concentration in the wastewater was also lowered, starting on day 122, to 3300 mg litre^{-1}. Significant recovery was soon achieved; the reactor was able to remove over 95% of propionate by day 155 and over 98% of propionate when its concentration in the wastewater was raised to 6500 mg litre^{-1} on day 156 (Fig. 7).
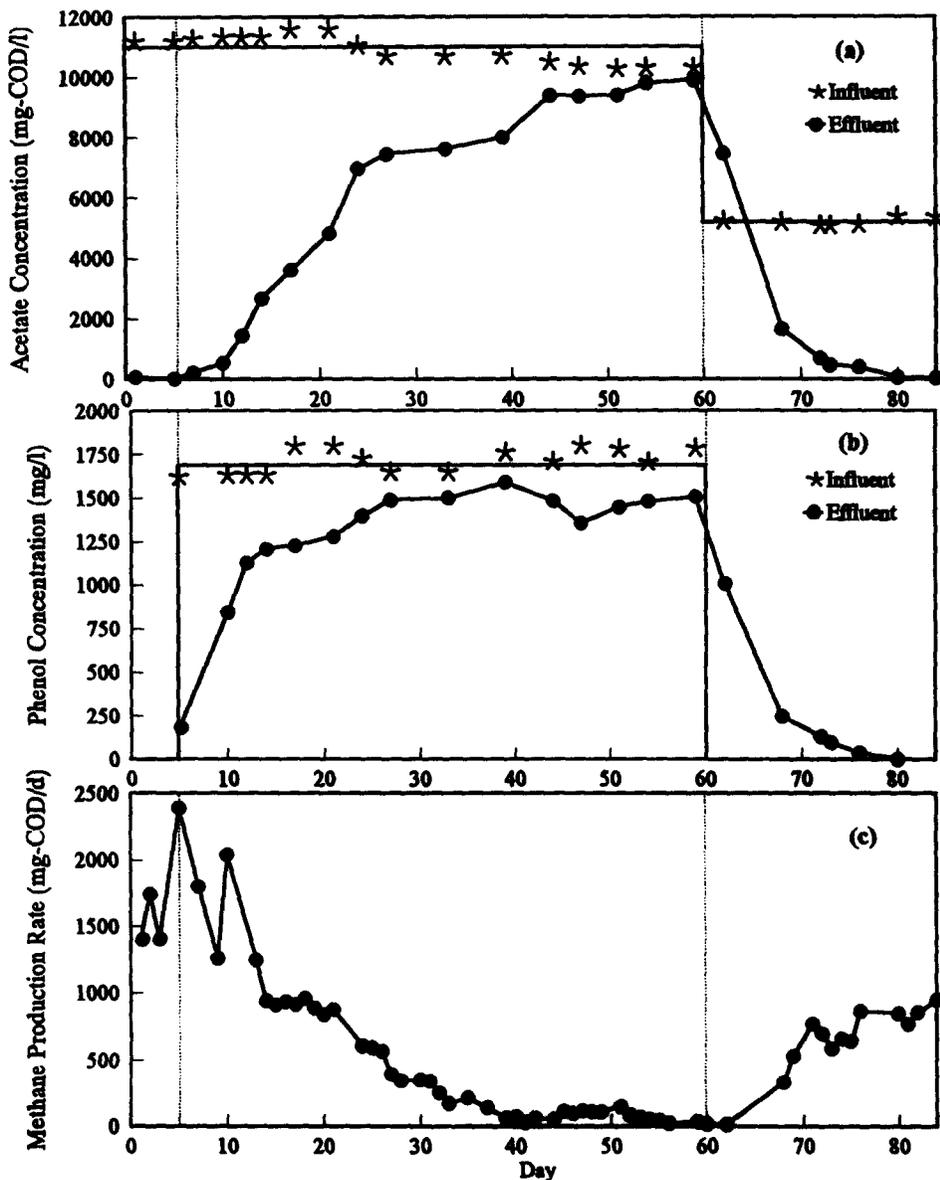

