UASB Treatment of Wastewater with Concentrated Mixed VFA

By Herbert H. P. Fang,[1] Member, ASCE, Yu You Li,[2] and Ho Kwong Chui[3]

Abstract: The upflow anaerobic-sludge blanket (UASB) process consistently removed 97–99% of chemical oxygen demand (COD) from wastewater containing concentrated mixed volatile fatty acids (VFA) at 37°C at loading rates of up to 24 g-COD/(L·d), corresponding to a food/microorganism ratio of 0.78 g-COD/[g–volatile suspended solids (VSS)·d]. It suggested that, with preacidification, the UASB process can be effective for a wide variety of wastewaters. The COD removal efficiency deteriorated at higher loading rates; there was no butyrate in the effluent, suggesting that butyrate degradation was not a rate-limiting step. Of the COD removed, 92.6% was converted to methane; the rest was converted to granular biomass with an average yield of 0.054 g-VSS/g-COD. The granules had a size of 1–2 mm and settled satisfactorily. Each gram of granule in the reactor was capable of converting a daily maximum of 0.86 g of COD into methane. The granules had a fluffy surface mostly composed of interwound filamentous *Methanothrix*-like bacteria. Syntrophic associations between *Methanothrix*-, *Methanospirillum hungatei*-, and *Syntrophobacter*-like bacteria were prevalent in the granule interior. The syntrophic relation between these species was elucidated by thermodynamics.

INTRODUCTION

Anaerobic degradation of complex pollutants in wastewater involves three phases: hydrolysis/acidification, acetogenesis, and methanogenesis. Complex pollutants are first hydrolyzed and acidified by acidogenic bacteria forming volatile fatty acids (VFA), which are then further converted to acetate and CO_2/H_2 by acetogenic bacteria. Finally, both acetate and CO_2/H_2 are converted by methanogenic bacteria into methane. Pohland and Ghosh (1971) suggested a two-phase anaerobic process treating complex wastewater in two separate reactors, one for hydrolysis/acidification and the other for acetogenesis/methanogenesis. It was found that such a two-phase process had higher efficiency (Aoki and Kawase 1991; Sutton and Li 1983), better stability to shock load (Bull et al. 1984), and less inhibition by lipids (Komatsu et al. 1991) as compared to the conventional single-phase treatment.

In a two-phase process, the VFA produced from the first reactor, which is mainly composed of butyrate, propionate, and acetate, become the substrates to the acetogenic and methanogenic bacteria in the second reactor. However, degradation of propionate and butyrate to acetate is thermodynamically unfavorable unless the other product, hydrogen, can be readily removed (Boone and Bryant 1980). In the case of propionate degradation, the hydrogen partial pressure must be kept under 10^{-4} atmosphere. Thus, degradation of propionate, and to a lesser degree, butyrate, requires syntrophic associations of hydrogen-producing acetogens in juxtaposition with the hydrogen-consuming methanogens (Thiele and Zeikus 1988). Furthermore, propionate is known to have the lowest tolerance level among the VFA for the anaerobic bacteria (Bajpai and Iannotti 1988). It is easily accumulated in anaerobic digesters during overloading and is difficult to remove during recovery (McCarty and Mosey 1991).

Although extensive research studies were conducted on the kinetics of VFA degradation (Ahring and Westermann 1987; Heyes and Hall 1983; Lawrence and McCarty 1969; Lin et al. 1986), as well as on the inhibitory effect of hydrogen, acetate, propionate and butyrate (Ahring and Westermann 1988; Fukuzaki et al. 1990; Stams et al. 1992; van Lier et al. 1993), little is known about the maximum loading rate of VFA in a high-rate anaerobic reactor. Among the high-rate anaerobic reactors, the upflow anaerobic-sludge blanket (UASB) process (Lettinga et al. 1980; Hulshoff Pol and Lettinga 1986; Fang et al. 1990) has gained popularity in recent years with over 200 installations worldwide (Lettinga and Hulshoff Pol 1991). In a UASB reactor, bacteria aggregate and form a granular ecosystem that, among its other advantages, may facilitate syntrophic association of various bacteria (Fang et al. 1994). However, only two studies have been reported so far on the treatment of mixed VFA in a UASB reactor under mesophilic

[1]Sr. Lect., Civ. and Struct. Engrg. Dept., The Univ. of Hong Kong, Pokfulam Rd., Hong Kong.
[2]Res. Assoc., Civ. and Struct. Engrg. Dept., The Univ. of Hong Kong; presently Assoc. Prof., Civ. Engrg. Dept., Tohoku University, Sendai, Japan.
[3]Res. Student, Civ. and Struct. Engrg. Dept., The Univ. of Hong Kong; presently Envir. Protection Ofcr., Envir. Protection Dept., Hong Kong.

