

ANAEROBIC DEGRADATION OF CORNSTARCH IN WASTEWATER IN TWO UPFLOW REACTORS

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ABSTRACT: Wastewater containing high concentrations of cornstarch was treated in two 8.5-L upflow reactors: upflow anaerobic sludge blanket (UASB) and a modified anaerobic filter (AF). Both reactors were operated in parallel at 37°C and pH 6.8–7.9 over 510 d with increased loadings of chemical oxygen demand (COD). Results showed that, in spite of having different sludge concentration profiles, both reactors, with 12 h of hydraulic retention time, removed an average of 95.3% of COD from wastewater containing up to 45,000 mg-COD·L⁻¹ (loading rates up to 90 g-COD·L⁻¹·d⁻¹). At loading rates up to 75 g-COD·L⁻¹·d⁻¹, only 0.42% and 0.80% of starch particulates remained unhydrolyzed and were washed out by the effluent from UASB and modified AF reactors, respectively. Of the COD removed, 85.9% was converted to methane and the rest was converted to biomass with a net sludge yield of 0.10 g-VSS·g-COD⁻¹. Both reactors failed at loading rates higher than 90 g-COD·L⁻¹·d⁻¹, because of severe washout of solids. The sludge granules had a methanogenic activity of 1.90–1.92 g-methane-COD·g-VSS⁻¹·d⁻¹ and consisted of various trophic bacteria in the order of 10⁸–10⁹ m/L.

INTRODUCTION

Since the introduction of the anaerobic filter (AF) by Young and McCarty (1969), anaerobic technology has gradually become accepted for the treatment of high-strength wastewaters from various industries, such as food/beverage, distillery, and agriculture (Lettinga and Hulshoff Pol 1991). Among the anaerobic treatment processes, the upflow anaerobic sludge blanket (UASB) (Lettinga et al. 1980) has become very popular in Western Europe and, more recently, in Asia. In a UASB reactor, biomass with high bioactivity and settleability develop into granules, which can be effectively separated from the effluent by a built-in internal gas-liquid-solid (GLS) separator and returned to the sludge bed in the reactor zone underneath.

On the other hand, organic suspended particulates often hinder the effective treatment of wastewater. They need to be hydrolyzed by enzymes, often a rate-limiting step, before anaerobic bacteria can degrade the solubilized products. Morris and Jewell (1981) showed that cellulose particulates (20–100 μm in size) could be effectively removed using an anaerobic attached-film expanded-bed reactor at chemical oxygen demand (COD) loading rates up to 8 g·L⁻¹·d⁻¹. They suggested that a wide range of complex organic particulates could be removed in anaerobic reactors. However, the UASB process has been perceived to be vulnerable to these suspended solids (SS) in the wastewater. The suspended particulates may inhibit the sludge granulation (Lettinga et al. 1980), impair the methanogenic activity of the sludge (Sayed 1987), and in some extreme cases, may cause a sudden acidification of the reactor content (Jans and de Man 1988) or even a sudden washout of the sludge bed (Lettinga et al. 1980). To avoid these potential adverse effects of SS to UASB reactors, Lettinga and Hulshoff Pol (1991) suggested installing a settling pretreatment system for SS removal, or restricting the volumetric loading rate to 2–8 g-COD·L⁻¹·d⁻¹. Souza (1986), on the other hand, suggested restricting the SS level to below

1,000 mg/L and the SS/COD ratio in the wastewater to below 0.5. Others suggested phase separation and preacidification (Jans and de Man 1988; Shin et al. 1992).

The present study was conducted to investigate the effect of biodegradable SS on the performance of two upflow anaerobic reactors: a conventional UASB reactor and a modified AF reactor packed with plastic rings but also featured with a built-in internal GLS separator as in a UASB reactor. The packed bed was aimed to increase the biomass retention capacity in the reactor and, hopefully, to reduce the adverse effect resulting from the high SS contents in the wastewater. Similar reactors have been used for treating wastewaters from sugar and yeast factories, as well as landfill leachate (Guiot and van den Berg 1984; Kennedy et al. 1988; van der Merwe and Britz 1993). Insoluble cornstarch was used as the model substrate for biodegradable SS. The performance of both reactors were closely monitored with increased loading of cornstarch.

MATERIALS AND METHODS

Both the UASB and the modified AF reactors were composed of a 8.5-L reactor zone and a 5.0-L built-in GLS separator, as illustrated in Fig. 1. Each reactor zone had an internal diameter of 104 mm and a height of 1,000 mm, on top of which was the GLS separator with an internal diameter of 144 mm and a height of 300 mm. The volumetric loadings in the present study were, however, calculated based on the 8.5 L of reactor volume alone, excluding volume of the GLS separator. In the modified AF, the whole reactor zone was filled with plastic rings (Flexiring, Koch, Inc., Wichita, Kans.) which are illustrated in Fig. 2. The rings with 25 mm in both diameter and length had a surface-to-volume ratio of 235 m²·m⁻³. Sampling ports were installed along the height of both reactors. Concentration profiles of volatile SS (VSS), total SS (TSS), and the protein contents in the SS were analyzed from samples taken from these ports. The amounts of biomass and starch retained in both reactors were estimated based on these profiles. Both reactors were kept inside a constant temperature chamber at 37°C. Neither reactor had effluent recycle.