Fig. 8. Operational parameters of reactor 4 treating acetate-containing wastewater: (a) benzoate concentrations, (b) phenol concentrations, (c) methane production rate.
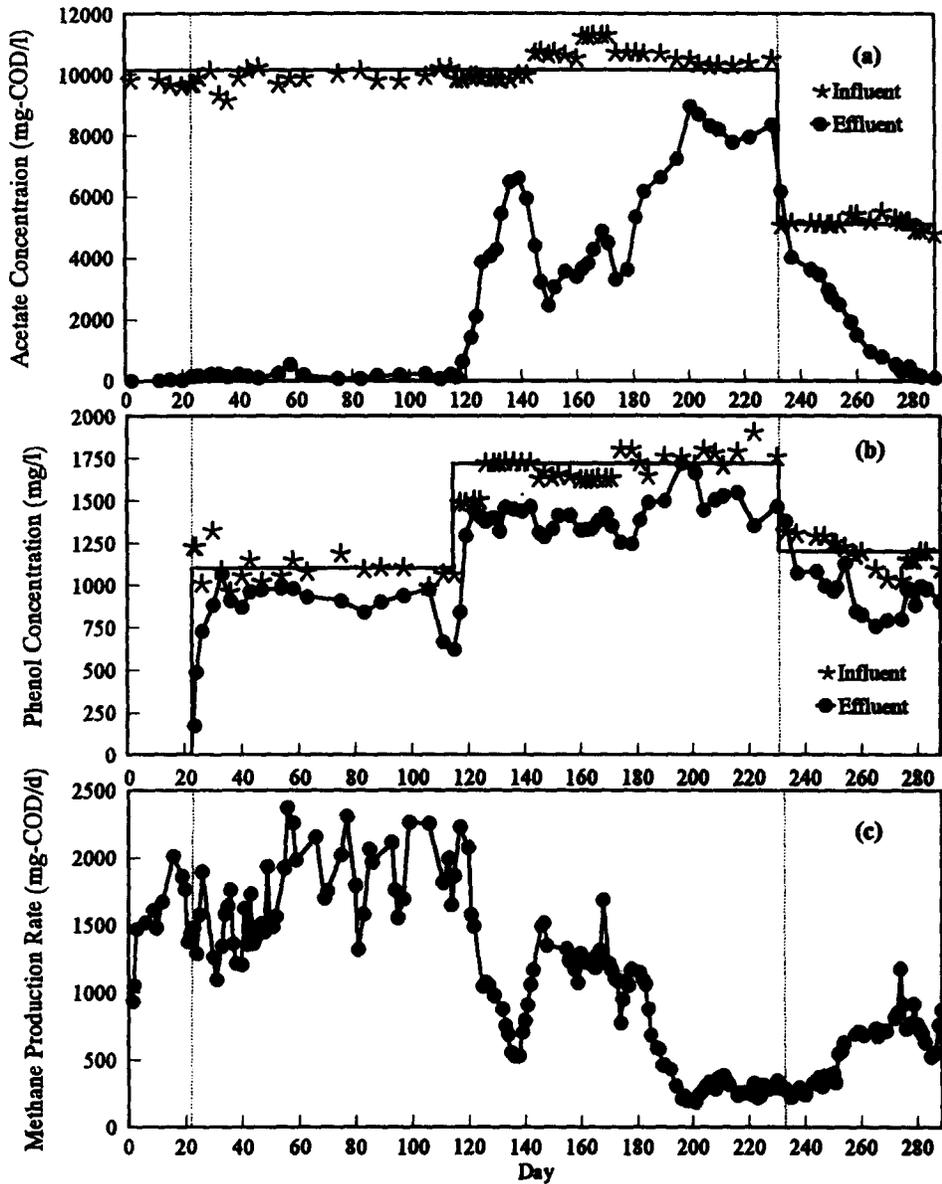
Fig. 9. Operational parameters of reactor 5 treating acetate-containing wastewater: (a) benzoate
concentrations, (b) phenol concentrations, (c) methane production rate.

