REMOVAL OF FORMATE FROM WASTEWATER BY ANAEROBIC PROCESS

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ABSTRACT: Formate in wastewater was effectively removed at 37°C by the upflow anaerobic sludge blanket (UASB) process. This was accomplished by keeping the recycle ratio at 3.0 and by lowering the pH of incoming wastewater to pH 3.8. The COD removal efficiencies were 97–98% at loading rates of 10–20 g-COD$\cdot$L$^{-1}\cdot$day^{-1}, but were reduced to 86–90% at 29–65 g-COD$\cdot$L$^{-1}\cdot$day^{-1}. When the loading was further increased to 77 g-COD$\cdot$L$^{-1}\cdot$day^{-1}, the reactor abruptly failed due to the sudden decrease of the pH. About 94% of COD of the formate removed was converted to methane with a sludge yield of 0.05 g-VSS$\cdot$g-COD^{-1}. The small sludge granules settled satisfactorily and had a maximum specific methane production rate (SMPR) of 2.06 g-COD$\cdot$VSS$^{-1}\cdot$day^{-1}. The specific methane activity (SMA) of granules using formate as substrate was 2.90 g-COD$\cdot$g-VSS$^{-1}\cdot$day^{-1}; but the granules were unable to degrade other fatty acids. Microscopic examinations indicated that the granules mainly comprised *Methanobacterium formicicum*–like bacteria, plus a small amount of *Methanococcus vannielii*–like bacteria, and an unknown species of bacterium.

INTRODUCTION

Interest in anaerobic-wastewater–treatment has increased considerably in the past decade. Through the pioneer work of Young and McCarty (1969), several high-rate anaerobic reactors have been developed (Henze and Harremoes 1983; Speece 1983), including the anaerobic filter (Young and McCarty 1969; Young and Yang 1989), the expanded/fluidized bed reactor (Switzenbaum and Jewell 1980; Sutton and Li 1983; Wang et al. 1986; Iza 1991), and the upflow anaerobic sludge blanket (UASB) process (Lettinga et al. 1980; Hulshoff Pol and Lettinga 1986; Fang et al. 1990; Fang and Chui 1993a; Weigant 1986). Among them, the UASB process has gained broad acceptance for the treatment of a variety of industrial wastewaters (Lettinga and Hulshoff Pol 1991).

Formate is one of the pollutants often found in industrial wastewater. This is due to the common uses of formic acid in various manufacturing industries, such as textile scouring, cattle-fodder preservation, chemicals/catalysts manufacturing, prevention of mold growth in silage, grain preservation, and leather processing. On the other hand, formate has been identified as one of the critical intermediates in anaerobic degradation, which involves acidogenesis, acetogenesis, and methanogenesis. In methanogenesis, methane is produced by bacteria using substrates including H$_2$/CO$_2$, formate, methanol, methylamines, and acetate. Of the 43 species of

methanogenic bacteria having been identified so far, 19 species can use either formate or H_2/CO_2 as substrate (Boone and Whitman 1988).

Furthermore, recent study has suggested that the transfer of formate from formate-producing bacteria to formate-consuming bacteria is critical in the anaerobic-degradation processes (Thiele and Zeikus 1988a). For instance, in the degradation of butyrate, the methanogenesis through interspecies formate transfer was estimated about five times higher than those through interspecies hydrogen transfer (Boone et al. 1989); in the degradation of ethanol, it could be eight times higher (Thiele and Zeikus 1988b).

However, despite formate's presence in industrial wastewaters and its critical role in anaerobic degradation, little has been studied on the removal of formate from wastewater by anaerobic processes. This study was carried out to investigate the removal of formate from wastewater by the UASB process. The key operational parameters, the maximum loading capacity, as well as the specific methanogenic activity (SMA) and the microbial composition of the granular sludge were examined.