Each reactor was seeded with 6.5 L of sludge taken from an anaerobic digester of the Shatin Sewage Treatment Works, Hong Kong. The wastewater was formulated initially using a mixture of sucrose and cornstarch as the organic substrates, but all the sucrose was later gradually replaced by cornstarch. The cornstarch had a solubility of less than 2%. It suspended as colloidal particulates in the synthetic wastewater with an

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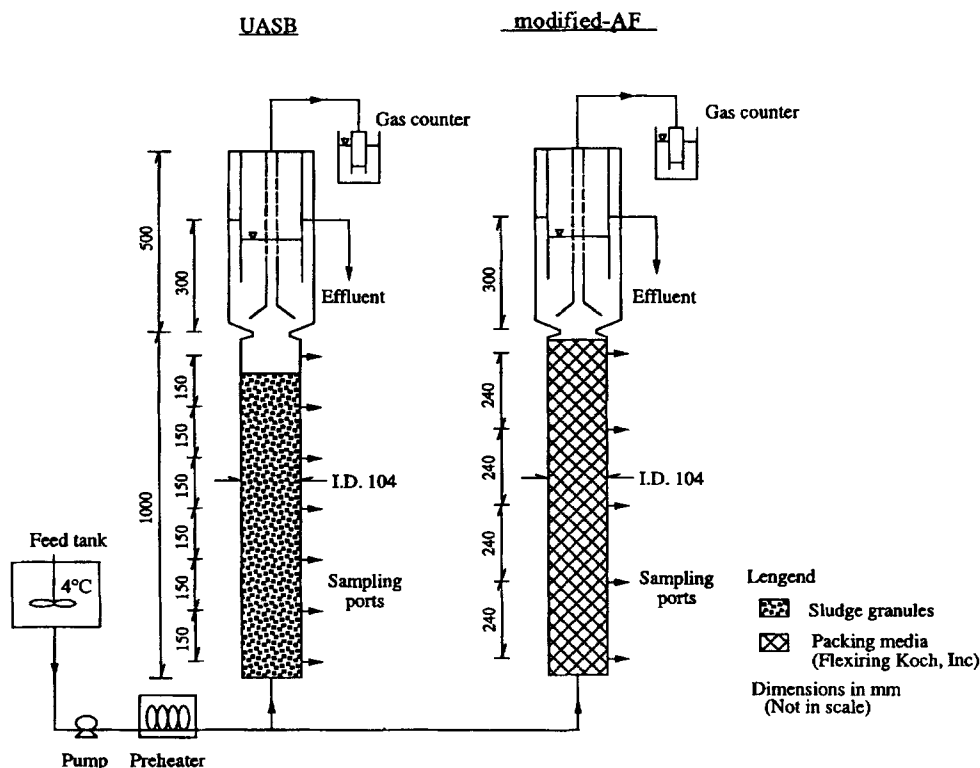


FIG. 1. Process Flow Diagram

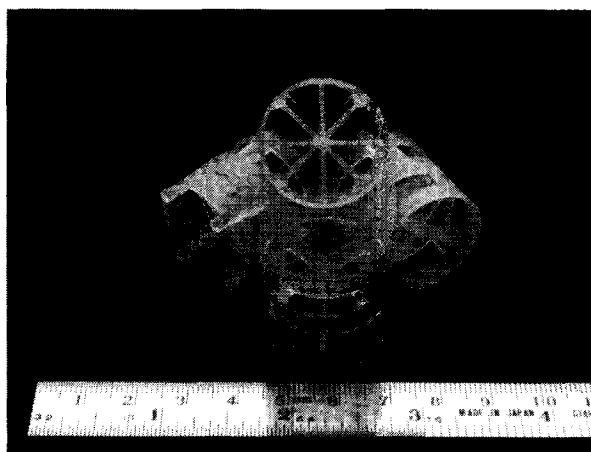


FIG. 2. Flexiring (Koch Inc.)

average size of $7.5 \mu\text{m}$ (80% within the range of $5.5\text{--}9.0 \mu\text{m}$), as estimated from Stokes' law according to their settling rates.

Trace metals and balanced nutrients were supplemented to the wastewater following the formulation used in a previous study (Fang and Chui 1993). Sodium bicarbonate was also added to supplement the alkalinity. The dosage of sodium bicarbonate was equal to the COD level, but not to exceed $8,000 \text{ mg/L}$ so as to avoid the potential toxic effect of concentrated sodium ion. Wastewater was made up once every two days and kept in a 4°C feed tank with constant mixing.

Gas production rate, gas composition, and sludge-bed height were measured daily. Volatile fatty acids (VFA), pH, COD, VSS, and TSS of the wastewater and effluent were measured twice a week. Detailed sampling strategies and the analytical procedures also followed a previous study (Fang and Chui 1993). A gas chromatograph (GC, Hewlett Packard, Palo Alto, Calif., Model 5890A) was used to measure the composition of VFA (from acetic to heptonic acid) in the effluent and the composition of the biogas. For VFA analyses, the

GC was equipped with a $10 \text{ m} \times 0.53 \text{ mm}$ 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 $0.45 \mu\text{m}$ 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 80°C for 5 min followed with a ramp of $10^\circ\text{C}/\text{min}$ and a final temperature of 130°C for 4 min. Volatile fatty acid standards (Supelco, Inc., Bellefonte, Pa.) were used for the calibration of the FID. For biogas analyses, the same GC was equipped with a thermal conductivity detector and a $2 \text{ m} \times 2 \text{ mm}$ (inside diameter) stainless-steel column packed with Porapak N (80–100 mesh) (supplied by Supelco, Inc., Bellefonte, Pa.). Injector and detector temperatures were kept at 130°C while column temperature was 50°C .

Protein contents in the sludge granules and the washed-out solids were measured using the method developed by Lowry et al. (1951). The difference in the protein content of the effluent and that of the filtered effluent represented the protein content in the washed-out solids of the effluent.

