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PERFORMANCE AND GRANULE CHARACTERISTICS OF UASB PROCESS TREATING WASTEWATER WITH HYDROLYZED PROTEINS

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ABSTRACT

UASB process consistently removed 84% of COD in wastewater with hydrolyzed proteins for loading rates up to 32 g-COD/L/day, corresponding to a food-to-microorganism ratio of 0.81 g-COD/g-VSS/day, at 37°C and a hydraulic retention time of 9 hours. Of all the COD in the wastewater, about 74% was converted to methane, 16% was unhydrolyzed proteins which remained refractory to degradation, and 10% converted to biomass. The average sludge yield was 0.079 g-VSS/g-COD. There was no noticeable foaming and sludge flotation. The maximum specific methane production rate in the reactor was 0.60 g-methane-COD/g-VSS/day, which was comparable to the specific methanogenic activity (SMA) of 0.59 g-methane-COD/g-VSS/day observed by the serum vial test using hydrolyzed proteins as substrate. The SMA using acetate as substrate was 0.89 g-methane-COD/g-VSS/day, higher than those (0.39–0.59 g-methane-COD/g-VSS/day) using formate, propionate and butyrate, individually, as substrate. The granules did not have a layered structure nor a predominant type of bacteria. Instead, it had a densely packed structure with interwined bacteria of diverse morphologies with scattered microcolonies of *Methanothrix*, *Methanosarcina*, and juxtapositioned syntrophic associations.

KEYWORDS

Anaerobic, granules, methanogenic activity, microbial structure, protein, syntrophic association, UASB.

INTRODUCTION

Since the introduction of the anaerobic filter (Young and McCarty, 1969), a number of anaerobic wastewater treatment processes have been developed. Among them, the upflow anaerobic sludge blanket (UASB) (Lettinga *et al.*, 1980; Fang *et al.*, 1990; Lettinga and Hulshoff Pol, 1991; Fang and Chui, 1993) has been successfully commercialized in the past decade. Over 300 full-scale reactors have been installed worldwide. Most of them are treating wastewaters from the sugar, starch and brewery industries; the main pollutants in these wastewaters are mainly carbohydrates.

However, when treating wastewaters with high levels of protein content, foaming and sludge flotation tend to occur (Lettinga and Hulshoff Pol, 1991), resulting in sludge washout and/or formation of scum in the gas–liquid–solid (G–L–S) separator. This problem significantly hinders the application of the UASB process to the treatment of certain wastewaters, such as those from the dairy and meat processing industries.

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The degradation of proteins is a complex process involving many different kinds of anaerobic microorganisms. In general proteins are hydrolyzed to peptides and amino acid which are fermented to volatile fatty acids (VFA), H_2 and CO_2 prior to being converted to methane (McInerney, 1988). The initial hydrolysis is the rate-limiting step with an apparent hydrolysis rate of 0.02–0.03 d^{-1} (Gujer and Zehnder, 1983). The hydrolysis of proteins, thus, requires long retention time and cannot be effectively carried out in the UASB reactor. Hulshoff Pol and Lettinga (1986) recommended a two-step process for treating wastewaters with high levels of protein, including a separate reactor for the hydrolysis of proteins followed by the UASB treatment.

This study was focused on the second step of such a process. The efficacy of the UASB process treating wastewater with hydrolyzed proteins was examined. In addition, the microstructure (Stams *et al.*, 1989, MacLeod *et al.*, 1990, Grotenhuis *et al.*, 1991, Macario *et al.*, 1991) and the specific methanogenic activity (SMA) (Dolfing, 1985, Dolfing and Mulder, 1985, Dolfing and Bloemen, 1985, Grotenhuis *et al.*, 1991) of the granules were also investigated.

MATERIALS AND METHODS

The UASB reactor used in this study 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 based on the profile of the volatile suspended solids (VSS) of the samples taken from the ports. On top of each reactor was a G-L-S 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 based on the reactor volume alone, excluding volume of the G-L-S separator. The reactor was water-jacketed and operated at a constant temperature of 37°C.