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conditions. The scopes of both studies were quite limited: one was conducted at a moderate loading rate of 7–9 g-chemical oxygen demand (COD)/[g-volatile suspended solids (VSS)·d] to examine the effect of sulfate and calcium (Thiele et al. 1990), and the other was conducted at low pH to control the sludge bulking (Brummeler et al. 1985).

This study was conducted to thoroughly investigate the performance of the UASB process in treating wastewater containing concentrated mixed VFA, including the efficiency of COD removal at high loading rates and the characteristics of the granules, such as the microstructure (MacLeod et al. 1990; Grotenhuis et al. 1991), the specific methane production rate (SMPR) inside the reactor, and the specific methanogenic activity (SMA) (Dolfing and Mulder 1985; Dolfing and Bloemen 1985).

MATERIALS AND METHODS

The UASB reactor used in this study, as illustrated in Fig. 1, was 2.8 L in volume with an internal diameter of 84 mm and a height of 500 mm. Five evenly distributed sampling ports were installed over the height of the column. Total biomass in the reactor was estimated periodically based on the profile of the VSS of the samples taken from these ports (Fang and Chui 1993a). On top of each reactor was a gas-liquid-solid separator with an internal diameter of 114 mm and a height of 250 mm, making a filled volume of 2.0 L. Volumetric loadings were calculated basing on the reactor volume alone, excluding volume of the separator. The reactor was water-jacketed and operated at a constant temperature of 37°C.

Prior to the present study, flocculent sludge from an anaerobic-sludge digester of the Shatin Wastewater Treatment Works, Hong Kong, was partially granulated in a 65-L UASB reactor for two months, using sucrose as the organic substrate. About 1.5 L of this partially granulated sludge was used to seed the reactor. The reactor was continuously fed with synthetic wastewater by a peristaltic pump. A mixture of acetate, propionate, and butyrate was used as the sole organic substrate. The COD levels in the wastewater ranged from 3,000–16,500 mg/L. At all levels of COD, the three VFA were kept at a constant COD ratio of acetate:propionate:butyrate = 2:1:1 throughout the study. Trace metals and balanced nutrients were added to the wastewater following the formula reported in a previous study (Fang and Chui 1993a). The pH of the wastewater was kept within the range of pH 6.0–6.5 by dosing sodium bicarbonate into the