The key operational parameters are summarized in Table 1. Both reactors were started with a wastewater feed containing $3,000 \text{ mg/L}$ of COD (10% starch and 90% sucrose) and a hydraulic retention time (HRT) of 24 h, giving a volumetric loading rate of $3 \text{ g-COD} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$. This ratio was kept constant while the loading rate was increased stepwise over 144 d until reaching $10 \text{ g-COD} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$. The loading rate was then kept at $10 \text{ g-COD} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ from day 145 to day 265, while the sucrose in the wastewater was gradually replaced by starch, until all organic substrate was solely composed of starch, i.e. starch fraction of COD was increased stepwise from 10% to 100%. From day 221 on, only starch was used as the sole organic substrate, while the COD loading rate was increased stepwise. At each loading rate, the reactor was operated for at least 10 d allowing the reactors to approach steady state, which was indicated by the constant rate

TABLE 1. Operational Conditions and Effluent pH

Day (1)	Waste-water COD (mg/L) (2)	HRT (hours) (3)	Starch in COD _{int} (%) (4)	COD loading rate (g·L ⁻¹ ·d ⁻¹) (5)	Effluent pH UASB (6)	Effluent pH modified-AF (7)
0-105	3,000	24	10	3.0	7.6	7.9
106-120	3,000	18	10	4.0	7.9	7.7
121-129	4,125	18	10	5.5	7.9	7.9
130-144	4,500	16	10	6.8	7.8	7.8
145-190	5,000	12	10	10.0	7.9	7.9
191-200	5,000	12	20	10.0	7.8	7.6
201-210	5,000	12	40	10.0	7.8	7.7
211-220	5,000	12	60	10.0	7.7	7.7
221-265	5,000	12	100	10.0	7.9	7.9
266-280	5,000	9.6	100	12.5	7.7	7.6
281-298	6,000	9.6	100	15.0	7.4	7.5
299-326	7,000	9.6	100	17.5	7.7	7.6
327-353	10,000	12	100	20.0	7.3	7.7
354-375	12,500	12	100	25.0	7.5	7.4
376-389	15,000	12	100	30.0	7.7	7.6
390-409	20,000	12	100	40.0	7.3	7.4
410-425	25,000	12	100	50.0	7.4	7.4
426-450	30,000	12	100	60.0	7.4	7.7
451-465	37,500	12	100	75.0	6.9	7.1
466-480	45,000	12	100	90.0	6.9	6.9
481-495	60,000	12	100	120.0	6.8	6.8
496-510	75,000	12	100	150.0	6.8	6.8

of biogas production. The loading rate was increased only when over 90% soluble COD was removed. The experiment was terminated on day 510 at 150 g-COD·L⁻¹·d⁻¹ because of the drastic deterioration of COD removal efficiency resulting from the severe washout of sludge.

Granules taken from the reactors on day 235, during which both reactors were operated at 10 g-COD·L⁻¹·d⁻¹ using starch as the sole organic substrate, were used for the analyses of specific methanogenic activity (SMA) and microbial populations. The SMA was measured in serum vials in duplicate by the method of Dolfig and Bloemen (1985) adapted from Owen et al. (1979) using VFA, sucrose, and starch as individual organic substrate. Enumeration of the bacterial population was performed by the most probable number (MPN) method in triplicate (Chartrain and Zeikus 1986; Li and Noike 1992) using individual VFA, CO₂/H₂, sucrose, and starch as substrate. The presence of starch-degrading bacteria was confirmed by the disappearance of the starch particulates (Barlaz 1988).

RESULTS AND ANALYSIS

As shown in Table 1, the two reactors were operated in parallel throughout the 510-d study, during which the loading rate was gradually increased from the initial 3 g-COD·L⁻¹·d⁻¹ to the final loading of 150 g-COD·L⁻¹·d⁻¹ [Fig. 3(c)]. In general, the two reactors exhibited different biomass concentration profiles, but showed little difference in overall performance, including COD removal efficiency [Fig. 3(a)] and biogas production [Fig. 3(b)], as well as net sludge yield and other granule characteristics. The pH of the effluent was within 7.3-7.9 for loading rates up to 60 g-COD·L⁻¹·d⁻¹. The pH was gradually reduced as loading rate further increased due to the increased VFA concentrations; at 120-150 g-COD·L⁻¹·d⁻¹ the pH was 6.8.

Granulation of Biomass

The appearance of granules was first observed on day 90 in both reactors at 3 g-COD·L⁻¹·d⁻¹ when starch was ac-

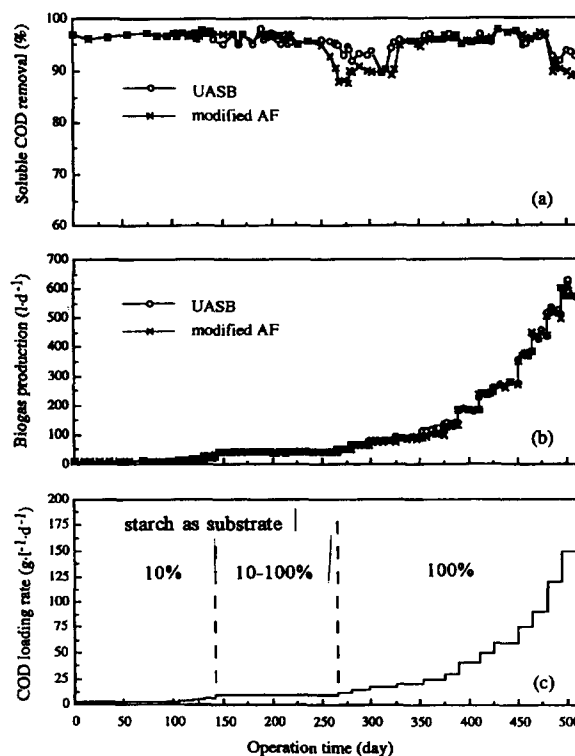


FIG. 3. Conditions and Performance of Reactors throughout Study: (a) Soluble COD Removal Efficiency; (b) Gas Production Rate; and (c) COD Loading Rate

counted for only 10% of the COD. The granules settled at the bottom of the reactor forming a sludge bed. The volume of granule bed in both reactors increased steadily even when the starch gradually became the sole substrate and the COD loading rate further increased. According to a number of previous reports, high levels of SS in the wastewater may inhibit the sludge granulation (Lettinga et al. 1980), impair the methanogenic activity of the sludge (Sayed 1987), and may cause a sudden washout of the sludge bed (Lettinga et al. 1980). None of these adverse effects was observed in the present study. This was probably due to the readily biodegradable nature of the cornstarch as well as the extended acclimation period for the development of sludge granules using sucrose as the cosubstrate.

