

UASB TREATMENT OF WASTEWATER CONTAINING CONCENTRATED BENZOATE

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ABSTRACT: The upflow anaerobic sludge blanket (UASB) process removed 97–99% of soluble chemical oxygen demand (COD) from wastewater containing concentrated benzoate at 37°C, pH 7.5, a hydraulic retention time of 9.8 h, and loading rates up to 30.6 g-COD/(L·day) based on the reactor volume. About 95.2% of the total COD removed was converted to methane; 0.034 g of volatile suspended solids (VSS) was yielded for each gram of COD removed. The highly settleable granules were 1–3 mm in size with a layered microstructure and were composed in abundance of bacteria resembling the benzoate-degrading *Syntrophus buswellii*. Two interesting observations have led to the postulation that the degradation of benzoate into acetate was probably conducted completely inside the cell of *Syntrophus buswellii*-like bacteria: (1) No fatty acids except acetate were found in the effluent; and (2) the granules showed very limited butyrate-degrading capability and could not degrade propionate. This study demonstrated the feasibility of removing aromatic pollutants in wastewater by anaerobic processes.

INTRODUCTION

Compared with conventional aerobic process in treating high-strength industrial wastewaters, anaerobic treatment has two intrinsic advantages: it saves aeration energy and produces a useful fuel, methane; and it has a low yield of sludge, which significantly reduces the cost of sludge disposal. The interest in anaerobic wastewater treatment has increased considerably in the past decade as a result of the successful development of high-rate reactors. Among them, the upflow anaerobic sludge blanket (UASB) reactor (Lettinga et al. 1980; Fang and Chui 1993) has probably received most commercial interests, especially in Europe and more recently in Asia. The UASB process has been found very effective for treating wastewaters from the food/beverage and agricultural industries, the pollutants from which are mainly composed of carbohydrates, proteins, and fatty acids. However, its application to wastewaters containing pollutants of other chemical natures has not yet been fully explored.

Benzoate is a common pollutant in the wastewaters from specialty chemical industry. It is also a key intermediate in the anaerobic degradation of many complex aromatic chemicals. Benzoate was detected as an intermediate in the degradation of phenol and chlorophenol by anaerobic consortia (Knoll and Winter 1989) and in the degradation of 3-hydroxybenzoate and 3-chlorobenzoate in defined syntrophic co-cultures or axenic cultures (Tschech and Schink 1986). Benzoate is, therefore, a sensible model substrate for studying the feasibility of anaerobic treatment of wastewater containing aromatic pollutants.

The objective of this study was to investigate the performance of a UASB reactor in treating wastewater containing concentrated benzoate. Key operational parameters, includ-

ing the maximum loading capacity, chemical oxygen demand (COD) removal efficiency, methane production, and growth yield were examined, along with the specific methanogenic activities (SMAs) of the granules in degrading volatile fatty acids (VFAs).

MATERIALS AND METHODS

A 2.8-L water-jacketed UASB reactor with a 2.0-L gas-liquid-solid separator on top (Fang et al. 1995) was used in this study. Volumetric loading rates were based on the reactor volume alone, excluding volume of the separator. The reactor was operated at pH 7.5 and a constant temperature of 37°C throughout the study.

Prior to this study, flocculent sludge from an anaerobic sludge digester of the Shatin Wastewater Treatment Works in Hong Kong was partially granulated in a 65-L UASB reactor for two months, using sucrose as substrate. About 1.5 L of this partially granulated sludge was used to seed the reactor. Wastewater was synthesized using (sodium) benzoate as the sole organic substrate. The addition of essential nutrients and trace metals to the wastewater, as well as the sampling strategies, followed those reported in a previous study (Fang and Chui 1993).

Throughout this study the hydraulic retention time (HRT) was kept constant at 9.8 h; thus, the loading rate of COD to the reactor was proportional to the COD level in the wastewater. The initial COD loading rate was 2.1 g/(L·day), corresponding to 870 mg/L of COD in the wastewater. The loading rate was increased stepwise by increasing the benzoate concentration in the wastewater. After reaching 13.1 g/(L·day), the COD loading rate was kept at this level for 150 days. Granule samples were taken at this loading rate on day 190 for microscopic examination and SMA analysis. The microstructure of the UASB granules was examined using a light microscope (LM) (Olympus, Model BH2) and transmission electron microscope (TEM) (JEOL 100SX). The sample preparation procedures were reported previously (Chui and Fang 1994).