Acetate as substrate. Wastewater treated in reactors 4 and 5 contained 10000–11000 mg COD litre⁻¹ of acetate. During days 6–60, phenol was added to the wastewater for reactor 4 at 1700 mg litre⁻¹, slightly lower than the $C_{I,50}$ of 1500 mg-phenol litre⁻¹ observed in the shock-loading tests. The reactor's capability to degrade acetate deteriorated immediately from the initial over 95% efficiency to less than 5% (Fig. 8a), with corresponding decreases of methane production (Fig. 8c). After day 61, the wastewater was phenol-free and the acetate concentration was lowered to 5000 mg-COD litre⁻¹ the biogranules gradually regained their

activity and removed 95% of acetate when the test ended on day 83.

For reactor 5, phenol was first dosed to the wastewater at 1100 mg litre⁻¹ during days 22–115. At this concentration, phenol did not show any inhibition effect to the acetate-degrading biogranules, and the reactor continued to remove over 95% acetate with steady effluent pH and methane production. The phenol concentration was then increased to 1700 mg litre⁻¹ during days 116–230. Immediately after day 116, the reactor's capability to remove acetate deteriorated, eventually lowering to about 20% by day 230. Starting day 231, the

concentrations of acetate and phenol were reduced, respectively, to 5000 and 1100 mg litre^{-1}. The reactor then gradually regained its capability and removed over 95% of acetate by day 285.

Phenol toxicity in continuous-loading conditions. Results in Figs 1–4 illustrated that under shock-loading conditions the toxicity of phenol towards individual types of biogranules increased with it concentration. However, results illustrated in Figs 5–9 indicate that, under continuous-loading conditions, phenol toxicity was not progressively increased with concentration. Instead, phenol had a threshold toxicity level for each individual biogranules. The threshold level for each type of biogranules was lower than the corresponding $C_{I,50}$ observed in the shock-loading tests. The phenol threshold toxicity levels were between 1050 and 1600 mg litre^{-1} for benzoate-, less than 850 mg litre^{-1} for propionate- and between 1100 and 1700 mg litre^{-1} for acetate-degrading biogranules. Below these threshold levels, phenol did not inhibit biogranule's activities; but above these levels, the inhibition was nearly 100%.

Results also indicate that the phenol toxicity was not cumulative. As long as the phenol concentration was below the threshold value, the methanogenic activity of the biogranules did not decrease over an extended period. Furthermore, the toxic effect of phenol to the biogranules was not permanent. Once the phenol concentration was lowered to below the threshold level, reactors 2–5 were able to gradually regain their substrate-degrading capabilities; even biogranules had been in contact with concentrated phenol for up to 210 days. Biogranules in reactor 5 fully recovered their acetate-degrading capability after being suppressed by phenol at 1100 mg litre^{-1} for 94 days and 1700 mg litre^{-1} for another 115 days. Lowering the substrate concentration seemed to expedite the recovering process. Biogranules in reactor 1 did not regain their activity, because the test was ended prematurely. Same type of biogranules in reactor 2 did not show sign of recovery until treating phenol-free wastewater for over 25 days.

CONCLUSIONS

1. Under batch shock-loading conditions, methanogenic activity of the biogranules decreased progressively with the increase of phenol concentration.

2. The $C_{I,50}$ values were 1750, 1000 and 1700 mg-phenol litre^{-1}, respectively, for acetate-, propionate- and benzoate-degrading biogranules. Propionate-utilizing acetogens appeared to be more vulnerable than other bacteria. Biosorption of phenol was unnoticeable.

3. Under continuous loading conditions, however, phenol toxicity was not progressive. Rather, it had a threshold toxicity level for each type of biogranules.

4. The phenol threshold levels were 1050–1600 mg litre^{-1} for benzoate-, less than 850 mg litre^{-1}

for propionate- and 1100–1700 mg litre^{-1} for acetate-degrading biogranules. Below them, phenol was not inhibitive to the activity of biogranules; but above these levels, the inhibition was nearly 100%. Phenol toxicity was neither cumulative nor permanent.

5. Once the phenol concentration was lowered to levels below the threshold, all reactors were able to gradually regain 100% of their bioactivity. Lowering substrate concentration appeared to expedite the recovering process.

6. Results of this study showed that anaerobic biogranules exhibited high degrees of tolerance towards phenol, not only under shock-loading conditions but also in upflow reactors treating wastewater continuously. A better understanding of the tolerance of biogranules to toxic chemicals should enhance the confidence in broadening the application of anaerobic technology to industrial wastewater treatment.

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