Biomass Concentration Profiles

The VSS measurements include both biomass and residual starch. Their individual contents could, however, be estimated from the protein content. The protein/VSS ratio was less than 0.005 for the cornstarch, but it averaged 0.68 for the granular biomass in the reactor. The latter ratio was comparable to previously reported values of 0.64-0.67 for UASB granules treating lactic acid (Fukuzaki et al. 1991) and 0.35-0.60 for granules treating various wastewaters (Dolfig et al. 1985). The ratio of protein/VSS of the reactor sludge would have decreased if starch particulates accumulated in the reactor. However, the protein/VSS ratio for the sludge in both reactors remained at a constant level of 0.68 from day 145 to day 480. This indicates that there was no accumulation of starch particulates in the reactors for loading rates up to 90 g-COD·L⁻¹·d⁻¹. Consequently, the VSS of the reactor sludge was considered as the measurement of the biomass.

The biomass concentration profiles were obtained by the VSS of sludge taken from sampling ports at various heights of the reactors. Figs. 4(a and b) illustrate the respective VSS profiles in UASB and modified-AF reactors obtained in three

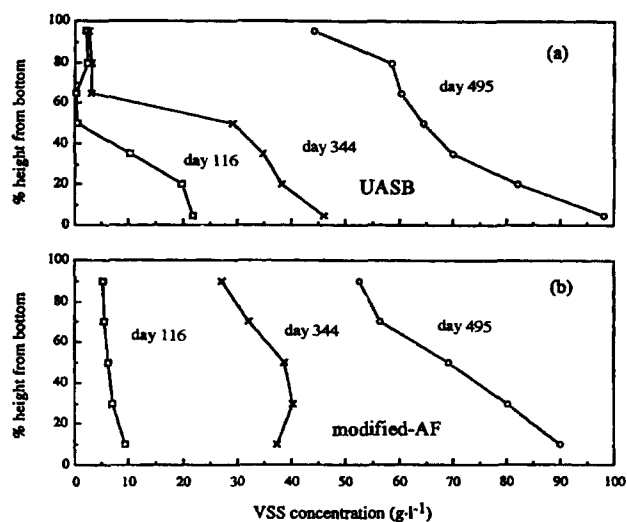


FIG. 4. Sludge Profile at Three Periods of Study: (a) UASB Reactor; and (b) Modified-AF Reactor

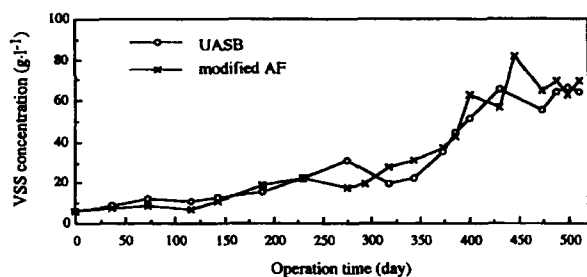


FIG. 5. Average Biomass Concentration in Reactors throughout Study

periods of the experiment. Fig. 4(a) illustrates that, on day 116, a dense sludge blanket containing approximately 20 g-VSS/L occupied the bottom 20% volume of the UASB reactor. As the experiment proceeded, the total amount of biomass in the reactor increased. On day 344, the sludge bed with an average of 37 g-VSS/L occupied about 50% of the reactor volume. By day 495, when the loading rate was 120 g-COD · L⁻¹ · d⁻¹, the sludge bed contained an average of 65 g-VSS/L occupying 80% of the reactor volume. On the other hand, the modified-AF reactor had no distinct sludge bed. The profiles illustrated in Fig. 4(b) indicate that the suspended VSS concentrations were rather uniform along the heights of the modified AF reactor (5.2–9.5 g-VSS/L on day 116; and 26.9–40.3 g-VSS/L on day 344). However, the concentration gradient along the height of the reactor was sharper on day 495 at 120 g-COD · L⁻¹ · d⁻¹, from 89.9 g-VSS/L at the bottom to 52.5 g-VSS/L on the top.

At the end of the experiment, the modified-AF reactor was dismantled. The packing rings were taken out for measuring the amount of VSS attached to the rings. It was found that the attached VSS amounted to only 8.3% of the total VSS in the reactor. The high shear caused by turbulent action of the mixed liquor, resulting from large quantities of biogas production, prevented the buildup of biofilm on the ring surface. Most of the active biomass in the modified-AF reactor were in suspension, as suggested in previous studies (Young and McCarty 1969; van den Berg and Lentz 1979).

Despite their difference in biomass profiles, the average concentration of biomass inside the reactors were similar, as illustrated in Fig. 5. The biomass concentrations in the reactors increased gradually with time, and finally stabilized at about 60 g/L at loading rate of 50 g-COD · L⁻¹ · d⁻¹ and higher. At this stage, the biomass produced balanced the sludge washed

out. At 60 g-COD · L⁻¹ · d⁻¹, the sludge retention time in both reactors was estimated as 18 d which was reduced to 5 d as loading rate increased to 120 g-COD · L⁻¹ · d⁻¹. There was a steady decrease of VSS/TSS ratio for the sludge in the reactors throughout the study, which was due to the increase of mineral content in the sludge as observed in previous studies (Fang and Chui 1993; Dolfig 1987).

VSS and Starch Washout

Figs. 6(a and b) illustrate, respectively, that the VSS and unhydrolyzed starch in the effluent increased with the COD loading rate. Of the VSS in the effluent, the large majority was the biomass and only a small fraction was the residual cornstarch particulates. During days 266–326, when the HRT was lowered from 12 h to 9.6 h at 12.5–17.5 g-COD · L⁻¹ · d⁻¹, 1.0–1.2% of starch in the wastewater remained unhydrolyzed in both reactors and were discharged along with the effluent. As HRT readjusted to 12 h on day 327, the quantity of residual starch in the effluent was readily reduced even as loading rate further increased. At 12 h of HRT and loading rates up to 75 g-COD · L⁻¹ · d⁻¹, the starch washout was only 0.42% in the UASB reactor and 0.80% in the modified-AF reactor. But, as illustrated in Figures 6(a and b), the washout of starch as well as VSS, increased as loading rate further increased due to the vigorous mixing action caused by the biogas generated. When loading rate was further increased to 120–150 g-COD · L⁻¹ · d⁻¹, the performance of the reactor declined rapidly. The whole experiment had to be terminated on day 510 at loading rate of 150 g-COD · L⁻¹ · d⁻¹, during which the unhydrolyzed starch in the effluent increased sharply to 6,750 mg/L for the UASB reactor and 4,690 mg/L for the modified-AF reactor, whereas the VSS contents in the effluent of both reactors exceeded 12,000 mg/L.