METHODS AND MATERIALS

A 2.8-L water-jacketed UASB reactor with an internal diameter of 84 mm and a height of 500 mm, as illustrated in Fig. 1, was used for this study. Five evenly distributed sampling ports were installed over the height of the column. Total biomass in the reactor was estimated based on the profile of the volatile suspended solids (VSS) of samples taken from the ports. On top of the 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,
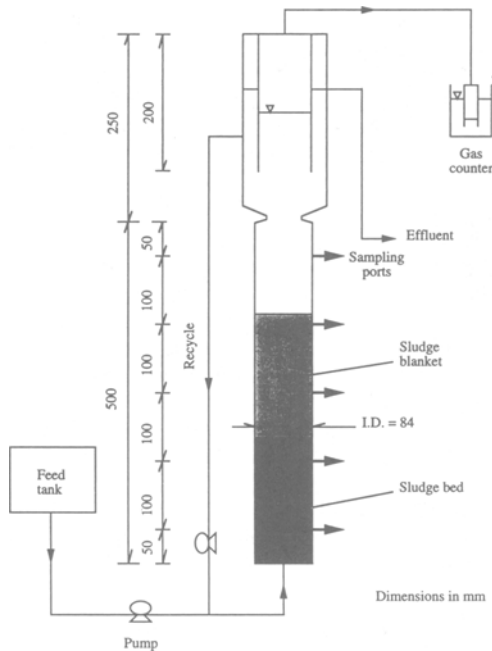

FIG. 1. Process Flow Diagram of UASB Reactor

however, based on the reactor volume alone, excluding volume of the separator. The reactor was operated at 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, Hong Kong, was partially granulated in a 65-L UASB reactor for two months, using sucrose as the substrate. About 1.5 L of this partially granulated sludge was used to seed the reactor. Wastewater was synthesized using formate—initially in the form of sodium formate and in a mixture of sodium formate and formic acid in later experiments—as the sole substrate. Essential nutrients and trace metals were also added to the wastewater, based on the formulation in a previous study (Fang and Chui 1993a). The sampling strategies and the analytical procedures, such as the methane content in biogas, the volatile–fatty-acids (VFA) contents in the effluent, etc., also followed those of in the same report. Part of the effluent was recycled to the bottom of the reactor, except in the first 45 days, to dampen the pH of the incoming wastewater.

The initial loading rate of chemical-oxygen demand (COD) to the reactor was $4.6 \, g \cdot L^{-1} \cdot day^{-1}$, corresponding to $2,200 \, mg \cdot L^{-1}$ of COD in the wastewater and 12 h of hydraulic retention time (HRT). The loading rate was increased stepwise, by either increasing the formate concentration or by decreasing the HRT, when the rate of methane production was consistent and the COD removal efficiency exceeded 80%.

Sludge samples were taken for SMA analyses and microbial examinations on day 190 when the reactor was operated at the steady-state condition at the loading rate of $10.5 \, g\text{-}COD \cdot L^{-1} \cdot day^{-1}$. The SMA of the granules was measured in duplicates in serum vials based on the method (Dolfing and Mulder 1985; Hwang and Cheng 1990) modified from the one proposed by Owen et al. (1979). It is an indicator of the methanogenic activity of the granules for a specific substrate when its supply is not a limiting factor; in this study, formate, acetate, propionate, and butyrate were used individually as the substrate for the SMA analyses. The microbial examinations were conducted by using scanning and transmission electron microscopies (SEM and TEM). The instruments and the sample preparation procedures were as reported previously (Fang and Chui 1993b).

RESULTS AND COMMENT

This study was carried out over a period of 340 days. The start-up phase took about 100 days, during which pH control was found most critical for the effective operation of the reactor. In the remaining period, the COD loading rate was steadily increased until a maximum loading rate was reached. Table 1 summarizes the wastewater characteristics and the operational conditions throughout the study.

Fig. 2 illustrates the operational conditions and the performance of the reactor during the start-up, including the efficiency of COD removal [Fig. 2(a)], production rate of biogas [Fig. 2(b)], methane content in biogas [Fig. 2(c)], acetate concentration in effluent [Fig. 2(d)], and the pH of wastewater and effluent [Fig. 2(e)]. Analyses by gas chromatography indicated that fatty acids other than acetate were not detectable in the effluent.