Prior to this study, flocculent sludge from an anaerobic sludge digester of the Shatin Wastewater Treatment Works, Hong Kong, was partially granulated in a 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. Peptone, which is composed of readily soluble, partially hydrolyzed proteins (China National Chemical Import/Export Corporation, Beijing), was the sole organic substrate in the wastewater. Laboratory test showed that each gram of hydrolyzed proteins was equivalent to 1.15 grams of Chemical Oxygen Demand (COD). The wastewater was also composed of trace metals and balanced nutrients using a formulation of a previous report (Fang and Chui, 1993). Likewise, the sampling strategies and the analytical procedures, such as the methane content in biogas, the VFA levels in effluent, etc., also followed those of the same report.

Throughout this study the hydraulic retention time (HRT) was kept at 9 hours. The initial COD loading to the reactor was 3.5 g/L/day with 1300 mg/L of COD in the wastewater. It increased stepwise, by increasing the hydrolyzed protein content in the wastewater, when the COD removal efficiency reached 80%. The concentrations of VFA (from acetate to valerate) were also closely monitored throughout this study to ensure that the acetic acid concentration did not exceed 500 mg/L before the COD loading was increased.

Granule samples were taken for structural examination, SMA analysis and bacterial counts on day 190 when the reactor was operated at 13–14 g-COD/L/day for over 5 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 as reported previously (Fang and Chui, 1993; Fang *et al.*, 1995a). The SMA of the granules were measured in duplicate in serum vials based on the method (Dolfing and Mulder, 1985; Hwang and Cheng, 1991) modified from the one proposed by Owen *et al.* (1979). The individual substrate used for the SMA measurements included formate, acetate, propionate, butyrate, and hydrolyzed proteins. The bacterial population of each trophic group in the granules was enumerated using the most probable number (MPN) method (Chartrain and Zeikus, 1986; Li and Noike, 1992).

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RESULTS AND DISCUSSION

COD Removal Efficiency

Fang and Chui (1993) reported that the COD removal efficiency of a UASB reactor 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, the HRT was kept constant at 9 hours. The COD loading rate was thus proportional to the COD level in the wastewater. Figure 1 illustrates (a) the efficiency of COD removal, (b) the total biogas production rate, and (c) the COD loading rate, throughout this study. The COD removal was calculated from the soluble COD in the effluent and the total COD in the wastewater which was attributed by the readily soluble hydrolyzed proteins. The loading rate was lowered from 14.3 to 6.7 g/L/day, during Days 227-240, hoping that the COD removal efficiency could be improved. It was resumed to 11.7 g/L/day on Day 241, and soon higher, when the attempt to improve the COD removal had failed.

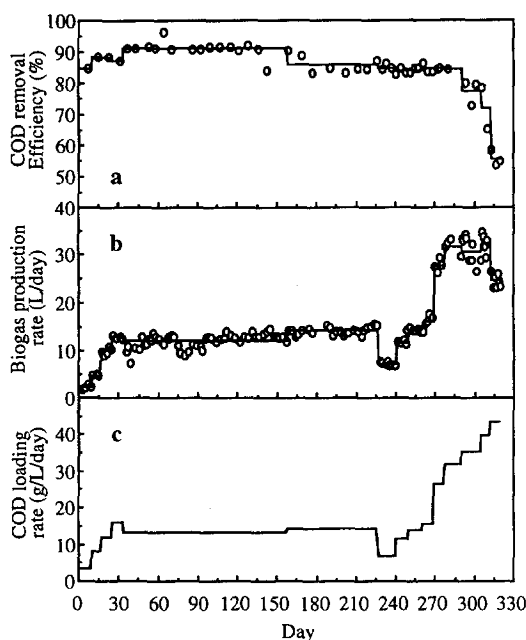

Fig.1. (a) COD removal efficiency, (b) biogas production rate, (c) COD loading rate, throughout the study.

