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DEGRADATION OF PHENOL IN WASTEWATER IN AN UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR

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Abstract—Phenol in wastewater could be effectively degraded in an upflow anaerobic sludge blanket (UASB) reactor. With a 1:1 effluent recycle ratio, over 97% of phenol was removed at 37°C and pH 6.9–7.5 with 12 h of hydraulic retention time for phenol concentration up to 1260 $mg \cdot l^{-1}$, corresponding to 3000 $mg \cdot l^{-1}$ of chemical oxygen demand (COD) and a loading rate of 6 $g\text{-}COD \cdot l^{-1} \cdot day^{-1}$. The seed sludge took about 7 wk to develop the phenol-degrading capability which was sensitive to shocks. The bioactivity deteriorated readily when the granules were exposed to sudden changes of temperature and loading. Although the damage was not permanent, the recovery of bioactivity was gradual and lengthy. At 6 $g\text{-}COD \cdot l^{-1} \cdot day^{-1}$, each gram of granules was able to convert 0.49 g of COD into methane daily. On the average, about 94.7% of the total COD removed was converted to methane, while the rest was converted to biomass with a net yield of 0.038 $g\text{-}VSS \cdot (g\text{-}COD\text{-}removed)^{-1}$. Electron micrographs show that the granules were composed of, among others, *Syntrophus buswellii*-, *Methanothrix*-, *Methanospirillum*- and *Methanobrevibacter*-like bacteria. Copyright © 1996 Elsevier Science Ltd

Key words—anaerobic, bioactivity, granule, loading, microstructure, phenol, shock, temperature, UASB, yield

INTRODUCTION

Phenol is the raw material 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 (Kirk-Othmer, 1978). Phenol is thus an organic pollutant often found in the wastewater of resin industries. In addition, phenol derivatives are also found in wastewaters from industries, such as synthetic chemicals, pesticides, coal conversion, pulp-and-paper etc. (Young and Rivera, 1985; Wang *et al.*, 1986).

Phenol is also a biocide and disinfectant (Kirk-Othmer, 1978). Therefore, it is often perceived as inhibitory to the bioactivity of microorganisms. Studies have been conducted recently to investigate the inhibition effect of phenol on the conversion of acetate to methane (Patel *et al.*, 1991; Sierra-Alvarez and Lettinga, 1991; Wang *et al.*, 1991). Young and Rivera (1985) found that phenol itself could be stoichiometrically converted to methane and carbon dioxide by anaerobic sludge from a municipal digester. Kobayashi *et al.* (1989) also found that phenol was biodegradable under anaerobic condition, but requiring dosing peptone as a co-substrate for the anaerobes. On the other hand, Wang *et al.* (1986) demonstrated that phenol could be removed in an expanded-bed reactor, but requiring activated carbon which served as adsorbent for phenol as well as carrier for the anaerobes. However, so far, removal of phenol in wastewater by conventional anaerobic processes has not been reported.

Anaerobic treatment of industrial wastewater has become a viable technology in recent years due to the rapid development of high-rate reactors, such as anaerobic filter (Young and McCarty, 1969) and upflow anaerobic sludge blanket (UASB) reactor. Since its introduction about 15 yr ago (Lettinga *et al.*, 1980), UASB has become popular in Western Europe and, more recently, in East Asia. It has been most commonly used for wastewaters from food/beverage/agricultural industries, in which the pollutants are mostly carbohydrates. However, recent studies have demonstrated that the UASB technology is applicable to treating wastewaters containing concentrated proteins (Fang *et al.*, 1994a–c), corn starch (Fang and Kwong, 1994) and aromatic chemicals, such as benzoate (Li *et al.*, 1996). It is thus warranted to investigate the feasibility of removing phenol in wastewater using the UASB process. Furthermore, it is also of engineering and scientific interest to investigate the effect of operational parameters on the reactor performance and the characteristics of the phenol-degrading granules.

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MATERIALS AND METHODS

Figure 1 illustrates the 2.8 l UASB reactor (Chui *et al.*, 1994) used in this study, which had an internal diameter of 84 mm and a height of 500 mm. Five evenly distributed sampling ports were installed over the height of the reactor. Total biomass in the reactor was estimated based on the profile of the volatile suspended solids (VSS) of the samples taken from these ports. On top of each reactor was a 2.0 l gas–liquid–solid separator with an internal diameter of 114 mm and a height of 250 mm. Volumetric loading rates were estimated, based on the reactor volume alone, excluding volume of the gas–liquid–solid separator. The reactor was water-jacketed and operated at a constant temperature of 37°C.