COD Removal Efficiency

Fig. 3 illustrates the overall performance of the reactors throughout the present study, including removal efficiencies

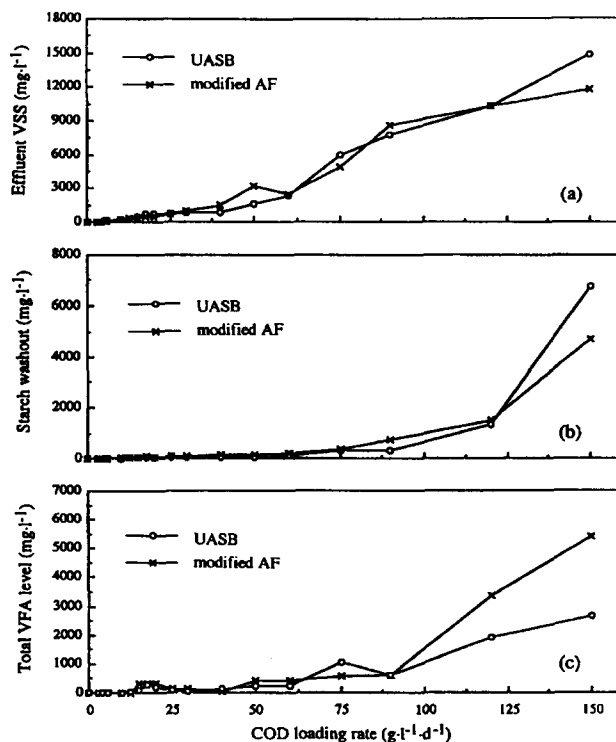


FIG. 6. Effluent Parameters at Various COD Loading Rates: (a) VSS; (b) Residual Cornstarch; and (c) VFA Levels in Effluent

of soluble COD, rates of biogas productions, and COD loading rates and the percentage of COD as starch. The soluble COD removal efficiency was defined as follows:

soluble COD removal efficiency

$$= (1 - \text{soluble COD in the effluent/wastewater COD}) \times 100\% \quad (1)$$

In general, both reactors performed comparably over a wide range of loading rate. The soluble COD removal efficiency exceeded 95% [Fig. 3(a)] throughout most part of the present study, and the biogas production increased with the COD loading rate [Fig. 3(b)]. From day 1 to day 190, when the wastewater COD was composed of 10% starch and 90% sucrose, the UASB and the modified-AF reactors, respectively, removed on average 96.9% and 96.8% of soluble COD, at loading rates ranging 3–10 g-COD·L⁻¹·d⁻¹. From day 191 to 265, both reactors removed consistently 96.4% of soluble COD at a constant loading rate of 10 g-COD·L⁻¹·d⁻¹, even though the sucrose in the wastewater was gradually replaced by starch during this period. Starting from day 266 until the end of the study, the reactors were fed solely with cornstarch as substrate. At loading rates up to 90 g-COD·L⁻¹·d⁻¹, both reactors on average removed 95.3% of soluble COD, except a 60-d period. Between days 266 to 326, the HRT in both reactors was lowered from 12 h to 9.6 h. During this period, the loading rate was increased from 10 g-COD·L⁻¹·d⁻¹ to 12.5–17.5 g-COD·L⁻¹·d⁻¹ and the removal efficiency of soluble COD in both reactors decreased considerably. The effect was more severe to the modified-AF reactor than to the UASB reactor, as illustrated in Fig. 3(a). As a result, the HRT was readjusted to 12 h on day 327, and the COD removal efficiency readily recovered to over 95%. The HRT was kept at 12 h for the rest of the present study.

As loading rate was increased further from 90 g-COD·L⁻¹·d⁻¹ to 120 g-COD·L⁻¹·d⁻¹ on day 481 and then to 150 g-COD·L⁻¹·d⁻¹ on day 496, the COD removal efficiency deteriorated, as illustrated in Fig. 3(a). But unlike the period between days 266 and 326, the reduction of COD at these high loading rates was accompanied by drastic increases of VSS [Fig. 6(a)] and starch washout [Fig. 6(b)]. As a result, the experiment was terminated on day 510 after two weeks at 150 g-COD·L⁻¹·d⁻¹.

Fig. 6(c) also illustrates that the decrease of COD removal efficiency were due to the increase of VFA in the effluent. Prior to day 265, the effluent of both reactors contained less than 50 mg/L of VFA. But during days 266–326, at 9.5 h of HRT and 12.5–17.5 g-COD·L⁻¹·d⁻¹, the VFA in the effluent of UASB and modified-AF reactors, respectively, increased to 340 mg/L and 270 mg/L; during this period the pH remained 7.4–7.7 due to the buffering capacity of the added sodium bicarbonate. From day 327 on, as the HRT was kept at 12 h, the VFA in the effluent increased, and thus the COD removal efficiency decreased, with COD loading. At COD loading rates higher than 60 g-COD·L⁻¹·d⁻¹, the alkalinity in wastewater was insufficient to provide the needed buffering capacity for the elevated VFA concentrations; the pH in the effluent decreased to 6.8–6.9 as a result, as shown in Table 1.