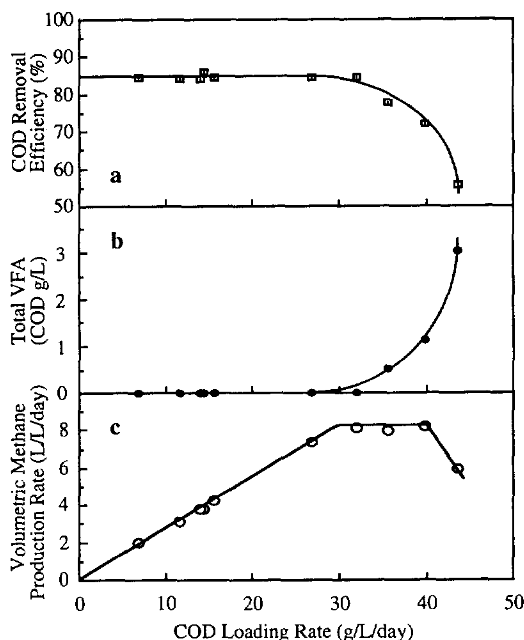

Fig.2. (a) COD removal efficiency, (b) VFA in effluent, (c) volumetric methane production rate, at various COD loading rate.

Figure 2 illustrates (a) the COD removal efficiency, (b) COD equivalent of VFA in the effluent, and (c) the methane production rate over COD loading rate ranging from 6.7 g/L/day to 43.7 g/L/day. Figure 2(a) illustrates that the COD removal efficiency was maintained at 84–90% over a wide range of COD loading rate (6.7–32 g/L/day), corresponding to 2500–12000 mg/L of COD in the wastewater. Beyond the maximum COD loading of 32 g/L/day, the COD removal efficiency rapidly deteriorated to 72% at 39.8 g/L/day, and further down to 56% at 43.7 g/L/day. At 32 g-COD/L/day, or lower, about 16% of the proteins in the wastewater was refractory to the anaerobic degradation. The refractory fraction was likely from the unhydrolyzed proteins.

At 32 g-COD/L/day, or lower, the effluent had less than 20 mg/L of acetate and negligible quantities of propionate, n- and i-butyrates and n- and i-valerates. At higher loading rates, the VFA concentrations in the

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effluent increased as the COD removal efficiency deteriorated, as illustrated in Figure 2(b). The residual VFA (from acetate to valerate) attributed a significant fraction of the soluble COD in the effluent from almost nil at the loading rate of 32 g-COD/L/day, to 17%, 29% and 42%, at loading rates of 35.5, 39.8 and 43.7 g-COD/L/day. The pH in the reactor was kept at a constant level of 7.2-7.8 throughout the study due to the chemical buffers in the wastewater.

Methane Production and COD Balance

The biogas production rate was measured daily using a gas meter based on the design of Mosey and Matthews (1982), whereas the methane content in the biogas was measured by gas chromatograph weekly. Based on these measurements the amounts of methane produced were calculated. Figure 2(c) illustrates that the volumetric methane production rate increased linearly with the COD loading rate, until reaching a maximum of 8.1 L/L/day at 32 g-COD/L/day. Since each gram of methane is equivalent to 4 grams of COD, the specific methane production rate (SMPR), expressed as grams of methane-COD produced daily by each gram of VSS, can thus be calculated. Figure 3 illustrates that the SMPR increased linearly with the food-to-microorganism (F/M) ratio with a slope of 0.74, until reaching a maximum of 0.60 g-COD/g-VSS/day at the F/M ratio of 0.81 g-COD/g-VSS/day.

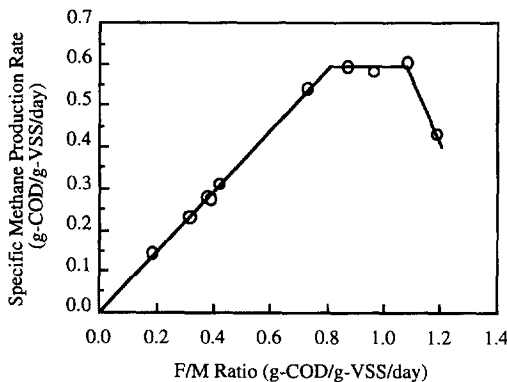

Fig.3. Specific methane production rate at various F/M ratios.