Adaptability of Using Formate as Sole Substrate

Initially, the reactor was operated at a HRT of 12 h with a wastewater COD of $2,200 \, mg \cdot L^{-1}$, corresponding to a loading of $4.5 \, g\text{-}COD \cdot L^{-1} \cdot day^{-1}$.

TABLE 1. Wastewater COD, HRT, COD Loading Rate, Wastewater pH, and Recycle Ratio During Experiment

Day (1)	Wastewater COD (mg/L) (2)	HRT (h) (3)	COD loading rate ($g \cdot L^{-1} \cdot day^{-1}$) (4)	Wastewater pH (5)	Recycle ratio (6)
1–7	2,200	12	4.5	6.5	0
8–24	5,250	12	10.5	6.5–4	0
25–45	5,250	12	10.5	7–5.5	0
46–69	5,250	12	10.5	4.3	1.5
70–235	5,250	12	10.5	3.8	3.0
236–242	7,500	12	15	3.8	3.0
243–258	10,000	12	20	3.8	3.0
259–268	14,500	12	29	3.8	3.0
269–296	14,500	10	35	3.8	3.0
297–325	14,500	7.5	46	3.8	3.0
326–335	14,500	5.5	63	3.8	3.0
336–340	14,500	4.5	77	3.8	3.0

The COD removal efficiency reached 80% within 7 days. Subsequently, the COD in wastewater was increased to 5,250 $mg \cdot L^{-1}$, corresponding to a loading rate of 10.5 g-$COD \cdot L^{-1} \cdot day^{-1}$. The COD removal efficiency was further increased to 92% on day 17. This indicated the seed sludge was readily able to adapt to formate as the sole substrate.

Control of pH in Mixed Liquor

Formic acid with a pK_a of 3.75 dissociates in wastewater of neutral pH forming formate. Anaerobic conversion of formate to methane consumes acidity, according to the two following equations:

$$4 \, HCOO^- + H^+ + H_2O \longrightarrow methane + 3 \, HCO_3^- \qquad (1)$$

$$HCO_3^- + H^+ \longrightarrow CO_2 + H_2O \qquad (2)$$
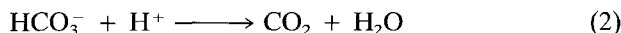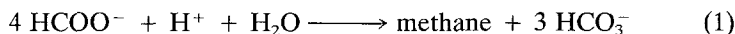
It thus results in an increase of pH in the mixed liquor. However, should the pH increase to the range above 8.0, the activity of the formate-consuming methanogenic bacteria would be suppressed, since the optimum pH for the growth of all but one formate-consuming methanogens is within the range of 6.1–8.0 (Boone and Whitman 1988). In this study, the pH of the mixed liquor was kept within the optimum range, except during the initial start-up, by keeping the wastewater acidic and by recycling part of the effluent with the incoming wastewater. Recycling of effluent is a technique that was applied to UASB process for reducing the wastewater toxicity (Koster 1989) and for supplementing the alkalinity requirement (Sam-Soon et al. 1991).