Prior to this study, flocculent sludge from an anaerobic sludge digester of the Shatin Wastewater Treatment Works, Hong Kong, was partially granulated in a 6.5 l UASB reactor for 2 months, using sucrose as the organic substrate. About 1.4 l of this partially granulated sludge and another 1.4 l of flocculent digester sludge were used to seed the reactor. Synthetic wastewater comprising phenol as the sole substrate, plus balanced nutrients and alkalinity, was used to feed the reactor using a peristaltic pump. The initial phenol concentration in the wastewater was 420 $mg \cdot l^{-1}$, corresponding to a chemical oxygen demand (COD) of 1000 $mg \cdot l^{-1}$. With 8 h of hydraulic retention time (HRT), the initial COD loading rate was 3 $g \cdot l^{-1} \cdot d^{-1}$. For each gram of COD, the wastewater was supplemented with 1 g of $NaHCO_3$, 260 mg of NH_4Cl, 42.5–64.4 mg of $MgSO_4 \cdot 7H_2O$, 24.8–37.5 mg of K_2HPO_4, 9.9–15.0 mg of KH_2PO_4, 13.0–17.2 mg of $CaCl_2$, 22.4–34.0 mg of sodium citrate, 5.3–8.0 mg of $NiSO_4 \cdot 7H_2O$, 4.1–6.2 mg of $FeCl_3 \cdot 6H_2O$, 1.1 mg of $MnCl_2 \cdot 4H_2O$, 0.6 mg of $ZnCl_2$, 0.6 mg of $CoCl_2 \cdot 2H_2O$, 0.4 mg of $(NH_4)_2MoO_4 \cdot 4H_2O$, 0.3 mg of $CuCl_2 \cdot 2H_2O$, and 0.2 mg of $NaBO_2 \cdot 10H_2O$. Details of the reactor design and analytical procedures, such as the biogas production and its methane content, can be found elsewhere (Fang and Chui, 1993; Chui *et al.*, 1994).

Compositions of volatile fatty acids (VFA; from acetic acid to heptoic acid), benzoate and phenol in the effluent were measured by a gas chromatograph (GC; Hewlett Packard Model 5890 Series II). The GC was equipped with a 10 m × 0.53 mm HP-FFAP fused-silica capillary column and a flame ionization detector (FID), using helium as the carrier gas. Injector and detector temperatures were 200 and 250°C, respectively. The fluid sample was filtered through a 0.45 μm membrane filter and acidified to pH 3 with concentrated phosphoric acid prior to injecting into the column using the fast injection technique. The initial temperature of the column was 70°C for 4 min followed with a first ramp of 10°C $\cdot$ min^{-1} to the temperature of 140°C for 2 min and a second ramp of 10°C $\cdot$ min^{-1} and a final temperature of 170°C for 2 min. Volatile fatty acid standards (Supelco, Inc., Bellefonte, PA) and reagent grade sodium benzoate and phenol (Merck) were used for the calibration of the FID.

Granule samples were taken on day 373 at 6 $g \cdot l^{-1} \cdot day^{-1}$ of loading rate for microscopic examination and analysis of specific methanogenic activity (SMA). The microstructure of the UASB granules was examined using scanning electron microscope (SEM, Cambridge Stereoscan 360) and transmission electron microscope (TEM, JEOL 100SX). The sample preparation procedures were as reported previously (Fang and Chui, 1993). The SMA measures the methanogenic activity of the granules in serum vials for a specific substrate at the concentration level where the availability of substrate is not a limiting factor. The SMA of the phenol-degrading granules were measured in duplicates in serum vials based on the method (Dolfin and Mulder, 1985; Hwang and Cheng, 1991) modified from the one proposed originally by Owen *et al.* (1979). Because the granules were composed of various species of bacteria, the SMA measurement is strictly dependent on the substrate chosen for the test. Six substrates including formate, acetate, propionate, butyrate, benzoate and phenol were used individually as the substrate for SMA measurements in this study.