The SMA measures the methanogenic activity of the granules in serum vials for a specific substrate at high concentrations at which level the availability of substrate is not a limiting factor. The SMA of the benzoate-degrading granules were measured in duplicate in serum vials based on a published method (Dolfing and Mulder 1985) that was 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. Five substrates, namely, formate, acetate,

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propionate, butyrate, and benzoate, were used individually as the substrate in this study.

The volatile suspended solids (VSS), which represented the biomass concentration, and the COD were measured according to the *Standard Methods* (1985). A gas chromatograph (GC) (Hewlett Packard model 5890 Series 2) was used to measure the composition of fatty acids (from acetic to heptonic acid) and benzoate in the effluent plus the composition of the biogas. For the analyses of VFA and benzoate, 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°C 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 ramp of 10°C/min and a second temperature of 140°C for 2 min. This was followed again with a ramp of 10°C/min and then a final temperature of 170°C for 5 min. VFA standards (Supelco, Inc., Bellefonte, Pa.) and sodium benzoate (Merck) solutions were used for the calibration of the FID. For biogas analyses, the same GC was equipped with a thermal conductivity detector and a 2 m × 2 mm (inside diameter) stainless-steel column packed with Porapak N (80–100 mesh). Injector and detector temperatures were kept at 130°C and column temperature was 50°C.

RESULTS AND COMMENTS

The 280-day experimental program was divided into three phases. Phase 1 was operated at low COD loading rate so that sludge could be gradually transformed into benzoate-degrading granules. Phase 2 was operated at a constant loading rate of 13.1 g-COD/(L·day) for a prolonged period to obtain mature granules for characterization. Phase 3 was op-

erated at elevated COD loading rates to investigate the maximum loading capacity of the reactor. Fig. 1 illustrates the operational conditions and the performance of the UASB reactor in all phases throughout this study, including COD loading rate, production rate of biogas, and efficiency of COD removal. In the wastewater, the COD was contributed solely by the highly soluble benzoate.

As illustrated in Fig. 1, start-up took less than 80 days, during which the COD loading rate was increased stepwise as the COD removal efficiency increased gradually. Initially, the UASB reactor was operated at a COD loading rate of 2.1 g/(L·day) with 870 mg/L of COD in the wastewater. The efficiency of COD removal increased from the initially observed 47% to 80% in two weeks. The loading was then increased to 6.0 g-COD/(L·day) for two more weeks, and then on day 28 to 10.3 g-COD/(L·day). When the loading was increased to 10.3 g-COD/(L·day), the efficiency of COD removal was immediately reduced to 59% but gradually recovered to over 97% by day 76. During this period, the biogas production rate increased steadily. A distinct granulated sludge bed was observed starting day 75. Because the reactor was operated at over 10 g-COD/(L·day), removed over 95% COD, and produced a distinct sludge bed, the start-up phase was considered completed on day 76.

Phase 2 started on day 77, during which the loading rate was kept at 13.1 g-COD/(L·day) for 150 days to ensure the uniformity of the granules under the steady-state condition. The efficiency of COD removal during this period was steadily 98–99% for soluble COD and 92–94% for total COD. Granules in the reactor were 1–3 mm in size and exhibited good settleability. Samples of granules were taken on day 190 for microbial analysis.

Phase 3 started on day 225, during which time the COD loading rate was increased stepwise to determine the maximum loading capacity of the reactor. There was no residual benzoate in the effluent for loading rates up to 25.8 g-COD/(L·day). Starting at 27.3 g-COD/(L·day), the residual benzoate in the effluent increased with COD loadings. Throughout this study, acetate in the effluent also increased with COD loadings, but there was no detectable propionate, butyrate, or valerate in the effluent.

Fig. 2 illustrates the efficiencies of soluble and total COD removal and the methane production rate at various loading