In the first 10 days of the experiment, the wastewater pH was kept at 6.5. During this period, the pH of the mixed liquor was about 9.0. The pH of the effluent of a UASB reactor approximates that of the mixed liquor, because UASB reactors are normally operated at a near complete-mix mode due to the vigorous mixing action caused by the biogas generated (Fang and Chui 1993a). The methane content in the biogas was 92–97%, because most of the by-product carbon dioxide was converted to bicarbonate due to the high pH in the reactor. Starting on day 11, the pH of the wastewater was lowered by replacing a fraction of sodium formate in the wastewater with
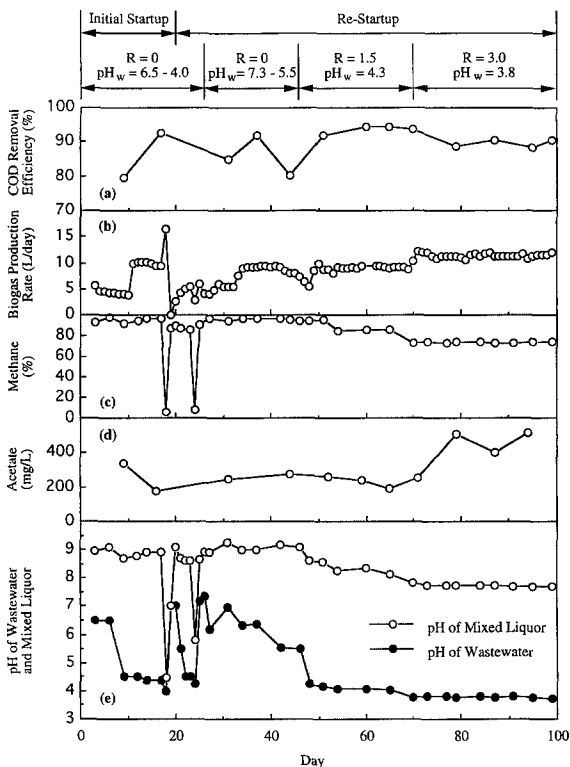
FIG. 2. Effect of Wastewater pH and Effluent Recycle on Reactor Performance during Startup: (a) COD Removal Efficiency; (b) Biogas Production Rate; (c) Biogas Methane Content; (d) Acetate Concentration in Effluent; (e) pH of Wastewater and Mixed Liquor

formic acid. However, the methanogenic activity of the sludge was abruptly impaired on day 18 when the pH of wastewater and mixed liquor reached pH 4.5 and pH 4.0, respectively. Within a day, the reactor ceased to produce gas even when the pH of the mixed liquor was readily adjusted to pH 7 by dosing sodium bicarbonate.

As a result, the reactor was restarted on day 20 by replacing two-thirds of the sludge in the reactor with partially granulated sludge from the same 65-L reactor. Again, the pH of the wastewater was gradually reduced. On day 24, when the pH of wastewater reached pH 4.2, the performance of the reactor deteriorated considerably. The pH of the mixed liquor was reduced to 5.8, while the gas production rate reduced by 50%. However, unlike in the previous disturbance, the reactor rapidly recovered when the pH of both the wastewater and the mixed liquor were adjusted to neutral. The methane content in the biogas in both occasions were less than 10%, reflecting the impairment of the methanogenic activity as well as the release of dissolved carbon dioxide due to the reduction of pH of the mixed liquor.

Starting on day 46, part of the effluent overflown from the top of the reactor was recycled along with the incoming wastewater. During days 46–69, the flow-rate ratio, R, between the recycled effluent and the incoming
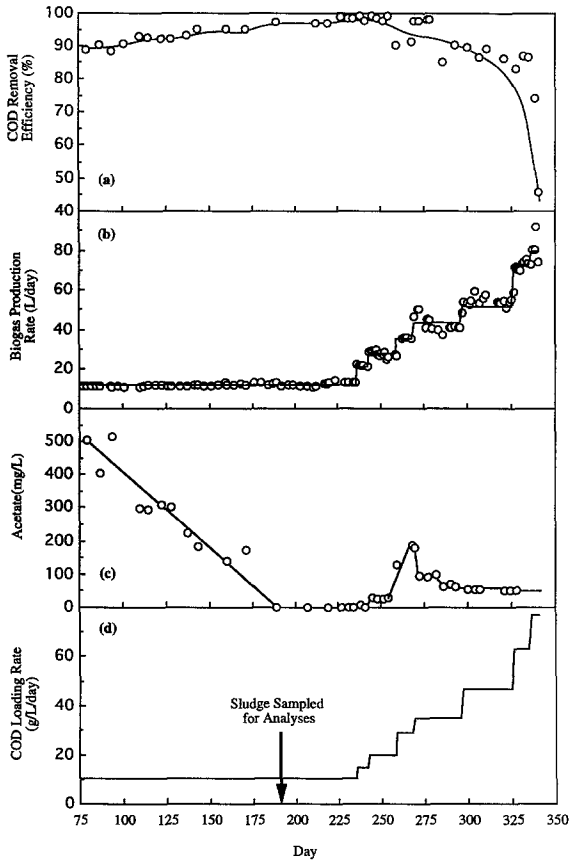
FIG. 3. Performance of UASB Reactor: (a) COD Removal Efficiency; (b) Biogas Production Rate; (c) Acetate Concentration in Effluent; (d) COD Loading Rate
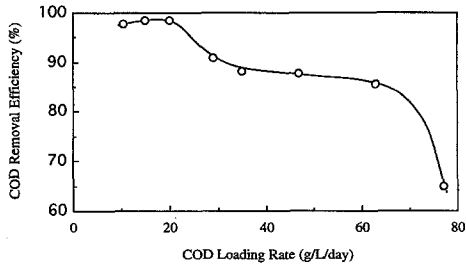