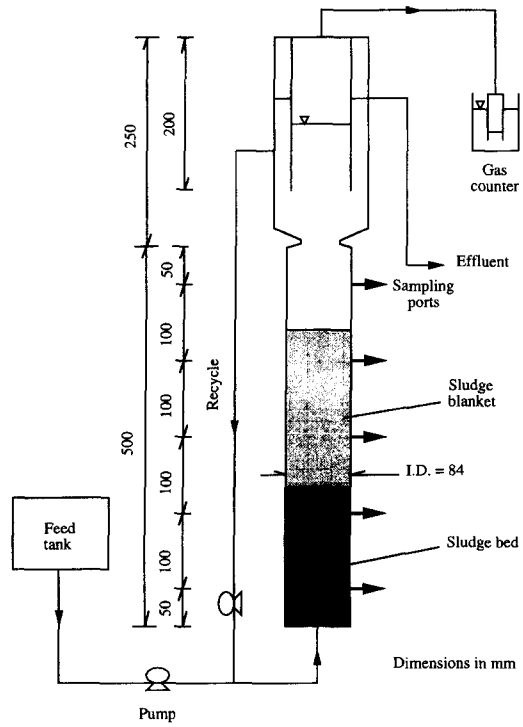

Fig. 1. UASB reactor setup.

RESULTS AND DISCUSSION

Table 1 summarizes the operational conditions throughout this 412 day study, including the COD level in the wastewater, HRT, COD loading and the effluent recycle. Fang and Chui (1993) demonstrated that the COD removal efficiency of a UASB reactor was mainly dependent on the COD loading rate, which could be adjusted by varying HRT and/or the COD level in the wastewater. The HRT in this study was 8 h initially, but was adjusted to 12 h, starting on day 62, and since then the loading rate was adjusted by the phenol concentration in the wastewater alone.

Table 1. HRT, wastewater COD and COD loading rates

Day	HRT (hr)	Wastewater COD ($mg \cdot l^{-1}$)	COD loading rate ($g\text{-}COD \cdot l^{-1} \cdot day^{-1}$)
1–45	8	1000	3
46–61	8	2000	6
62–80	12	2000	4
81–84	12	2500	5
85–205	12	2000	4
206–222	12	2600	5.3
223–341	12	3500	7
242–267	12	4500	9
268–307	12	2000	4
308–325	12	2600	5.3
326–412	12	3000	6

Starting day 125, part of the effluent from the reactor was recycled to dilute the phenol concentration in the incoming wastewater in order to lower its toxicity to the biomass. The flowrate of the recycle stream was maintained at the same level as that of the incoming wastewater. Figure 2 illustrates: (a) removal efficiency of soluble COD; (b) COD levels of the filtered effluent; (c) biogas production rate; and (d) the corresponding COD loading rates throughout this study.

The COD loading rate was slowly increased stepwise when the reactor removed over 80% of soluble COD from the wastewater. However, the smooth operation was interrupted three times. The interruptions were due to the deterioration of the reactor performance, as reflected by the lowering of biogas production and the COD removal efficiency. In each of these occasions, the COD loading rate was reduced from a higher rate to 4 $g \cdot l^{-1} \cdot day^{-1}$ and kept at that level until the biogas production and COD removal efficiency were fully recovered before the loading rate was increased once more.

A typical phenol-degrading granule was 1–2 mm in diameter with satisfactory settleability. The sludge washout was steady. Over a 3-month period, during days 326–412 when the reactor was operated at a constant loading of 6 g-COD $\cdot l^{-1} \cdot day^{-1}$, the effluent contained an average of 60 $mg \cdot l^{-1}$ of VSS.

COD removal efficiency

(a) Startup. Prior to the experiment, the partially granulated sludge in the reactor was first fed with wastewater containing sucrose as substrate at a loading rate of 10 g-COD $\cdot l^{-1} \cdot day^{-1}$. During this period,