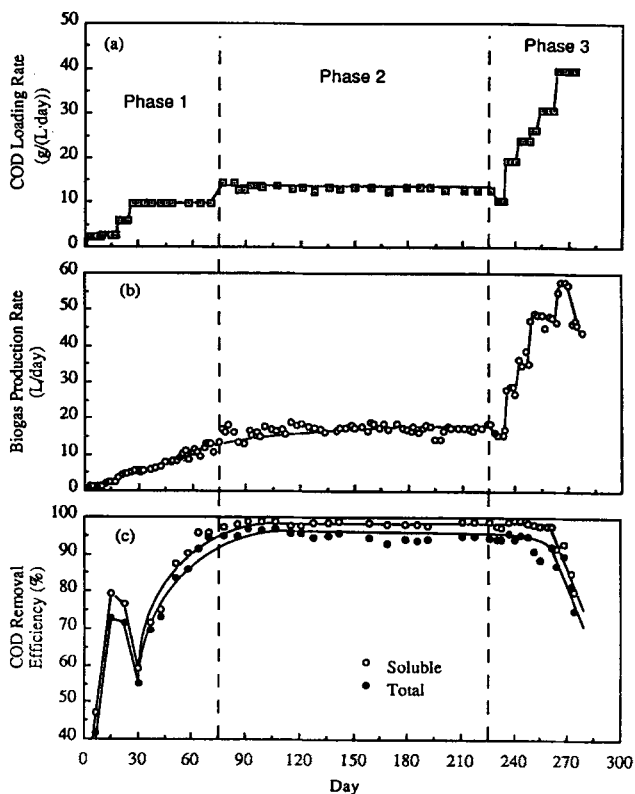


FIG. 1. Operational Conditions and Performance of UASB Reactor throughout Study: (a) COD Loading Rate; (b) Total Biogas Production; (c) Efficiencies of Soluble and Total COD Removal

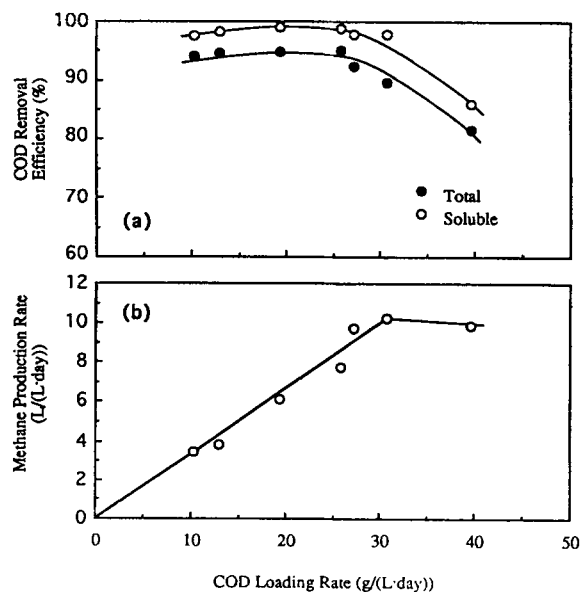


FIG. 2. Performance of UASB Reactor at Various COD Loading Rates: (a) Efficiencies of Soluble and Total COD Removal; (b) Volumetric Methane Production Rate

rates, which were based on the reactor volume alone, after start-up. Fig. 2(a) illustrates that the efficiencies of soluble COD removal were maintained at 97–99% up to the loading rate of 30.6 g-COD/(L·day), based on reactor volume only. The corresponding COD level in wastewater at that loading was 12,500 mg/L. Beyond this loading rate, the performance of the reactor deteriorated rapidly. Fig. 2(b) illustrates that the amount of methane production increased linearly with the COD loading rate up to the maximum loading of 30.6 g-COD/(L·day). The increased gas production at 27.3 g-COD/(L·day) and higher loading rates caused more biomass wash-out, resulting in a reduction of removal efficiency of total COD. The experiment was terminated after being operated at 39.7 g-COD/(L·day) for 10 days because the removal efficiency of soluble COD deteriorated to less than 80% and continued to worsen.

Five months after start-up, the sludge bed consistently occupied 42% of the reactor volume with a VSS level ranging from 55 g/L to 58 g/L. At 30.6 g-COD/(L·day), the effluent on average had 380 mg/L of VSS.

The methane content in the biogas collected was 64–68% throughout the study. As illustrated in Fig. 2(b), the methane production rate increased linearly with the COD loading rate until it reached 10.2 L/(L·day) at 30.6 g-COD/(L·day). Fig. 3 illustrates that the capacity of the biomass to convert benzoate into methane was proportional to the COD removed. The specific methane production rate increased linearly with the specific substrate utilization rate with a slope of 0.952. The slope indicates that, of all the COD removed, an average of 95.2% was converted to methane and the remaining 4.8% was, presumably, utilized for the bacterial growth. Because the biomass washed out had an average of 1.41 g-COD/g-VSS, the average net yield for benzoate-degrading biomass was thus estimated as 0.034 g-VSS/g-COD, which was comparable to those of formate (0.050 g-VSS/g-COD) reported by Chui et al. (1994); those of mixed VFA (0.054 g-VSS/g-COD) reported by Fang et al. (1995); and those of acetate (0.040–0.054 g-VSS/g-COD), propionate (0.042–0.051 g-VSS/g-COD), and butyrate (0.047 g-VSS/g-COD) reported by Lawrence and McCarty (1969).