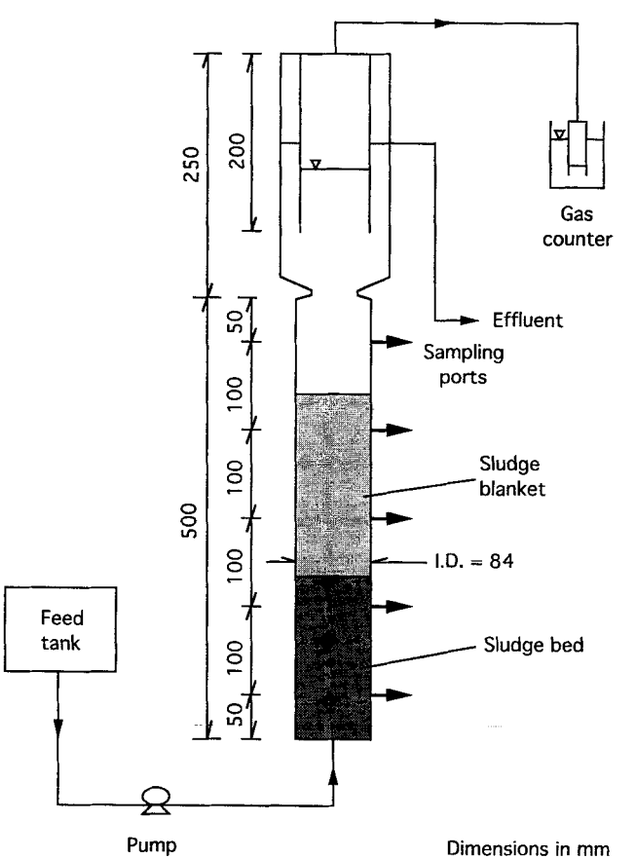

FIG. 1. UASB Reactor

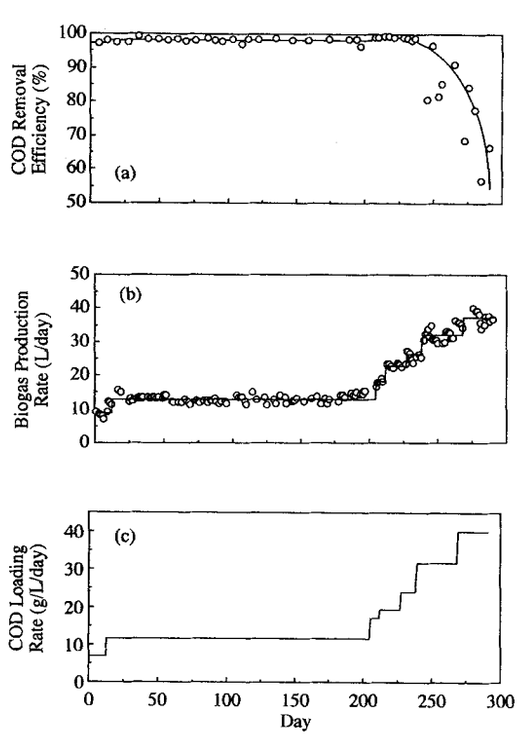

FIG. 2. Performance of UASB Reactor: (a) COD Removal Efficiency; (b) Biogas Production Rate; (c) COD Loading Rate

wastewater. The sampling strategies and analytical procedures, such as the biogas content, the VFA levels in effluent, etc., also followed those reported previously (Fang and Chui 1993a).

Throughout the present study, the hydraulic retention time (HRT) was kept within the range of 10–12.5 h, and the VFA concentrations in the effluent were closely monitored. The COD loading rate to the reactor was 7 g/(L·d) initially, with 3,000 mg/L of COD in the wastewater. The loading rate was increased only when the COD removal efficiency reached 80% and the acetate concentration was maintained below 500 mg/L. Granule samples were taken for microstructure examination and for SMA analysis on day 190 after the reactor had been operated at 12 g-COD/(L·d) for about six months.

The microstructure of the UASB granules was examined by scanning and transmission electron microscopies (SEM and TEM). The instruments and the sample preparation procedures were reported previously (Fang and Chui 1993b). The SMA of the granules were measured in duplicate based on the method (Dolfing and Mulder 1985; Hwang and Cheng 1991) modified from the one proposed originally by Owen et al. (1979). The SMA measures the methanogenic activity of the granules in serum vials for a specific substrate at a high concentration at which level the availability of substrate is not a limiting factor. 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, propionate, butyrate, and mixed VFA, were used individually as the substrate in this study.

RESULTS AND COMMENTS

COD Removal Efficiency

Fang and Chui (1993a) reported that the COD removal efficiency of a UASB reactor was mainly dependent on the COD loading rate, and was not sensitive to either the hydraulic retention time (HRT) or the wastewater COD level alone. In this study, COD loading rate was increased by increasing the COD level in wastewater, or by reducing the HRT which was kept within 10–12.5 h. Because the reactor was seeded with partially granulated sludge, sludge in the reactor was readily granulated during the startup. A sludge bed appeared within 30 days.