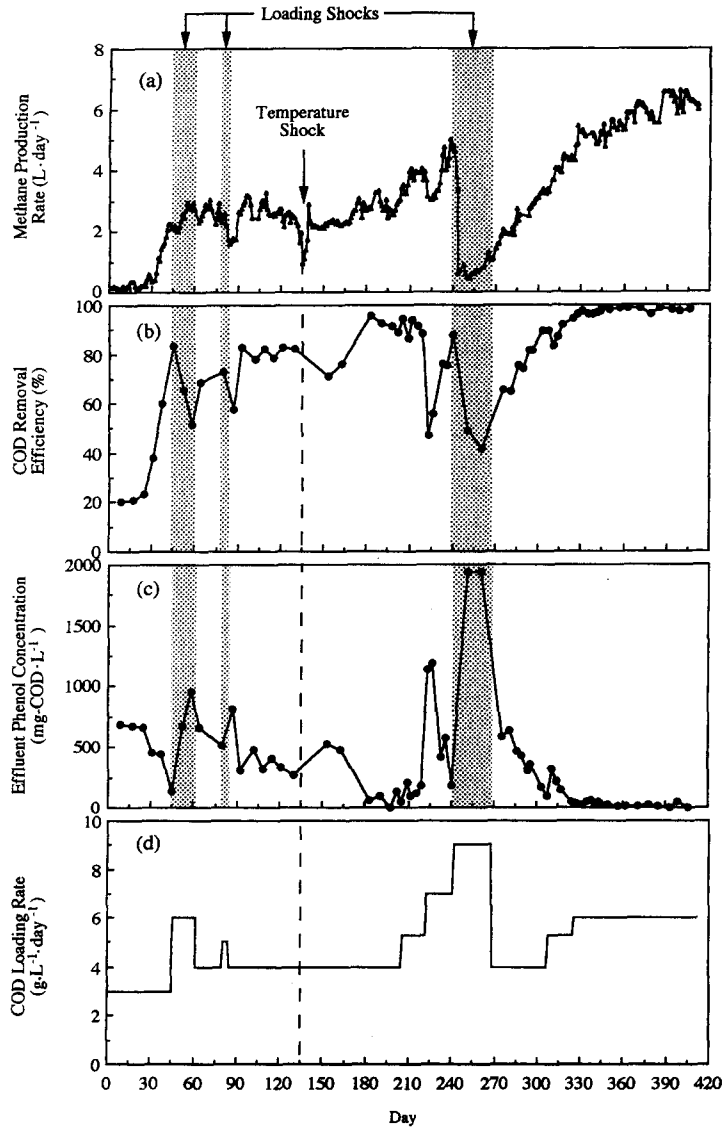

Fig. 2. (a) Removal efficiency of soluble COD. (b) COD levels of the filtered effluent. (c) Biogas production rate. (d) The corresponding COD loading rates.

the biogas production rate was about 20 l·day^{-1} and the COD removal efficiency was over 95%. However, starting day 1 when the substrate was switched to phenol at 3 g-COD·l^{-1}·day^{-1}, the biogas production rate dropped immediately to 0.3 l·day^{-1} and the COD removal efficiency decreased to less than 20%. Biomass in the reactor took a long time to become adapted to the new substrate. Only after about 30 days of acclimation, the gas production rate and the COD removal efficiency began to recover. By day 45, the reactor was capable of producing over 2.8 l·day^{-1} of biogas and removing over 83% of COD.

(b) Effect of effluent recycle. During days 85–125, when the reactor was operated at 4 g-COD·l^{-1}·day^{-1} with an influent COD of 2000 mg·l^{-1}, the COD removal efficiency maintained steadily at 81%. The inability to further increase the COD removal efficiency was attributed to the toxic effect of the high concentration of phenol in the wastewater to the granular biomass in the reactor. In order to lower the toxicity of the incoming wastewater, starting day 125, part of the effluent was recycled to dilute the phenol concentration in the incoming wastewater. With 1:1 recycle ratio, the COD removal efficiency steadily improved to an average of 92.5% during days 180–205 at the same loading of 4 g-COD·l^{-1}day^{-1}. The combined stream entering the reactor contained about 430 mg-phenol·l^{-1} during this period.

(c) Response to temperature shock. On days 132–134, the temperature of the reactor dropped from the normal 37 to 20°C for about 48 h, due to the breakdown of the water heater. Although the temperature change was not severe, it adversely affected the bioactivity of the phenol-degrading granules. As illustrated in Fig. 2, the biogas production reduced readily by 64%, from 2.5 to 0.9 l·day^{-1}. Even though the reactor recovered about 80% of its biogas production rate 5 days after the temperature returned to 37°C, the full recovery of bioactivity took about 40 days. Figure 2 illustrates that the biogas production rate, the phenol concentration in the effluent and the COD removal efficiency did not return to the pre-shock levels until day 173. During this time, several UASB reactors using other individual substrates, including mixed VFA, peptone and benzoate, were operated in parallel to this study. However, the temperature change from 37 to 20°C had only mild effect to the reactors; all of them recovered fully within 2 days when the temperature returned to 37°C. Only the reactor of this study treating phenolic wastewater exhibited high sensitivity to the temperature shock.