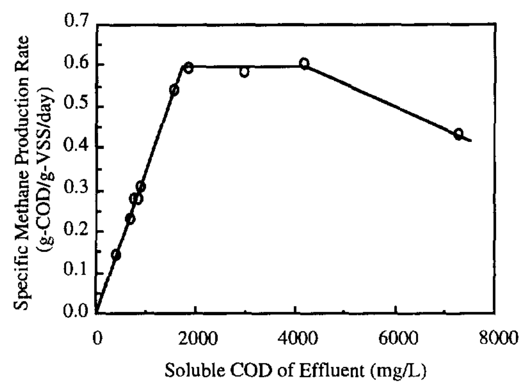

Fig.4. Specific methane production rate at various COD levels of effluent.

Of all the COD in the wastewater, about 74% was converted to methane, judging from the slope in Figure 3, 16% was unhydrolyzed protein which remained refractory to degradation, and the remaining 10% was presumably converted to biomass. The biomass in the reactor had a COD/VSS ratio of 1.50. Accordingly, the sludge yield was estimated to be 0.079 g-VSS/g-COD, which is comparable to the 0.10–0.12 g-VSS/g-COD for long-chain fatty acids estimated by Novak and Carlson (1970), but smaller than the 0.18 g-VSS/g-COD estimated by Henze and Harremoes (1983).

Absence of Foaming and Sludge Flotation

The degrees of foaming and sludge flotation in the UASB reactor caused by proteinaceous substrate depend on a number of factors, such as the presence of other compounds, COD loading rate, protein concentration, temperature, and the extent to which the sludge was adapted (Lettinga and Hulshoff Pol, 1991). However, throughout this study, there was no foaming observed at all. The absence of foaming was likely due to the pre-hydrolysis treatment of the proteins, and the slow, controlled pace of startup which allowed bacteria to acclimate properly. It was noted that sludge in this study settled less well as compared to those in other UASB reactors operated in parallel for treating wastewater comprising fatty-acids, carbohydrates and

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aromatic chemicals (results yet to be published); it was however not severe enough to cause serious sludge washout.

Specific Methane Production Rate of Granules in the UASB Reactor

Figure 4 illustrates that the SMPR of the granules as a function of the soluble COD levels in the reactor, which equalled those in the effluent because of the vigorous mixing action generated by the biogas (Fang and Chui, 1993). At soluble COD levels below 1860 mg/L, the SMPR increased linearly with the soluble COD in the reactor, representing a first order reaction, until it reached the maximum level of 0.60 g-methane-COD/g-VSS/day. However, between 1860 mg/L and 4200 mg/L of soluble COD, the SMPR was constant at the level 0.60 g-methane-COD/g-VSS/day, representing a zeroth order reaction. Furthermore, as the soluble COD reached 7300 mg/L when operated at the COD loading rate of 43.7 g/L/day, the SMPR was reduced to 0.43 g-methane-COD/g-VSS/day. During this period, the pH was 7.7 and the ammonium nitrogen was below 1700 mg/L. The suppression of SMPR of the granules was thus probably caused by the accumulated VFA, which exceeded the COD equivalent level of 3000 mg/L.

The maximum SMPR of granular biomass in UASB reactors using sucrose as the main substrate was reported as 1.7 g-methane-COD/g-VSS/day (Fang and Chui, 1993). The maximum SMPR of granules observed in this study using hydrolyzed proteins as substrate was only 35% of that using sucrose as substrate. This is probably due to the fact that the degradation of hydrolyzed proteins is more complex than the degradation of sucrose, involving a larger number of different bacteria. Thus the methanogenic activity per unit mass of total protein-degrading bacteria was less than that of sucrose-degrading bacteria. For comparison, Breure *et al.* (1985) reported that specific biomass activity for sludge degrading gelatin was only 50% of that for sludge degrading glucose.

Specific Methanogenic Activity and Bacterial Population

TABLE 1. Specific Methanogenic Activities (SMA) of Granules using Fatty Acids and Hydrolyzed Proteins as Substrates

Sludge treating wastewater of	SMA (g-methane-COD/g-VSS/day)					Source
	formate	acetate	propionate	butyrate	hyd.prtn.	
hydrolyzed proteins	0.58	0.89	0.39	0.46	0.59	this study
brewery	1.26	0.49	0.13	0.12		Fang et al., 1994
sucrose	1.22	1.20	0.52	0.61		Fang et al., 1994
sucrose	0.76	0.30	0.22			MacLeod et al., 1990
sugar		0.58	0.23			Dolfing, 1985
sugar	1.01	0.30	0.26			Dolfing and Mulder, 1985
sugar	1.01	0.90	0.41			Dolfing and Bloemen, 1985
sugar		0.30	0.31	0.28		Grotenhuis et al., 1991
starch		0.18-0.88				Dubourguier et al., 1988
Maize starch	0.74	0.09	0.12			Stams et al., 1989