FIG. 4. COD Removal Efficiency at Various COD Loading Rates

wastewater, was kept at 1.5, while the wastewater pH was kept at pH 4.3. During this period, the COD-removal efficiency was about 90–92% and the pH of the effluent was reduced from pH 9.1 to pH 8.3.

After day 70, the recycle ratio was increased to 3.0 while the wastewater pH was lowered to pH 3.8. The reactor performed satisfactorily under this condition in the next 30 days, as illustrated in Fig. 2; it consistently removed

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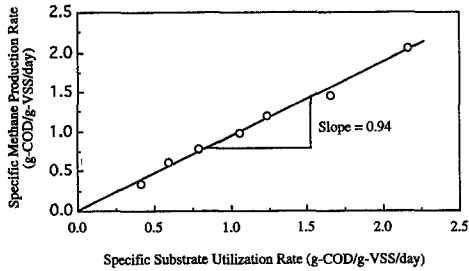
FIG. 5. Specific Methane Production Rate at Various Specific Substrate Utilization Rates
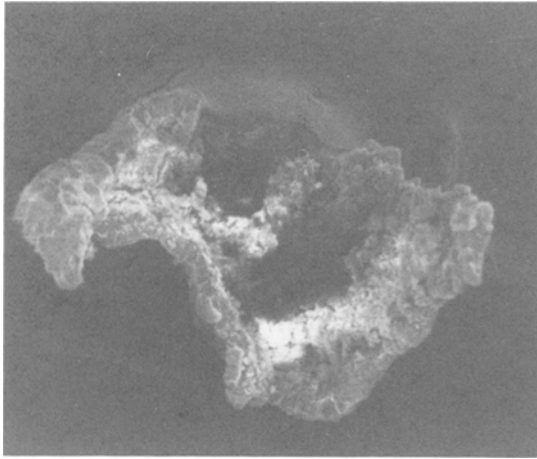

FIG. 6. Typical Sludge Granule under SEM

88–90% of COD, and the pH of the effluent was reduced to a stable level of pH 7.7. At this pH level, the methane content in the biogas was reduced to the constant level of 70%, as illustrated in Fig. 2(c), because more carbon dioxide was released to the gas phase at the lower pH. Acetate with concentrations ranging 180–450 mg·L^{-1} attributed significantly to the residual COD in the effluent. Since formate was the sole substrate in the wastewater, acetate was produced presumably by some homoacetogenic bacteria, which were capable of producing acetate using formate as substrate (Li et al. 1994).

Performance of UASB Process at Increased COD Loading Rates

Since the pH of the mixed liquor was under control after day 70, the recycle ratio of 3.0 and the wastewater pH of 3.8 were adopted for the rest of the experiment. Fig. 3 illustrates the performance of the reactor at the various COD loading rates. During days 70–235, the reactor was operated at a loading rate of 10·5 g-COD·L^{-1}·day^{-1}, corresponding to 5,250 mg·L^{-1} of COD in wastewater and 12 h of HRT. The biogas production rate was stable during this period, as illustrated in Fig. 3(b). Initially, the COD removal efficiency was 88–90%, mainly due to high levels of acetate in the mixed liquor, as illustrated in Figs. 3(a) and 3(c). As the biomass in the

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reactor became acclimated, the COD-removal efficiency gradually increased to 95–97%, as the acetate level reduced from the 500 mg·L^{-1} level to nil.