Fig. 2 illustrates the following throughout this study: (1) The efficiency of COD removal; [Fig. 2(a)]; (2) the total biogas production rate [Fig. 2(b)]; and (3) the COD loading rate [Fig. 2(c)]. The removal of COD was calculated from the soluble COD in the effluent and the total COD in the wastewater, which was solely due to the dissolved mixed VFA. Throughout the study, the pH of the reactor was within pH 7.4–8.0, as compared to pH 6.0–6.5 in the wastewater. The increase of pH was due to the conversion of stronger acids, namely mixed VFA, to a weaker acid, carbonic acid. The COD loading increased stepwise from the initial 7 g-COD/(L·d) to 40 g-COD/(L·d). The amount of biogas produced increased with COD loading rates. In the first 240 days, 97–99% of COD was removed consistently. The efficiency of COD removal deteriorated after day 240 as the loading rate further increased. The experiment was terminated on day 290, when the COD removal efficiency decreased to the level of about 70%.

Fig. 3 illustrates at various loading rates: (a) The COD removal efficiency; (b) concentration

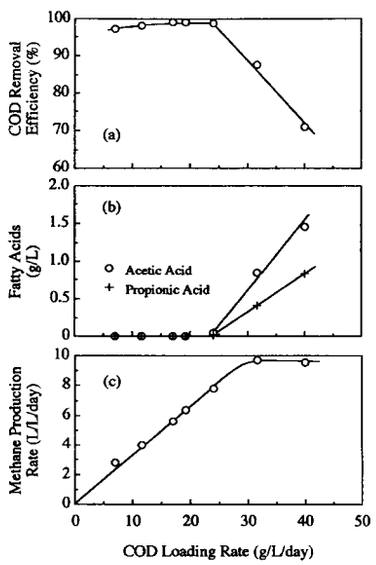

FIG. 3. Performance of UASB Reactor at Various COD Loading Rates: (a) COD Removal Efficiency; (b) VFA Concentrations in Effluent; (c) Methane Production Rate

of VFA in the effluent; and (c) the methane production rate. Fig. 3(a) illustrates that the COD removal efficiency was maintained at 97–99% up to 24 g-COD/(L·d), which corresponded to a food-to-microorganism (F/M) ratio of 0.78 g-COD/(g-VSS·d). Beyond this loading rate, the efficiency decreased as loading rate increased. It decreased to about 70% at 40 g-COD/(L·d), corresponding to an F/M ratio of 1.30 g-COD/(g-VSS·d). Fig. 3(b) illustrates that the acetate and propionate levels in the effluent were below 50 and 20 mg/L, respectively, for loading rates up to 24 g-COD/(L·d); but at 40 g-COD/(L·d), the residual acetate and propionate both increased sharply to the levels of 1,500 mg/L and 800 mg/L, respectively. Fukuzaki et al. (1990) reported that inhibition of propionate on its own degradation to methane was dependent on the pH; at pH 7.6, propionate had no inhibition effect up to approximately 3,500 mg/L. Thus the accumulation of residual acetate and propionate in the effluent was likely, not due to the inhibition of propionate, but due to the high loadings of mixed VFA exceeding the methanogenic activity of the biomass. Butyrate was not detected in the effluent throughout the experiment. The absence of butyrate in the effluent at loading rate as high as 40 g-COD/(L·d) suggested that degradation of butyrate to acetate was not a rate-limiting step.

Methane Production and COD Balance

The biogas collected throughout this study was composed of 78–85% methane, 2% nitrogen, and CO_2, which made up the balance. Fig. 3(c) illustrates that the volumetric methane production rate increased linearly with the COD loading rate, until it reached a maximum level of 9.7 L/(L·d) at 30 g-COD/(L·d). The SMPR was calculated from the COD equivalent of the daily methane production (each gram of methane is equivalent to four grams of COD) and the total biomass inside the reactor estimated from the VSS profile. It reflected the overall bioactivity of the granules in the reactor under a given operating condition. Fig. 4 illustrates that the SMPR increased linearly with the specific substrate utilization rate (SSUR) with a slope of 0.926, until it reached a maximum level of approximately 0.86 g-COD/g-VSS/d). Of all the COD removed, an average of 92.6% was converted to methane, judging from the slope in Fig. 4, and the remaining 7.4% was presumably converted to biomass. Since the biomass in the reactor was measured to have a COD/VSS ratio of 1.38, the sludge yield was estimated as 0.054 g-VSS/g-COD (0.074/1.38 = 0.054), which was comparable to the yield values on 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), as reported by Lawrence and McCarty (1969). The methane conversion and the yield observed in this study were also comparable to the corresponding data, i.e., 94% and 0.05 g-VSS/g-COD, respectively, reported in a recent study on the UASB treatment of formate in wastewater (Chui et al. 1994).