Overall, both reactors were effective for removing COD from cornstarch wastewater at loading rates up to 90 g-COD·L⁻¹·d⁻¹ and 12 h of HRT. Although a high loading rate was reported (Fang and Chui 1993), that was conducted using soluble carbohydrate as substrate. The maximum volumetric loading rate of 90 g-COD·L⁻¹·d⁻¹ demonstrated in the present study was considerably higher than those in literature treating wastewaters containing insoluble solids (Na-

gano et al. 1992; Shin et al. 1992; Oleszkiewicz and Romanek 1989; Ng 1989; and Robertson and Green 1988). The success of achieving such a high loading rate in the present study was probably due to the readily biodegradable nature of the cornstarch, the balanced nutrients supplemented in the wastewater, and the systematic acclimation strategy during the start-up.

The high loading rate achieved in the present study can also be attributed to the effective design of the built-in UASB GLS separator. A separate study for the treatment of cornstarch was conducted for an upflow sludge blanket filter (UBF) reactor, in which a packed bed replaced the GLS separator of the UASB reactor (Fang and Kwong 1995). Although it had comparable efficiency for soluble COD removal as the two reactors in the present study, the packed-bed separator failed to retain sludge granules in the reactor at loading rates higher than 40 g-COD·L⁻¹·d⁻¹. The superior design of the UASB GLS separator was evident.

Hydrolysis of Starch Particulates and Methane Production

Biodegradation of starch particulates is a complex process. Starch particulates are organic polymers that cannot be degraded by microorganisms unless they are hydrolyzed by enzymes forming soluble carbohydrates (usually monomers or dimers), which can then pass the cell membrane (Pavlostathis and Giraldo-Gomez 1991). Soluble carbohydrates are then degraded by bacteria forming alcohols and VFA, which are further degraded by acetogenic bacteria forming acetate, carbon dioxide, and hydrogen. Finally, both acetate and H₂/CO₂ are converted to methane by the methanogenic bacteria (Speece 1983; Vogels et al. 1988). A syntrophic relationship exists between the hydrogen-forming acetogens and the hydrogen-consuming methanogens. The partial pressure of hydrogen must be kept at an extremely low level, say in the order of 10⁻⁶ kPa, to enable favorable thermodynamic conditions for the formation of acetate from some VFA, such as propionate and butyrate (Fang et al. 1995a,b).

In most of the literature studying the anaerobic degradation of complex substrates (Henze and Harremoes 1983) the conversion of complex substrate to methane was usually assumed to be a single-step process. However, from those studying the kinetics of the hydrolysis step, it was usually found that the hydrolysis was the rate-limiting step in the overall conversion of complex substrates to methane (Pavlostathis and Giraldo-Gomez 1991).

The final biogas product is mainly composed of methane and carbon dioxide. The solubility of methane in water is usually negligible; at 25°C, the Henry's law constant for methane is 1.34 × 10⁻³ mole·L⁻¹·atm⁻¹ as compared to 1.28 × 10⁻⁵ mole·L⁻¹·kPa⁻¹ for oxygen (Manahan 1984). Whereas, the solubility of carbon dioxide is strictly dependent on the pH. Carbon dioxide dissolved in water is converted to carbonic acid, which is further dissociated, forming bicarbonate and carbonate ions plus protons. The degrees of carbonic acid dissociation, and, consequently, the overall solubility of carbon dioxide, increase with pH. As the COD loading increased, the pH of the mixed liquor decreased and thus more carbon dioxide was dissolved. As a consequence, the methane content in the biogas decreased as COD loading increased, from about 60% at 10 g-COD·L⁻¹·d⁻¹ to about 52% at 120 g-COD·L⁻¹·d⁻¹, in both reactors.

Sludge Yield

The net sludge yield in a reactor is usually estimated by monitoring the COD removal as well as the VSS contents in both the reactor and the effluent. The amount of VSS ac-

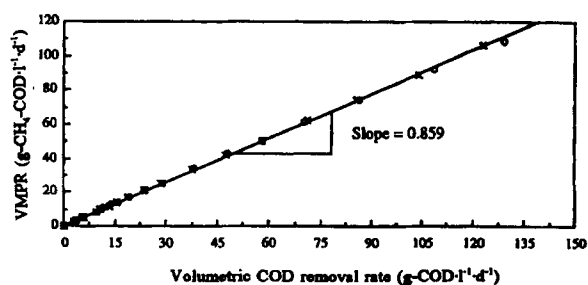


FIG. 7. VMPR versus Volumetric COD Removal Rate. [UASB (○); modified-AF reactor (×)]

accumulated inside the reactor plus those washed out divided by the total COD removed in the same period equals the net sludge yield. However, the accuracy of this conventional means of estimation is strongly dependent on the reliability of the VSS data. In reality, obtaining accurate data of VSS in both reactor and effluent is not easy. However, the net sludge yield in an anaerobic reactor can be estimated by another means without any VSS measurements. One may assume that during anaerobic degradation all the COD removed are converted to either methane, carbon dioxide or biomass. The COD equivalent for the biomass inside the reactor can be directly measured, while the COD equivalent is 4 g-COD/g for methane, and nil for carbon dioxide. Thus, the net sludge yield can thus be estimated from three sets of data, i.e. methane production, the total COD removed, and the COD equivalent of the biomass, all of which can be accurately measured. This method has been used to estimate the net sludge of various fatty acids (Chui et al. 1994; Fang et al. 1995a,b) and hydrolyzed proteins (Fang et al. 1994b).

Fig. 7 illustrates that the volumetric methane production rate (VMPR) increased linearly with the volumetric COD removal rate with a slope of 0.859 for both reactors. This slope indicates that of all the COD removed, consistently 85.9% was converted to methane, over an extended period covering a wide range of loading rate. The remaining 14.1% could only be converted to biomass. Since each gram of starch-degrading granules in the reactors was found equivalent to 1.40 g of COD, the net sludge yield was estimated as 0.10 g-VSS/g-COD (0.141/1.40), which is comparable to the 0.13 g-VSS/g-COD of the UASB granules treating wastewater containing sucrose estimated by Hulshoff Pol (1989), and the 0.08–0.23 g-VSS/g-COD for the anaerobic sludge treating wastewater of carbohydrates estimated by Henze and Harremoës (1983).