(d) Response to loading shocks. Phenol-degrading granules also exhibited high sensitivity to the increase of COD loading. In this study, the loading was lowered from a higher rate to 4 g-COD·l^{-1}·day^{-1} on three occasions when the reactor performance deteriorated badly. When the loading rate was increased from 3 to 6 g-COD·l^{-1}·day^{-1} on day 46, the COD removal efficiency steadily decreased from 83 to 52%. Thus, on day 62 the loading rate was lowered to 4 g-COD·l^{-1}·day^{-1}, at which the COD removal efficiency recovered gradually. When the loading was increased to 5 g-COD·l^{-1}·day^{-1} on day 81, the COD removal efficiency readily dropped again. Once more, the COD removal efficiency was recovered when the loading was lowered to 4 g-COD·l^{-1}·day^{-1}, as illustrated in Fig. 2.

After the reactor performed steadily at 4 g-COD·l^{-1}·day^{-1} for a long period, the loading rate was raised for the third time, to 5.3 g-COD·l^{-1}·day^{-1} on day 206. After 2 wk of steady satisfactory performance, the loading was raised further to 7 g-COD·l^{-1} day^{-1}. At this loading, the COD removal efficiency dropped to 47%; but it readily recovered to 88% in about 2 wk. The effluent during days 223–226 contained 481–499 mg·l^{-1} of residual phenol, which did not appear to have any inhibitory effect for the further degradation of phenol. However, when the loading was further increased to 9 g-COD·l^{-1}·day^{-1} on day 242, the COD removal efficiency dropped drastically, and did not recover after 25 days. During this period, the effluent contained 812–816 mg·l^{-1} of residual phenol, which appeared to have inhibitory effect to further degradation of phenol. On day 268 the loading was lowered to 4 g-COD·l^{-1}day^{-1}; at this level the COD removal efficiency recovered gradually from about 15% to over 90% by day 307.

COD removal, methane production and sludge yield at steady state

For nearly 3 months during days 326–412, the reactor was operated at a steady-state condition. At 6 g-COD·l^{-1}·day^{-1}, corresponding to 12 h of HRT and a wastewater containing 3065 mg-COD·l^{-1} (i.e. 1290 mg·l^{-1} of phenol), the reactor removed an average of 97.7% of soluble COD. The effluent contained on average 68.2 mg·l^{-1} of soluble COD. Based on GC analyses, the effluent had an average of 10.2 mg·l^{-1} of phenol and its concentrations of benzoate and VFAs were below detectable levels. The experiment was terminated on day 412. No attempt was made to further increase the loading rate beyond 6 g-COD·l^{-1}·day^{-1}.

During this period, the sludge bed steadily occupied 42% of the reactor volume with a constant VSS level of 27.5 g·l^{-1}. The total biomass inside the reactor was estimated to be 32.0 g, judging from the sludge bed's volume and VSS content. The VSS level in the effluent was 69 mg·l^{-1}, and, thus, the sludge in the reactor had an average retention time of 84 days. During this period, the reactor produced daily 8.4 l of biogas with 69% of methane content. Each gram of methane is equivalent of 4 g of COD. Thus, the biomass inside the rector had the specific methane production rate of 0.49 g-methane-COD·g-VSS^{-1}·day^{-1} and 94.7% of the COD removed was converted to methane. The remaining 5.3% of the COD removed was, presumably, converted to biomass. Since each gram of biomass had 1.41 g of

Table 2. Bioactivity of phenol-degrading granules

Substrate	COD ($mg \cdot l^{-1}$)	SMA ($g\text{-}COD \cdot g\text{-}VSS^{-1} \cdot day^{-1}$)
Formate	1500	0.98
Acetate	2500	0.64
Propionate	2500	nil
Butyrate	2500	nil
Benzoate	2400	0.24
Phenol	1000	0.23
Phenol	2000	0.16

COD, the sludge yield based on COD balance was estimated to be 0.038 $g\text{-}VSS \cdot g\text{-}COD^{-1}$.

Specific methanogenic activity

Kobayashi *et al.* (1989) proposed a degradation pathway of phenol under anaerobic conditions, in which phenol is initially converted to benzoate. The latter is then dearomatized forming cyclohexane carboxylic acid, the ring structure of which is then cleaved to form heptanoate. Heptanoate is further degraded either through β-oxidation to form valerate, propionate and acetate (Keith *et al.*, 1978), or directly to form propionate and butyrate, both of which can be further oxidized to acetate (Fina *et al.*, 1978).