Specific Methanogenic Activity

Table 1 summarizes the SMA of the mixed-VFA-degrading granules measured in serum vials in duplicate using five different substrates, i.e., formate, acetate, propionate, butyrate, and mixed VFA. The SMA is an indicator of the methanogenic activity of the biomass under a condition in which the supply of substrate is not a limiting factor. Corresponding literature data of SMA of UASB granules treating various types of wastewaters are also shown for comparison. As shown in Table 1, each gram of the mixed-VFA-degrading granules was capable of producing daily 0.81 g of methane-COD from butyrate, but only 0.35 g of methane-COD from propionate, suggesting that butyrate has a higher rate of degradation than propionate. This is consistent with the observation that, at the loading rate of 30 g-COD/(L·d) or higher, residual propionate was found in the effluent [as illustrated in Fig. 3(b)], but there was no detectable residual butyrate.

The SMA using acetate as substrate was 1.17 g-methane-COD/(g-VSS·d), indicating that the granules had a high acetate-degrading capability. This was evidenced by the absence of acetate in the effluent at loading rates up to 24 g-COD/(L·d). However, residual acetate was found in the effluent at the loading rates of 30 g-COD/(L·d), or higher. This was because the acetate concentration was significantly higher than the propionate and butyrate concentrations in the

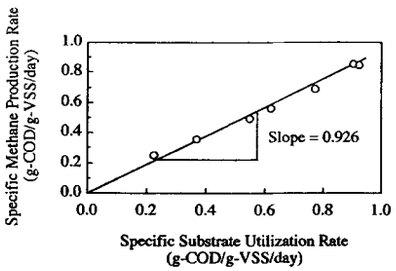

FIG. 4. Specific Methane Production Rate at Various Specific Substrate Utilization Rates

TABLE 1. SMA of Granules Using Fatty Acids as Substrate

Substrate/ wastewater (1)	SMA (g-methane-COD/g-VSS/day)					Reference sources (7)
	Formate (2)	Acetate (3)	Propionate (4)	Butyrate (5)	Mixed VFA (6)	
Mixed VFA	0.73	1.17	0.35	0.81	1.03	Present study
Formate	2.90	n/d[a]	n/d[a]	n/d[a]	n/d[a]	Chui et al. (1994)
Propionate	n/a[b]	1.89	1.78	1.32	n/a[b]	Grotenhuis et al. (1991)
Propionate	n/a[b]	0.55	0.52	n/a[b]	n/a[b]	Dolfing and Bloemen (1985)
Sugar	1.01	0.90	0.41	n/a[b]	n/a[b]	Dolfing and Bloemen (1985)
Sucrose	0.76	0.30	0.22	n/a[b]	n/a[b]	MacLeod et al. (1990)
Brewery	1.26	0.49	0.13	0.12	n/a[b]	Fang et al. (1993)
Maize starch	0.74	0.09	0.12	n/a[b]	n/a[b]	Stams et al. (1989)

[a]n/d = not detectable.
[b]n/a = not available.