Fang and Chui (1993a) reported that the COD-removal efficiency of a UASB reactor treating sucrose as substrate was mainly dependent on the COD loading rate, and was insensitive to either the HRT or the wastewater's COD level, individually. In this study, starting on day 236, the COD loading was increased stepwise, as illustrated in Fig. 3(d). Prior to day 268, the COD loading increased with the COD level in wastewater, while the HRT was kept at 12 h. At loading rate of 29 g-COD·L^{-1}·day^{-1}, the wastewater had a COD of 14,500 mg·L^{-1} and a corresponding sodium level of 15,000 mg·L^{-1}. To avoid the inhibitory effect on methanogenesis by the increased sodium concentration, COD loadings of 35 g·L^{-1}·day^{-1} and higher were
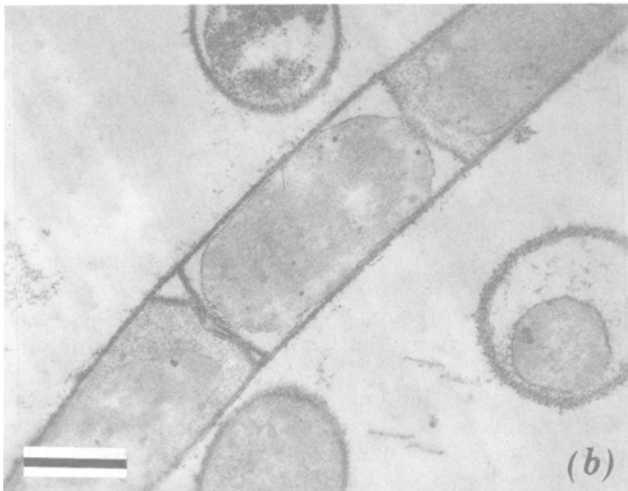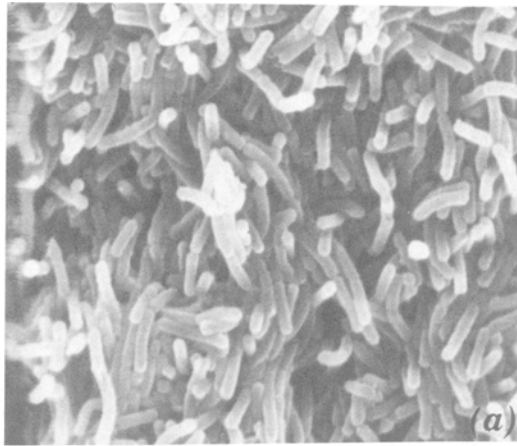
FIG. 7. Microphotographs of Methanobacterium formicicum-like Bacteria: (a) under SEM; and (b) under TEM (Bar = 0.5 μm)

controlled by reducing the HRT while keeping the COD at 14,500 mg·L^{-1} and, thus, the sodium concentration at 15,000 mg·L^{-1}.

At loading rate of 20 g·L^{-1}·day^{-1} or less, the reactor consistently removed 97–98% of COD, while little acetate was found in the effluent after day 190. As the loading rate increased from 29 g-COD·L^{-1}·day^{-1} to 63 g-COD·L^{-1}·day^{-1}, the COD removal efficiency gradually deteriorated, while the pH of the reactor was maintained at pH 7.8–8.0; about 50–200 mg·L^{-1} of acetate were found in the effluent. At 77 g-COD·L^{-1}·day^{-1}, the reactor failed to remove substantial amounts of formic acid resulting in an abrupt decrease of pH, which further suppressed the methanogenic activity. The experiment was stopped in three days at this condition when the pH of the mixed liquor was reduced to pH 5 and less than 40% of the COD in the wastewater was removed.