Table 2 summarizes the SMA data of the phenol-degrading granules using six individual substrates, including formate, acetate, propionate, butyrate, benzoate and phenol. The phenol-degrading granules exhibited a SMA of 0.23 $g\text{-}COD \cdot g\text{-}VSS^{-1} \cdot day^{-1}$ when the feed contained 420 $mg \cdot l^{-1}$ of phenol (corresponding to 1000 $mg \cdot l^{-1}$ of COD) but only 0.16 $g\text{-}COD \cdot g\text{-}VSS^{-1} \cdot day^{-1}$ when the feed contained 840 $mg \cdot l^{-1}$ of phenol. This is consistent with what was observed in the continuous reactor, i.e. phenol concentration at about 800 $mg \cdot l^{-1}$; equivalent to about 2000 $mg \cdot l^{-1}$ of COD, was inhibitory to granules' bioactivity.

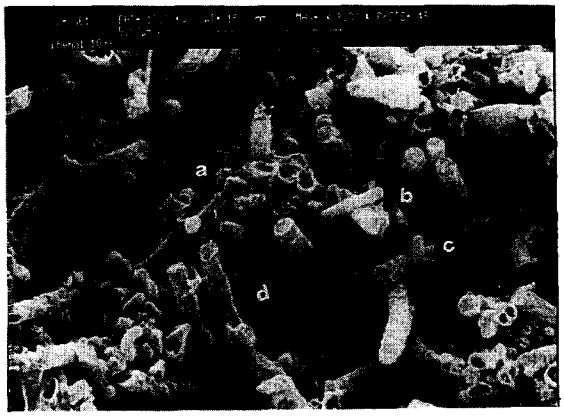

Fig. 3. SEM micrograph of a syntrophic microcolony consisting of *Syntrophus buswellii*-like bacteria (a) in association with two hydrogenotrophic *Methanospirillum hungatei*- (b) and *Methanobrevibacter*- (c), and the acetotrophic *Methanothrix*-like (d) bacteria.

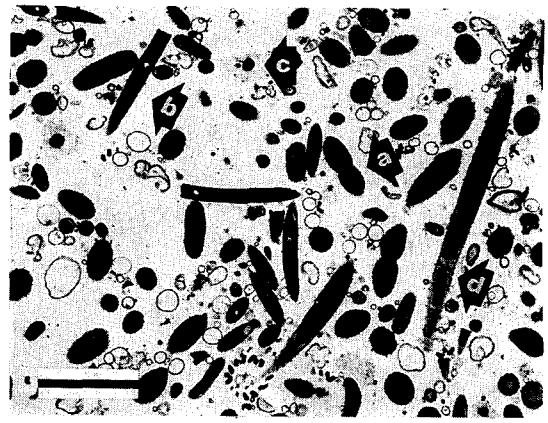

Fig. 4. TEM micrograph of a syntrophic microcolony consisting of *Syntrophus buswellii*-like bacteria (a) in association with two hydrogenotrophic *Methanospirillum hungatei*- (b) and *Methanobrevibacter*- (c), and the acetotrophic *Methanothrix*-like (d) bacteria (bar = 3 μm).

The phenol-degrading granules were also found capable of degrading benzoate. Using benzoate as the sole substrate, the granules had a SMA of 0.24 $g\text{-}COD \cdot g\text{-}VSS^{-1} \cdot day^{-1}$ which was comparable to the 0.23 $g\text{-}COD \cdot g\text{-}VSS^{-1} \cdot day^{-1}$ using phenol as the sole substrate. This seems to support the Kobayashi's proposed pathway that phenol was degraded by first converting to benzoate. This was further confirmed by the presence in large quantity of *Syntrophus buswellii*-like bacteria in the granules, as to be discussed in the next section.

Table 2 also shows that the granules' SMA using phenol as substrate was significantly less than those using formate (0.98 $g\text{-}COD \cdot g\text{-}VSS^{-1} \cdot day^{-1}$) and acetate (0.64 $g\text{-}COD \cdot g\text{-}VSS^{-1} \cdot day^{-1}$) as substrates. This, combining with the fact that there was no detectable benzoate in the effluent, seems to suggest that the initial conversion from phenol to benzoate was likely the rate-limiting step in the anaerobic degradation process of phenol. In addition, the phenol-degrading granules did not exhibit any

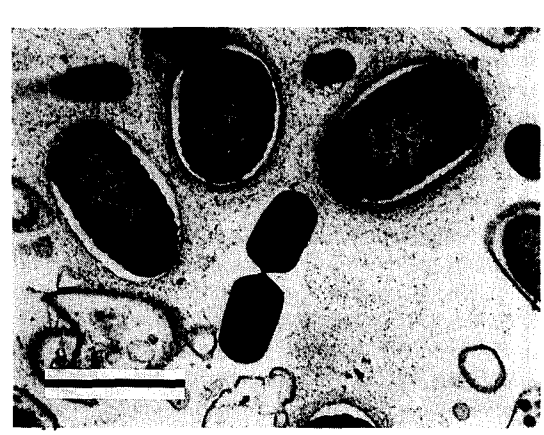

Fig. 5. TEM micrograph showing *Syntrophus buswellii*-like bacteria in juxtaposition with *Methanobrevibacter*-like bacteria (bar = 1 μm).