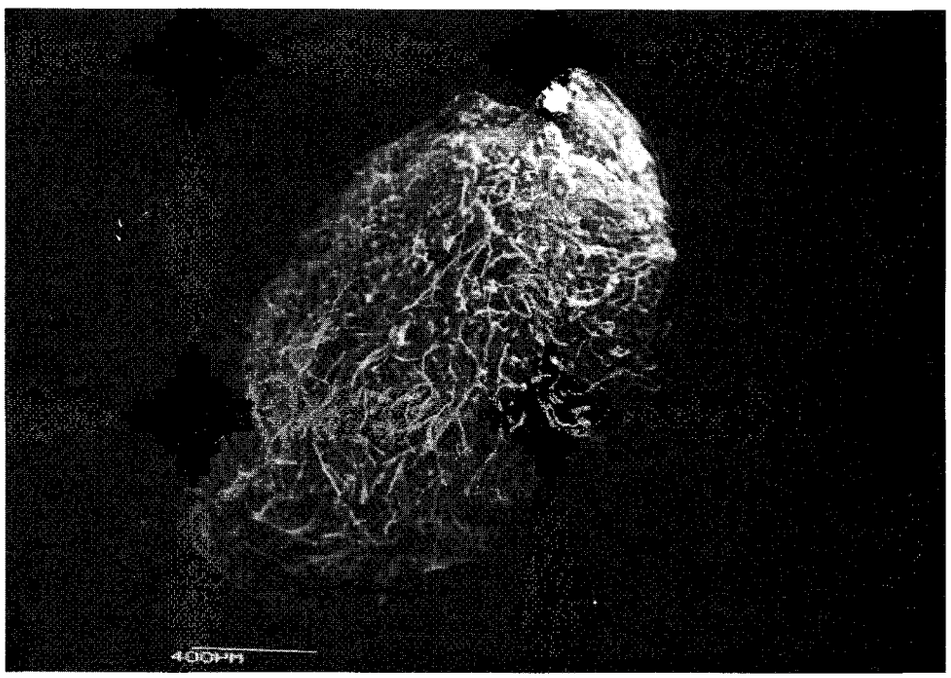

FIG. 5. *Methanothrix*-Like Bacteria at Granule Surface under SEM

wastewater; furthermore, degradation of propionate and butyrate produced additional quantities of acetate.

Table 1 shows that the SMA using mixed VFA as substrate was 1.03 g-methane-COD/(g-VSS·d), which was slightly higher than the maximum SMPR of 0.86 g-methane-COD/g-VSS/d in the reactor, as illustrated in Fig. 4. The difference is likely due to the fact that the solution used in the SMA tests in serum vials had high concentration of substrate and, thus, the supply of substrate was not a limiting factor. On the other hand, the SMPR reflected the methanogenic activity of the granular biomass inside the UASB reactor, in which the substrate concentration in the mixed liquor was lower.

Microstructure of Granules

Granules treating mixed VFA had a size of 1–2 mm and settled satisfactorily. Unlike granules treating sugar (MacLeod et al. 1990) and brewery wastewater (Fang et al. 1993) which exhibited a layered structure, granules from this study did not exhibit any patterned structure. Also unlike the granules treating sugar (Fang and Chui 1993a) and brewery wastewater (Fang et al. 1993) that exhibited a densely packed surface, granules from this study exhibited a fluffy surface comprised mostly of interwound filamentous *Methanothrix*-like bacteria, as illustrated in Fig. 5. *Methanothrix* is an acetoclastic bacterium, which can use acetate solely as substrate. With a very low half-rate constant of 30 mg-COD/L (Gujer and Zehnder 1983), *Methanothrix* outcompetes other methanogenic bacteria, such as *Methanosarcina*, when the acetate concentration is low, as in the mixed liquor of the UASB reactor and the interior of the granule in this study. This was also evidenced by the absence of *Methanosarcina* in the granules.

Thermodynamic analysis (Thiele and Zeikus 1988) has indicated that the degradation of