Table 1 summarizes the SMA of the benzoate-degrading

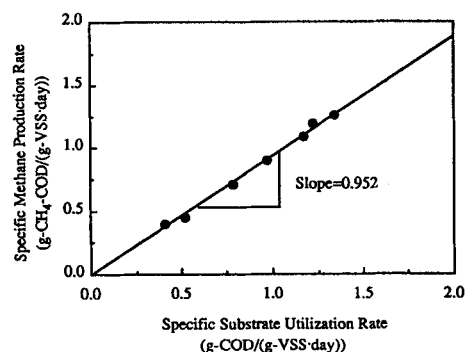


FIG. 3. Relationship between Specific Methane Production Rate and Specific Substrate Utilization Rate

TABLE 1. Specific Methanogenic Activity of Benzoate-Degrading Granules

Substrate (1)	SMA [g-methane-COD/(g-VSS·day)] (2)
Benzoate	1.05
Formate	1.15
Acetate	1.32
Propionate	nil
Butyrate	0.27

granules measured using benzoate and four VFAs, individually, as substrate. The SMA using benzoate as substrate was 1.05 g-methane-COD/(g-VSS·day), which was slightly lower than 1.32 g-methane-COD/(g-VSS·day) using acetate and 1.15 g-methane-COD/(g-VSS·day) using formate as substrate. The SMA using butyrate as substrate was 0.27 g-COD/(g-VSS·day), which was considerably lower than those using benzoate, acetate, and formate as substrate. The SMA using propionate as substrate was not detectable.

A typical benzoate-degrading granule exhibited a layered structure (MacLeod et al. 1990; Fang et al. 1994) with acidogens concentrated near the granule surface. On the other hand, methanogens that were fluorescent under excitation at 350 nm and 420 nm (Edwards and McBride 1975; Chui and Fang 1994) were concentrated in the interior, as illustrated in Fig. 4. The granules were composed in abundance of bacteria resembling the benzoate-degrading *Syntrophus buswellii* (Tarvin and Buswell 1934), as illustrated in Fig. 5.

It has been suggested that, in anaerobic degradation, benzoate is first dearomatized forming cyclohexane carboxylic acid, which is then converted to heptanoate. The latter is either further degraded through β -oxidation to form valerate, propionate, and acetate (Keith et al. 1978), or degraded directly to form propionate and butyrate, both of which can be further oxidized to acetate (Fina et al. 1978). It is interesting to note that, in this study, there was neither propionate, butyrate, valerate, nor heptanoate found in the effluent based on GC analysis, even when the reactor was operated at the loading of 39.7 g-COD/(L·day). Furthermore, the SMA data show that the benzoate-degrading granules had only limited butyrate-degrading capability and were unable to degrade propionate at all. One could postulate, based on these observations, that the degradation of benzoate into acetate is probably conducted completely inside the cell of *Syntrophus*

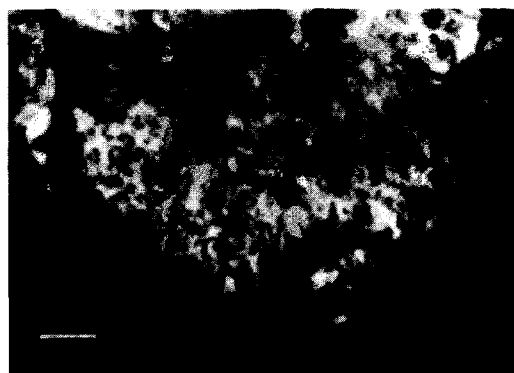


FIG. 4. Distribution of Methanogens Examined under Epifluorescent Excitation (LM, Bar = 0.15 mm)



FIG. 5. *Syntrophus buswellii*-like Bacteria (TEM, Bar = 6.5 μ m)

buswellii-like bacteria. Not only was there no intermediate VFA, except acetate, released to the outside of the cell, the benzoate-degrading bacteria were also unable to degrade the VFA other than acetate in the mixed liquor. Such a postulation is highly speculative, but appears to warrant further scrutiny.

CONCLUSION

Results from this study show that benzoate in wastewater could be effectively removed by the UASB process. At 37°C, pH 7.5, and a HRT of 9.8 h, a laboratory-scale UASB reactor was capable of removing 97–99% of soluble COD and 91–95% of total COD for loading rates up to 30.6 g-COD/(L·day) based on reactor volume only. About 95.2% of the total COD removed was converted to methane, and the rest was converted to biomass with an average net growth yield of 0.034 g-VSS/g-COD. The benzoate-degrading granules were 1–3 mm in size and exhibited good settleability. Results of this study suggest the feasibility of treating industrial wastewater containing aromatic pollutants by anaerobic processes.

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