Anaerobic degradation of hydrolyzed proteins is a complex process, involving complex biochemical reactions. Fatty acids of low molecular weight, i.e. formate, acetate, propionate and butyrate, are among the key intermediates (Thiele and Zeikus, 1988). Table 1 shows the SMA of the granules measured in the serum vials using individual fatty acids and the hydrolyzed proteins as the sole organic substrate. Corresponding literature data of SMA of UASB granules treating wastewaters composing of carbohydrates as substrate are also shown for comparison. The SMA using hydrolyzed proteins was 0.59 g-methane-COD/g-VSS/day,

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which was comparable to the maximum SMPR of 0.60 g-methane-COD/g-VSS/day observed in the UASB reactor. The SMA using acetate (0.89 g-methane-COD/g-VSS/day) was slightly higher than those using other substrates (0.39–0.59 g-methane-COD/g-VSS/day). However, generally speaking, the granules were able to degrade the hydrolyzed proteins and the key intermediates at similar rates. This indicates that, for the 84% of the degradable hydrolyzed proteins in the wastewater, there is no particular rate-limiting step. It also seems to imply that, for the 16% of proteins which was refractory to degradation, the initial hydrolysis was most likely the rate-limiting step.

The bacterial population of each trophic group in the granules was enumerated using the MPN method. Results showed that for each gram of granular biomass the populations of bacteria capable of using H_2/CO_2, acetate, propionate, butyrate, and hydrolyzed proteins, individually, as the sole substrate were $3.4x10^7$, $1.3x10^8$, $3.4x10^7$, $6.1x10^7$, $1.3x10^8$, respectively. Although they were of the same order of magnitude, the population of acetotrophic bacteria about equalled the combined populations of those using H_2/CO_2, propionate and butyrate.

Microstructure of Granules

The microstructure of UASB granules depend on the nature of the substrate and the temperature of the reactor (Fang *et al.*, 1995a, Macario, 1991). Fang and Chui (1993) found that UASB granules treating sucrose as substrate had a dense layer of diverse bacterial population, while the interior was mainly composed of *Methanothrix*. MacLeod *et al.* (1990) proposed a three-layered microstructure for the UASB granules treating sucrose as substrate. They also found that the core of the granule was mainly composed of *Methanothrix*. Grotenhuis *et al.* (1991), on the other hand, found that there was no evidence of a layered structure for UASB granules treating propionate, ethanol, and sugar refinery wastewaters. The propionate-degrading granules were found to be composed of two types of colonies: one consisted of *Methanothrix* and the other consisted of small electron-opaque *Methanobrevibacter arboriphilus* in juxtaposition with large, spherical electron-translucent bacteria.

Granules from treating hydrolyzed proteins in this study were 1–2 mm in size with satisfactory settleability. The surface of these granule had a coarse and loosely packed structure with multiple cracks, unlike the smooth, densely packed surface of those granules treating carbohydrates as substrate (Fang and Chui, 1993). Figure 5 is the plan view of the granule surface under SEM, illustrating the loose network of filamentous bacteria on the surface. Figure 6 is the ultrasection of the granule under TEM, which also illustrates the loose network of filamentous bacteria near the surface.

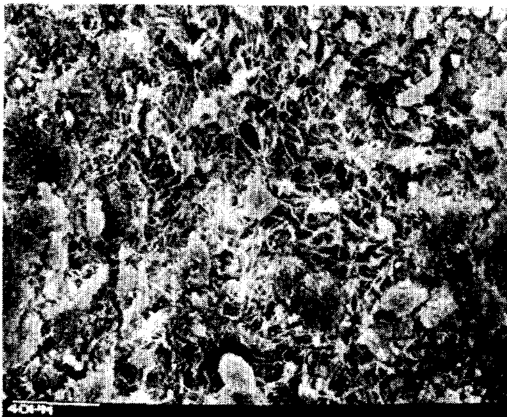

Fig.5. Plan view of the granule surface under SEM.