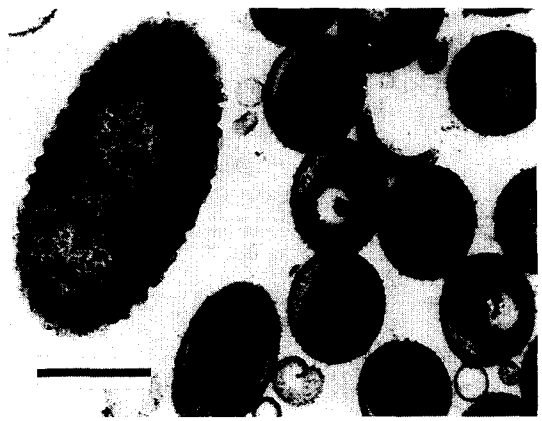

Fig. 6. TEM micrograph showing *Syntrophus buswellii*-like bacteria in juxtaposition with *Methanospirillum*-like bacteria (bar = 0.5 μm).

methanogenic activity when propionate and butyrate were used as substrate. This further suggests that the degradation of benzoate into acetate was probably conducted completely inside the cell of the *Syntrophus buswellii*-like bacteria; acetate was then released to the mixed liquor to be degraded by acetotrophic methanogens. Such a postulation was supported by the observation that there was no detectable propionate and butyrate in the effluent.

Microscopic examinations of granules

Recent studies have shown that most granules, such as those treating brewery wastewater (Fang *et al.*, 1995) and those degrading sucrose (Fang *et al.*, 1994b) exhibit a three-layered structure (MacLeod *et al.*, 1990). Such a microstructure is dependent on the rate of degradation and diffusion of the substrates and metabolites. The overall anaerobic degradation involves at least three steps, i.e. hydrolysis/acidogenesis, acetogenesis and methanogenesis. Substrate in the mixed liquor was normally first hydrolyzed and converted by acidogens in the outer layer of the granules to fatty acids, which then diffuse inward due to concentration gradients and became substrates to the syntrophic acetogens and acetotrophic methanogens in the middle layer. Acetate which produced in the middle layer diffuse further inward and was converted by the acetotrophic methanogens in the center core.

However, the phenol-degrading granules, based on electron-microscopic examinations, did not exhibit such a layered microstructure. This could be attributed to the slow reaction rate of the initial acidogenesis process converting phenol to benzoate. Significant fraction of phenol was thus likely to diffuse into the granules, instead of being converted near the surface. Thus the distribution of bacterial species was rather uniform and the granules lacked a distinct layered microstructure. Similar observation was also recently reported for granules degrading hydrolyzed proteins (Fang *et al.*, 1994a); in that case, the initial hydrolysis of proteins was also the rate-limiting step and thus proteins were degraded throughout the granules, instead of just near the surface.

Bacterial composition and phenol degradation in granules

Although there was no predominant bacterial species in the phenol-degrading granules, four familiar species were noticeable, including those resemble the benzoate-degrading *Syntrophus buswellii* (Mountfort and Bryant, 1982), acetotrophic *Methanothrix* (Zehnder *et al.*, 1980), and two hydrogenotrophic *Methanospirillum hungatei* (Zeikus and Bowen, 1975) and *Methanobrevibacter* (Mah and Smith, 1981). The presence of *Syntrophus buswellii*-like bacteria in abundance provided additional supporting evidence for Kobayashi's proposal that, in its methanogenic degradation, phenol was first converted to benzoate.