Fig. 4 illustrates the average COD-removal efficiency at various COD loading rates. At loading rate of 29–63 g-COD·L^{-1}·day^{-1}, the UASB reactor removed 86–90% COD, significantly lower than the 97–98% at 20 g-COD·L^{-1}·day^{-1} or less. The reduction of COD removal efficiency was likely attributed to the inhibitory effect of sodium concentration, which was increased from 10,000 mg·L^{-1} to 15,000 mg·L^{-1}. Similar inhibitory effect of sodium on the degradation of acetate to methane had been reported, but at lower sodium concentrations. Rinzema et al. (1988) reported that at neutral pH, sodium concentrations of 5,000; 10,000; and 14,000 mg·L^{-1} resulted in 10%, 50%, and 100% inhibitions of the acetoclastic methanogenic activity, respectively.

COD Balance and Sludge Yield

The specific methane production rate (SMPR) shows the methanogenic activity of the granules under the specific operating conditions of the reactor. Fig. 5 illustrates that the SMPR increased linearly with the specific substrate utilization rate until reaching the maximum of 2.06 g-methane-COD·g-VSS^{-1}·day^{-1}, which is slightly higher than the 1.7 g-methane-COD·g-VSS^{-1}·day^{-1} reported for UASB granules treating sucrose as substrate (Fang and Chui 1993a). The slope of 0.94 indicates that 94% of COD removed was converted to methane, with the remaining 6% converting to biomass. The average ratio between the insoluble COD level in the effluent and its VSS was 1.16 g-COD·g-VSS^{-1}. Accordingly, the sludge yield was estimated as 0.05 g-VSS·g-COD^{-1}, which is comparable to the 0.04–0.10 g-VSS·g-COD^{-1} for the pure culture of *Methanobacterium formicicum* (Schauer and Ferry 1980).

Specific Methanogenic Activity of Sludge

Sludge samples were taken for SMA analysis on day 190 when the reactor was operated at 10.5 g-COD·L^{-1}·day^{-1} with 98% COD removal and without detectable VFA in the effluent. The SMA of the sludge was measured to be 2.90 g-COD·g-VSS^{-1}·day^{-1} on the degradation of formate at the concentration of 3,000 mg·L^{-1}. However, there was no detectable SMA using acetate, propionate and butyrate, individually, as substrate. This indicates that the sludge was fully adapted to using formate as the sole substrate, but was unable to degrade other fatty acids. The SMA value on the degradation of formate was significantly higher than the corresponding SMA of 0.76–1.26 g-COD·g-VSS^{-1}·day^{-1} for granules treating carbohydrates as substrate (Dolfing and Mulder 1985; Fang et al. 1993; MacLeod et al. 1990; and Stams et al. 1989). This is probably because the sludge of this
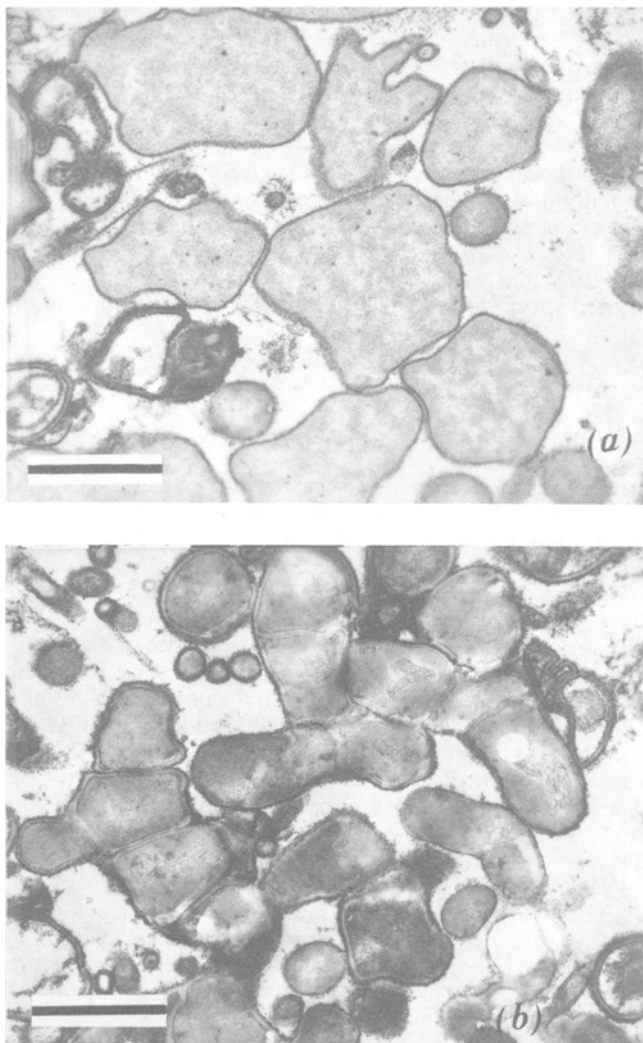
FIG. 8. Microphotographs of TEM: (a) Methanococcus vannielii-like Bacteria; (b) Unknown Bacterium (Bar = 1 μm)