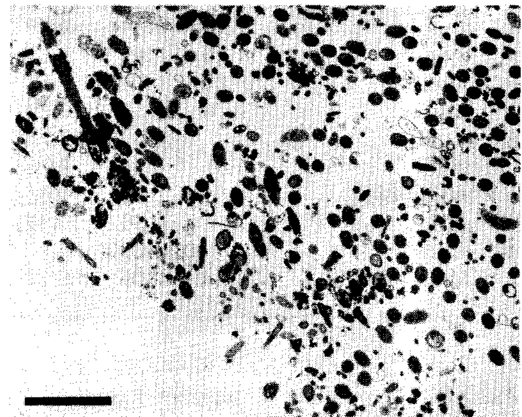

Fig.6. Ultrasection of the granule near the surface under TEM, bar = 5 μm.

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Furthermore, there was neither a layered structure nor noticeable predominant bacteria in these granules. Other than the porous surface, the granule had a densely packed structure with interwined bacteria of different morphologies, as illustrated in Figures 7 and 8, including not only cocci and filaments, but also bacilli, sarcina and spirochetes. This reflects the complex nature of protein degradation requiring a large number of different microorganisms. In granules treating sucrose as substrate (Fang and Chui, 1993), spirochetes and *Methanosarcina* were found only near the surface, while *Methanothrix* were the predominant bacteria in the interior. In granules degrading hydrolyzed proteins, however, spirochetes, *Methanosarcina* and *Methanothrix* were distributed throughout the granule. Spirochetes were probably responsible for the degradation of amino acids and fatty acids (Johnson, 1981), whereas *Methanosarcina* converts H_2/CO_2, methanol, methylamines and acetate to methane and *Methanothrix* converts acetate only at low concentration (Oremland, 1988).

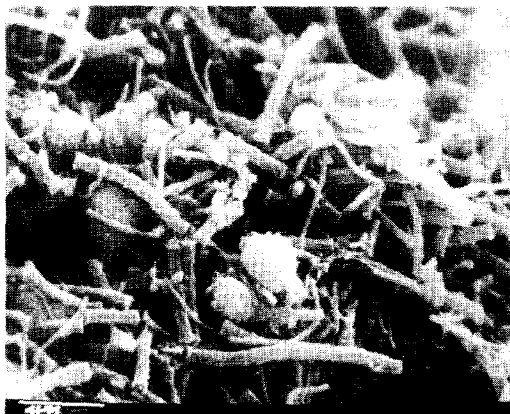

Fig.7. The granule interior under SEM.

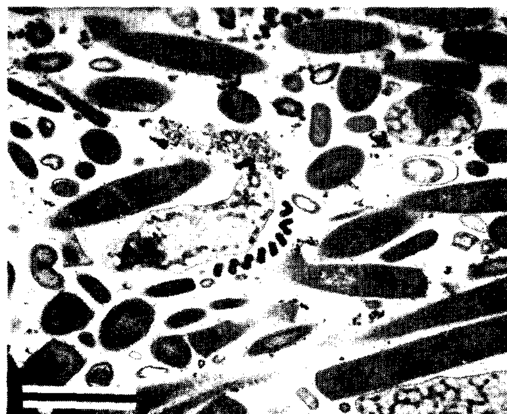

Fig.8. The granule interior under TEM, bar = 2 μm

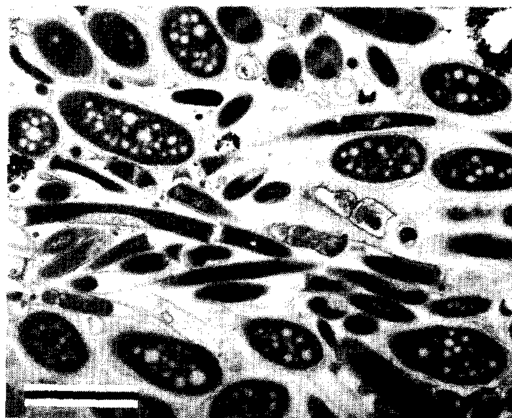

Fig.9. Micrograph of juxtapositioned syntrophic association - 1, bar = 2 μm.