The methanogenic degradation of benzoate was first reported by Tarvin and Buswell (1934). Its degradation pathway was later studied using enriched methanogenic consortia (Fina *et al.*, 1978; Ferry and Wolfe, 1976; Keith *et al.*, 1978) before strains of syntrophic bacteria were isolated (Mountfort and Bryant, 1982), and acetate, H_2 and CO_2 were identified as the intermediates, as shown in the following equation:

$$C_7H_5O_2^- + 6H_2O \rightarrow 3CH_3COO^- + 3H_2 + CO_2 + 2H^+ \quad (1)$$

$$\Delta G^{0'} = +53.0 \text{ kJ/reaction},$$

where $\Delta G^{0'}$ represents the change of standard Gibbs free energy at neutral pH. With 53.0 kJ/reaction of $\Delta G^{0'}$, the reaction is thermodynamically unfavourable unless intermediates, i.e. acetate, H_2 and CO_2, can be readily removed. This could be accomplished when the benzoate-degrading bacteria are syntrophically associated with the hydrogen-consuming bacteria, such as *Methanospirillum hungatei* and *Methanobrevibacter*. The hydrogenotrophic methanogenesis reaction is as follows:

$$12H_2 + 3HCO_3^- + 3H^+ \rightarrow 3CH_4 + 9H_2O, \quad (2)$$

$$\Delta G^{0'} = -406.8 \text{ kJ/reaction}.$$

Thus, the overall reactions can be expressed as:

$$4C_7H_5O_2^- + 19H_2O \rightarrow 3CH_4 + 12CH_3COO^- + HCO_3^- + 9H^+, \quad (3)$$

$$\Delta G^{0'} = -49.2 \text{ kJ/reaction}.$$

The acetate produced became the substrate for the acetrophic *Methanothrix*, which converted acetate to methane and carbon dioxide according to the following reaction:

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (4)$$

$$\Delta G^{0'} = -135.6 \text{ kJ/reaction}.$$

In phenol-degrading granules, an abundance of syntrophic microcolonies could be observed. Figures 3 and 4 illustrate a microcolony in which *Syntrophus buswellii*-like bacteria (with a mark **a**) are in syntrophic association with two hydrogenotrophic *Methanospirillum hungatei*- (**b**) and *Methanobrevibacter*- (**c**), and the acetotrophic *Methanothrix*-like (**d**) bacteria. *Syntrophus buswellii* has a rod-shaped cell (0.8×1.0–$2.0\,\mu m$) with rounded-ends and an ultrastructure with undulatory outer membrane, and occurs either singly or in pairs (Mountfort *et al.*, 1984). *Methanobrevibacter*, on the other hand, has a rod-shaped cell (0.5–0.75×1.8–$3.5\,\mu m$) which occurs singly, in pairs or in chains, (Mah and Smith, 1981). *Methanospirillum hungatei* has filamentous cell (0.3–$0.66\,\mu m$) with square-off appearance, cell spacer, and a continuous outer cell wall (Zeikus and Bowen, 1975). *Methanothrix*, which is commonly found in different kinds of anaerobic granules, can be identified by its fluorescence-emitting, bamboo-shaped filament, rod-shaped cell (0.6–0.8×2.0–$3.5\,\mu m$) with flat-ends, and an ultrastructure with outer and inner cell walls (Zehnder *et al.*, 1980). Figures 5 and 6 are two close-up micrographs showing *Syntrophus buswellii*-like bacteria in juxtaposition with *Methanobrevibacter*- and *Methanospirillum*-like bacteria, respectively.

CONCLUSION

Phenol in wastewater was effectively degraded in an upflow anaerobic sludge blanket (UASB) reactor at loading rate up to $6\,g\text{-}COD \cdot l^{-1} \cdot day^{-1}$. With a 1:1 recycle ratio, over 97% of phenol was removed at 37°C, pH 6.8–7.5 and an HRT of 12 h for phenol concentrations up to $1260\,mg \cdot l^{-1}$, corresponding to $3000\,mg \cdot l^{-1}$ of chemical oxygen demand (COD). Granules in the reactor took a lengthy acclimation period to develop the phenol-degrading capability. Such a capability deteriorated when the granules were exposed to sudden changes of temperature and loading; although the damage was not permanent, the recovery was gradual and lengthy. At $6\,g\text{-}COD \cdot l^{-1} \cdot day^{-1}$, each gram of granules was capable of converting 0.49 g of COD into methane daily. On the average, about 94.7% of the total COD removed was converted to methane, while the rest was converted to biomass with a net yield of $0.038\,g\text{-}VSS \cdot (g\text{-}COD\text{-}removed)^{-1}$. Electron micrographs show that the granules did not exhibit a layered structure and were composed of, among others, *Syntrophus buswellii*-, *Methanothrix*-, *Methanospirillum*- and *Methanobrevibacter*-like bacteria.

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