Specific Methanogenic Activity and Microbial Populations

Formate, acetate, propionate, and butyrate are among the key intermediates in anaerobic degradation of complex sub-

strate (Thiele and Zeikus 1988). Table 2 summarizes the SMA of the granules using these individual VFA, as well as sucrose and starch, as the sole organic substrate. Corresponding data in literature on UASB granules treating carbohydrates are also included for comparison.

Using individual VFA, sucrose, or starch as substrate, the SMA of the starch-degrading granules obtained from the UASB and modified-AF reactor were similar, as one would have expected, since the operating conditions applied to the reactors were identical. The SMA of the starch-degrading granules, as well as other sugar-degrading granules, were substantially higher than those of the seed sludge (digester sludge). This was due to the densely populated active biomass inside the granules, which had been acclimated to using starch and other intermediates as substrates.

Sayed (1987) suggested that the adsorption of colloidal materials on the surface of granules would hamper the supply of substrate to the bacteria at the interior of the granules, which might, as a result, deteriorate the methanogenic activity of the granules. In Sayed (1987), the SMA of granular sludge decreased from 0.3 to 0.1 g-methane-COD·g-VSS⁻¹·d⁻¹ after treating slaughterhouse wastewater with coarse suspended solids. In the present study, however, there was no noticeable adverse effect by the starch particulates on the SMA. This may, again, be attributed to the readily biodegradable nature of the cornstarch.

The specific methane production rate of the granules inside the reactor could be calculated from the methane production rate and the corresponding VSS concentration. At 120 g-COD·L⁻¹·d⁻¹, the biomass in both reactors had a specific methanogenic activity of 1.3 g-methane-COD·g-VSS⁻¹·d⁻¹, which was about only two-thirds of the SMA (1.90–1.92 g-methane-COD·g-VSS⁻¹·d⁻¹) measured in serum vials using starch as substrate. The discrepancy was due to the fact that the SMA was measured in serum vials in which the substrate supply, unlike in the reactor, was not a rate-limiting factor.

Table 3 shows that the microbial populations in the granules of the present study are, in general, comparable to those in literature for UASB granules treating wastewaters containing carbohydrates. In the granules of the present study, the three distinct groups of bacteria—namely acidogens, syntrophic acetogens, and methanogens—were in the order of 10⁸–10⁹ per mL (MPN). Like SMA measurements, the microbial populations of the granules obtained in both reactors of the present study were nearly identical.

Microstructure of Granules

SEM and TEM micrographs illustrate that there was little difference on the microstructure of the granules obtained from the two reactors in the present study. The granules had a layered microstructure (Fang et al. 1994a; MacLeod et al. 1990). The outer layer was approximately 10–20 μm in thick-

TABLE 2. Specific Methanogenic Activity (SMA) of Granules Using VFA and Carbohydrates As Substrates

Sludge origin (1)	SMA (g-CH ₄ -COD·g-VSS ⁻¹ ·d ⁻¹) Using Substrate						Reference (8)
	Formate (2)	Acetate (3)	Propionate (4)	Butyrate (5)	Sucrose (6)	Starch (7)	
Digester sludge	0.11	0.16	0.07	0.20	0.16	0.19	This study
UASB	1.94	1.91	0.18	2.05	0.95	1.92	This study
AF-USAB	1.86	1.80	0.18	2.00	0.94	1.90	This study
UBF	1.98	2.26	0.19	2.33	0.99	1.96	Fang and Kwong (1995)
Sucrose	1.22	1.20	0.52	0.61	0.85	—	Fang et al. (1994)
Brewery	1.26	0.40	0.13	0.12	0.32	—	Fang et al. (1994)
Sugar	1.01	0.90	0.41	—	—	—	Dolfing et al. (1985)
Maize starch	0.74	0.09	0.12	—	—	—	Stams et al. (1989)
Starch	—	0.18–0.88	—	—	—	—	Dubourguier et al. (1988)

TABLE 3. Bacterial Counts on Granules in Reactors of This Study and Those Treating Soluble Carbohydrate

Metabolic group (1)	Substrate (2)	Number of Organisms per mL of Granular Sludge				
		UASB (3)	Modified-AF (4)	Use A ^a (5)	Use B ^b (6)	Use C ^c (7)
Acidogens	starch particulates	4.5×10^8	4.5×10^8	—	—	—
—	Sucrose	4.5×10^8	4.5×10^8	1.1×10^9	10^{10}	9.5×10^9
Methanogens	H ₂ /CO ₂	4.5×10^8	4.5×10^8	2.5×10^8	10^9	4.5×10^9
—	acetate	4.5×10^8	4.5×10^8	2.5×10^8	10^8	4.5×10^9
—	formate	1.5×10^9	9.5×10^8	—	—	—
Syntrophs	propionate	9.5×10^7	9.5×10^7	2.5×10^8	10^7	4.5×10^9
—	butyrate	1.5×10^8	1.5×10^8	1.1×10^8	10^7	2.0×10^{10}

^aUASB granules treating starch industry wastewater (Dubourgier et al. 1988).

^bUASB granules treating liquid waste from a sugar factory (Dolfing et al. 1985).

^cUSAB granules treating synthetic wastewater of sucrose (Fang et al. 1994).