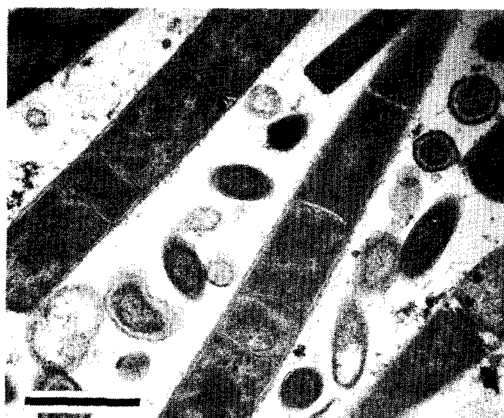

Fig.10. Micrograph of juxtapositioned syntrophic association - 2, bar = 1 μm.

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Although there were no noticeable predominant bacteria, four types of microcolonies could be found scattered in the interior of the granules. Two were *Methanothrix* and *Methanosarcina*, respectively. The other two seemed to show juxtapositioned syntrophic associations. Figure 9 illustrates one type of microcolony in which *Syntrophobacter*-like bacteria (1.5–3 μm) are in juxtaposition with *Methanospirillum hungatei* (0.4 μm) and an unknown group of bacteria (0.8 μm). *Methanospirillum hungatei*, which has an unique ultrastructure under TEM (Zeikus and Bowen, 1975; Zeikus, 1977), converts either formate or H_2/CO_2 to methane. *Syntrophobacter* is able to degrade propionate to acetate and hydrogen, only if there are hydrogen-consuming bacteria, such as *Methanospirillum hungatei*, in the immediate vicinity, keeping the hydrogen pressure below 10^{-4} atm as required according to thermodynamic analysis. This type of juxtapositioned syntrophic association has also been found in granules treating carbohydrates as substrate (Fang *et al.*, 1995b; MacLeod *et al.*, 1990).

Figure 10 illustrates another type of microcolony in which *Methanothrix* seemed to be in juxtapositioned with some bacteria of unknown identities. *Methanothrix*, which uses only acetate as substrate, was normally found forming microcolonies of its own. Figure 10 seems to suggest that *Methanothrix* may also syntrophically associate with some acetate-forming bacteria which require low concentrations of acetate.

CONCLUSION

The following conclusions could be drawn regarding the UASB treatment of wastewater containing hydrolyzed proteins at 37°C and a HRT of 9 hours.

1. The UASB process consistently removed 84% of COD in wastewater for loading rates up to 32 g-COD/L/day, corresponding to a F/M ratio of 0.81 g-COD/g-VSS/day. Of all the COD in the wastewater, about 74% was converted to methane, 16% was unhydrolyzed proteins which remained refractory to degradation, and 10% converted to biomass. The sludge yield was about 0.079 g-VSS/g-COD. There was no noticeable foaming and sludge flotation problem in the reactor.

2. The maximum SMPR was 0.60 g-methane-COD/g-VSS/day, about 35% of that for treating sucrose as substrate. The SMPR was suppressed at a loading rate of 43.7 g-COD/L/day, probably due to the accumulated VFA in the effluent which exceeded the level equivalent to 3000 mg/L of COD.

3. The SMA using hydrolyzed proteins as substrate was 0.59g-methane-COD/g-VSS/day, comparable to the maximum SMPR in the UASB reactor. The SMA using acetate as substrate was 0.89 g-methane-COD/g-VSS/day, slightly higher than those (0.39–0.59 g-methane-COD/g-VSS/day) using formate, propionate and butyrate, individually, as substrate. It seemed, however, that there was no particular rate-limiting step for converting the degradable hydrolyzed protein to methane.

4. Bacteria capable of converting H_2/CO_2, acetate, propionate and butyrate were of similar populations, although acetotrophs were slightly more populous than the others.

5. The microstructure of granules treating hydrolyzed proteins was different from those reported previously. It had neither a layered structure nor a predominant species of bacteria. Instead, it had a densely packed structure with interwined bacteria of diverse morphologies with scattered microcolonies of *Methanothrix*, *Methanosarcina*, and juxtapositioned syntrophic associations.

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