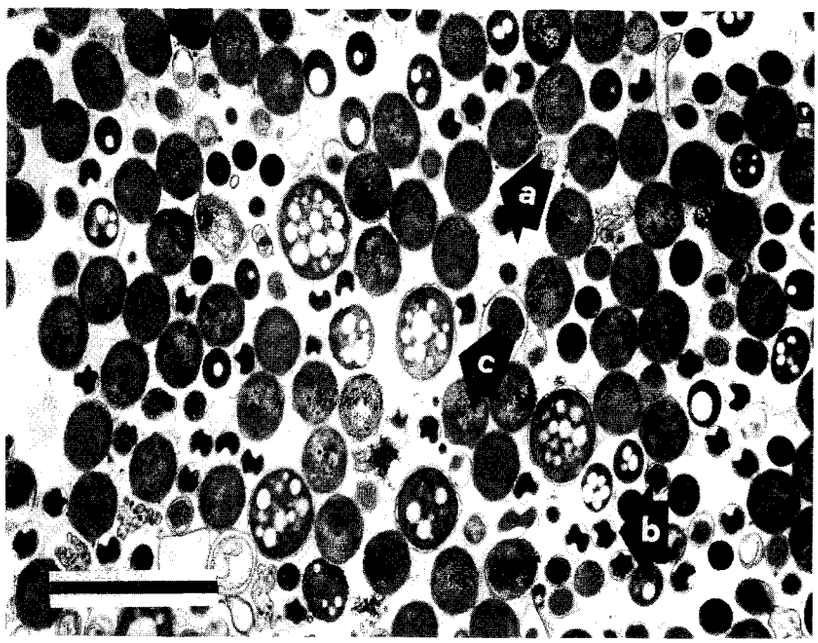

FIG. 6. Juxtapositioned Syntrophic Microcolonies of *Methanothrix*-Like Bacteria in Juxtaposition with *Methanospirillum hungatei*- and *Syntrophobacter*-Like Bacteria under TEM (Bar = 2 μm)

butyrate and propionate requires the syntrophic associations between the hydrogen-producing acetogens and the hydrogen-consuming methanogens. For the degradation of propionate in particular, in order to minimize the possible local buildup of hydrogen, juxtapositioned syntrophic association between these two groups of bacteria is required (Grotenhuis et al. 1991). Such a syntrophic relationship between different species of bacteria was evident in the granules obtained in this study. Juxtapositioned syntrophic associations involving three different species, as illustrated in Fig. 6, were prevalent. Presumably, the *Syntrophobacter*-like bacteria (arrow c in Fig. 6) degraded propionate to form acetate, hydrogen, and CO_2; the adjacent *Methanothrix*-like bacteria (arrow a) readily consumed the acetate, whereas the *Methanospirillum hungatei*–like bacteria (arrow b) readily consumed hydrogen and CO_2, to form the final product, methane.

Syntrophobacter wolinii (Boone and Bryant 1980), which has a width of 0.6–1.0 μm and a length of 1.0–4.5 μm, is the only propionate-degrading syntrophic bacteria identified so far. As illustrated in Fig. 6, the *Syntrophobacter*-like bacteria had the size of 1.0–1.4 μm. The aceto-clastic *Methanothrix*-like bacteria (0.6 μm) had a bamboo-shaped morphology (Zehnder et al. 1980). The hydrogen-consuming *Methanospirillum hungatei*–like bacteria (0.3 μm), also illustrated in Fig. 6, exhibited its well-documented features, such as the spacer between adjacent cells, structural elements in the cell spacer, flagella through the end plug, and the cell division mechanism (Zeikus and Bowen 1975; Beveridge et al. 1987; Southam et al. 1990).

CONCLUSION

The following conclusions are drawn regarding the UASB treatment of wastewater containing mixed VFA at 37°C:

1. Results from this study suggest that, with preacidification, the UASB process can be effective for a wide variety of wastewaters. It consistently removed 97–99% of the mixed VFA at loading rates up to 24 g-COD/(L·d), corresponding to an F/M ratio of 0.78 g-COD/(g-VSS·d).
2. The COD removal efficiency deteriorated at loading rates of 30 g/(L·d) and higher. At these loading rates, only residual acetate and propionate, and no butyrate, were found in the effluent. The absence of butyrate suggested that butyrate degradation was not a rate-limiting step.
3. Of all the COD removed, 92.6% was converted to methane. The rest was converted to biomass, with an average yield of 0.054 g-VSS/g-COD. The SMPR analysis showed that each gram of granule in the reactor was capable of converting a daily maximum of 0.86 gram of COD in wastewater into methane.
4. Using the same mixed VFA as a substrate, the SMA measured was 1.03 g-methane-COD/(g-VSS·d). The SMA measurement was higher than the SMPR, because the substrate concentration in the serum vials was higher than that in the UASB reactor. The

SMA using formate, acetate, propionate, and butyrate, individually, as substrate were 0.73, 1.17, 0.35, and 0.81 g-methane-COD/g-VSS/d, respectively.

5. Degradation of mixed VFA granules produced a fluffy surface that was mainly made of interwound filamentous acetoclastic *Methanothrix*-like bacteria. Juxtapositioned syntrophic associations between *Methanothrix-*, *Methanospirillum hungatei*–, and *Syntrophobacter*-like bacteria were prevalent in some microcolonies. The syntrophic relation between these species was elucidated by thermodynamics.

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