FIG. 8. Streptococci on Surface Layer of Granule (SEM, Bar = 4 μm)

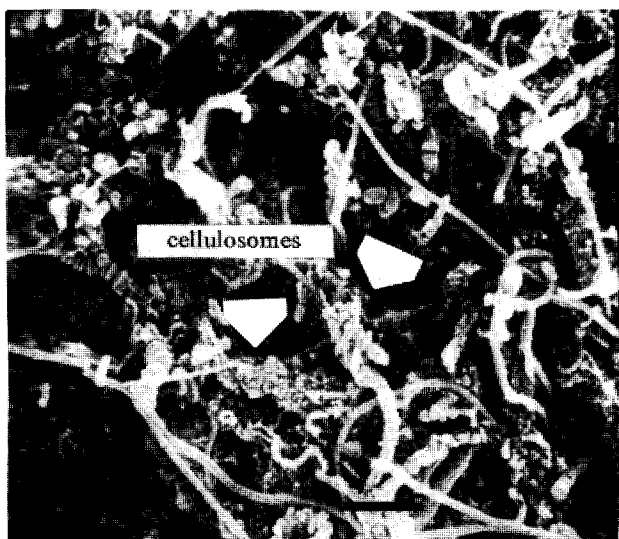


FIG. 9. Plan View of Granule Showing Presence of Cellulosome (Marked with Arrows; SEM, Bar = 4 μm)

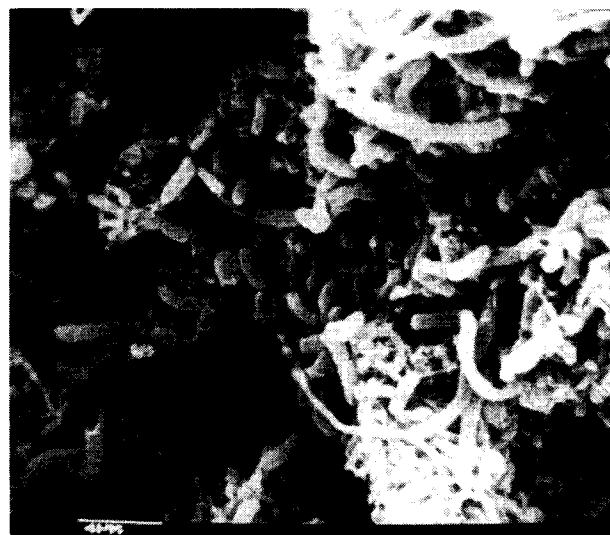


FIG. 10. Syntrophic Associations of Bacteria under SEM (Bar = 4 μm)



FIG. 11. Methanotrix in Granule Interior (SEM, Bar = 4 μm)

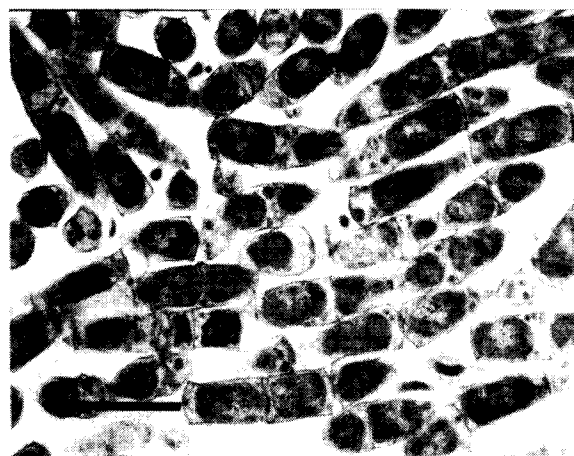


FIG. 12. Methanotrix in Granule Interior (TEM, Bar = 2 μm)

ness and was predominantly composed of streptococci ($\approx 0.7 \mu\text{m}$ in diameter) (Fig. 8), which was, presumably, responsible for carrying out the initial hydrolysis of starch particulates. Plan view of the surface of granules (Fig. 9) shows the presence of cellulosome-like aggregates (marked by arrows), which

are discrete multicomponent enzymatic complex that effect the hydrolysis of cellulose and its derivatives. These cellulosomal aggregates locate on the cell surface where they promote adherence of the bacterial cells to the cellulose (Lamed et al. 1987; Coughlan and Ljungdahl 1988; Wu and Demain 1988; Wu et al. 1988). Their presence in the starch-degrading granules suggests that cellulosomal aggregates are likely also required to promote the adherence of bacteria and starch.

The middle layer, which had a thickness of 100–200 μm , was composed of long thin filaments, small rods, cocci, syntrophic microcolonies, along with scattered streptococci, as illustrated in Fig. 10. The interior core of the granule was predominantly composed of *Methanothrix*, as illustrated in Figs. 11 and 12, which could be identified by its size (0.8 μm in diameter and 1.8–2.0 μm in length) and its bamboo-shaped morphology.

CONCLUSION

The following conclusions can be drawn regarding the treatment of wastewater containing high concentrations of corn starch particulates in a UASB and a modified-AF reactors at 37°C and pH 6.8–7.9:

1. The two reactors exhibited different biomass concentration profiles in the reactor, but had little difference on the COD removal efficiency and on the characteristics of sludge granules. The cornstarch particulates in the wastewater, despite its insoluble nature, did not inhibit the sludge granulation nor impair the COD removal efficiency in both reactors.

2. The UASB and the modified-AF reactors both removed 95.3% of soluble COD from wastewater containing starch as the sole organic substrate at loading rates up to 90 g-COD $\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. There was no noticeable accumulation of starch particulates in the reactor. At loading rates up to 75 g-COD $\cdot\text{L}^{-1}\cdot\text{d}^{-1}$, only 0.42% of starch particulates remained unhydrolyzed and were washed out in the effluent in the UASB reactor, and 0.80% in the modified AF reactor. Of all the COD removed in both reactors, about 85.9% was converted to methane, and the remaining 14.1% was converted to biomass with a sludge yield of 0.10 g-VSS/g-COD.

3. The achievement of effective COD removal at high loading rates in the present study was probably due to the readily biodegradable nature of the starch, the balanced nutrients, the systematic acclimation strategy during start-up, and the built-in GLS separator, which effectively retained sludge granules in both reactors.

4. The granules from both reactors exhibited similar SMA, microbial populations, and microstructures. The SMA using either individual VFA or sucrose as substrate were in general higher than those of granules treating soluble carbohydrates. The bacterial populations of acidogens, syntrophic acetogens, and methanogens were in the order of 10^8 – 10^9 (MPN) per mL of granules.

5. The granules exhibited a layered microstructure with a surface covered with large amount of cellulosome. The outer layer of the granules were composed of large amount of streptococci, which were likely responsible for the formation of cellulosome to facilitate the starch hydrolysis and also for the subsequent acidogenesis of starch.

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