study comprised nearly 100% formate-consuming methanogens, whereas sludges in other studies comprised not only methanogens but also other bacteria, such as acidogens and acetogens.

The maximum SMPR of 2.06 g-COD·g-VSS^{-1}·day^{-1} was only 70% of the measured SMA. It implies that the sludge in the UASB reactor was potentially capable of treating wastewater at higher loading rates.

Microbial Composition

Unlike other UASB granules (Fang et al. 1993), sludge granules of this study were not only small (0.5 mm or less) but also of irregular shape, as illustrated in Fig. 6. However, despite their small size, the sludge granules

settled satisfactorily. The amount of sludge washout was insignificant throughout the experiment, even when the reactor was operated at 77 g-COD·L^{-1}·day^{-1}.

A typical granule was found to be mainly composed of *Methanobacterium formicicum*–like bacteria, judging from its specific filamentous rod-shaped morphology under SEM [Fig. 7(a)] and TEM [Fig. 7(b)], and its capability of converting formate to methane (Zeikus and Bowen 1975; Mah and Smith 1981). A small number of *Methanococcus vannielii*–like bacteria were also found in the sludge granules; it was identified by its specific irregular shape under TEM [Fig. 8(a)], as reported by Stadtman and Barker (1951) and Jones et al. (1977) and, also, its formate-consuming capability. Furthermore, an unidentified species of bacterium [Fig. 8(b)] was also found in the granules but at much lower quantities.

CONCLUSION

The following conclusions were drawn on the removal of formate in wastewater by UASB process at 37°C:

Successful removal of formate in wastewater by the UASB process was demonstrated with an effective control of the mixed liquor pH, which was accomplished by keeping the recycle ratio at 3.0 and by lowering the pH of the incoming wastewater to pH 3.8.

The COD removal efficiencies were 97–98% at loading rates of 10–20 g-COD·L^{-1}·day^{-1}, but were reduced to 86–90% at 29–65 g-COD·L^{-1}·day^{-1}. The reduction was likely due to the inhibitory effect of increased sodium concentration. About 94% of COD of formate removed from the wastewater was converted to methane with a sludge yield of 0.05 g-VSS·g-COD^{-1}. When the loading was further increased to 77 g-COD·L^{-1}·day^{-1}, the reactor abruptly failed due to the sudden decrease of the pH resulting from the accumulation of formic acid.

Sludge granules were small but had good settleability. The maximum SMPR in the UASB reactor was 2.06 g-COD·g-VSS^{-1}·day^{-1}. The SMA of granules as measured in serum vials using formate as substrate was 2.90 g-COD·g-VSS^{-1}·day^{-1}; but, the granules were unable to degrade other fatty acids. Microscopic examination indicated that the granules were mainly composed of *Methanobacterium formicicum*–like bacteria, plus small amounts of *Methanococcus vannielii*–like bacteria and an unknown species of bacterium.

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