Environmental Technology, Vol. 16. pp 13-23
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ANAEROBIC DIGESTION OF STARCH PARTICULATES IN AN UPFLOW SLUDGE BLANKET FILTER REACTOR

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(Received 6 May 1994; Accepted 18 August 1994)

ABSTRACT

The effect of starch particulates in wastewater on an anaerobic upflow sludge blanket filter (UBF) reactor was evaluated at 37°C and pH 7.0-7.5 over a period of 415 days, during which the volumetric chemical oxygen demand (COD) loading was increased from 3 $kg.m^{-3}.d^{-1}$ to 50 $kg.m^{-3}.d^{-1}$. The initial organic substrate was a mixture of sucrose and starch. Upon reaching the constant loading rate of 10 $kg\text{-}COD.m^{-3}.d^{-1}$, starch gradually replaced sucrose in the wastewater and eventually became the sole substrate at loading rates of 10 $kg\text{-}COD.m^{-3}.d^{-1}$ and higher. Results showed that, despite the insoluble nature of the starch, only about 1.2% of starch in wastewater remained unhydrolyzed and about 5.9% became soluble COD in the effluent for loading rates up to 40 $kg\text{-}COD.m^{-3}.d^{-1}$. Of the COD removed, 86.1% was converted to methane and the rest was presumably converted to biomass with a sludge yield of 0.10 $kg\text{-}VSS.kg\text{-}COD^{-1}$. The suspended solids in the effluent were mostly composed of the washed-out biomass, the amount of which increased with loading rate, because of the turbulence generated by the increased gas production and the insufficient depth of the reactor. There was no accumulation of starch in the reactor throughout this study. The starch-degrading granules had a higher methanogenic activity than granules in the previous studies degrading soluble carbohydrates. The bacterial populations of acidogens, syntrophic acetogens, and methanogens, were about 10^8-10^9 per ml.

Keywords: Anaerobic, methanogenic, particulate, starch, UBF

INTRODUCTION

Most industrial wastewaters, such as food processing industry, distillery and piggery etc., as well as raw domestic sewage contain considerable amount of biodegradable suspended solids (SS). Although it has been successfully applied for the treatment of many industrial wastewater, the upflow anaerobic sludge blanket (UASB) process has been perceived to be vulnerable to high levels of SS. The SS in wastewater may inhibit the sludge granulation (1) and impair the methanogenic activity of the sludge (2), or, in some extreme cases, may cause a sudden acidification of the reactor content (3) or even a sudden washout of the sludge bed (1).

To avoid the adverse effect of high SS in wastewater, Lettinga and Hulshoff Pol (4) suggested restricting the volumetric loading rate of chemical oxygen demand (COD) to 2-8 $kg.m^{-3}.d^{-1}$ for UASB reactors. Souza (5), on the other hand, suggested restricting the SS level to below 1000 $g.m^{-3}$ and the SS/COD ratio in the wastewater to below 0.5. Others suggested phase separation and pre-acidification (3, 6, 7).

The upflow sludge blanket filter (UBF) reactor is a modification of the UASB reactor. In the UBF reactor, a packed bed is used to replace the gas-liquid-solid separator on the top of the sludge bed in a typical UASB reactor. The packed bed is filled with a plastic medium, similar to that used in an anaerobic filter (AF) (8), to retain the active biomass, while the sludge bed underneath allows the development of granular biomass with good settling characteristics. Such a hybrid reactor may incorporate the benefits of both UASB and AF reactors (9, 10). It has been used for treating wastewaters from sugar and yeast factories, as well as landfill leachate (9, 11, 12). The objectives of this study were (a) to investigate the effect of biodegradable SS on the performance of a UBF reactor using insoluble starch as the model substrate, and (b) to evaluate the efficiency of COD removal, sludge yield, bacterial populations and the methanogenic activities of the starch-degrading granules.

MATERIALS AND METHODS

Figure 1 illustrates the process flow diagram of the UBF reactor. The 8.5 litre reactor, which had an internal diameter of 84 mm and a height of 1550 mm, was kept inside a constant temperature chamber at 37°C. The top 500 mm of the reactor was packed with plastic rings (Flexiring, Koch, Inc.), illustrated in Figure 2, forming a fixed bed. The diameter and the length of the packed rings were both 25mm. The surface-to-volume ratio was 235 $m^2.m^{-3}$. The sludge bed occupied the bottom 1050 mm of the reactor. Seven sampling ports were installed along the height of the reactor. The sludge quantities in the reactor were estimated periodically based on the concentration profiles of sludges sampled from these ports.

The reactor was seeded with 6.5 litres of digester sludge taken from an anaerobic digester of the Shatin Wastewater Treatment Works, Hong Kong. The seeded sludge contained 6.7 $kg.m^{-3}$ of total suspended solids (TSS) and 6.0 $kg.m^{-3}$ of the volatile suspended solids (VSS). The wastewater was formulated initially using a mixture of sucrose and corn starch as the organic substrates. The sucrose was totally soluble, whereas the corn starch was over 98% insoluble and suspended as colloidal particulates in the

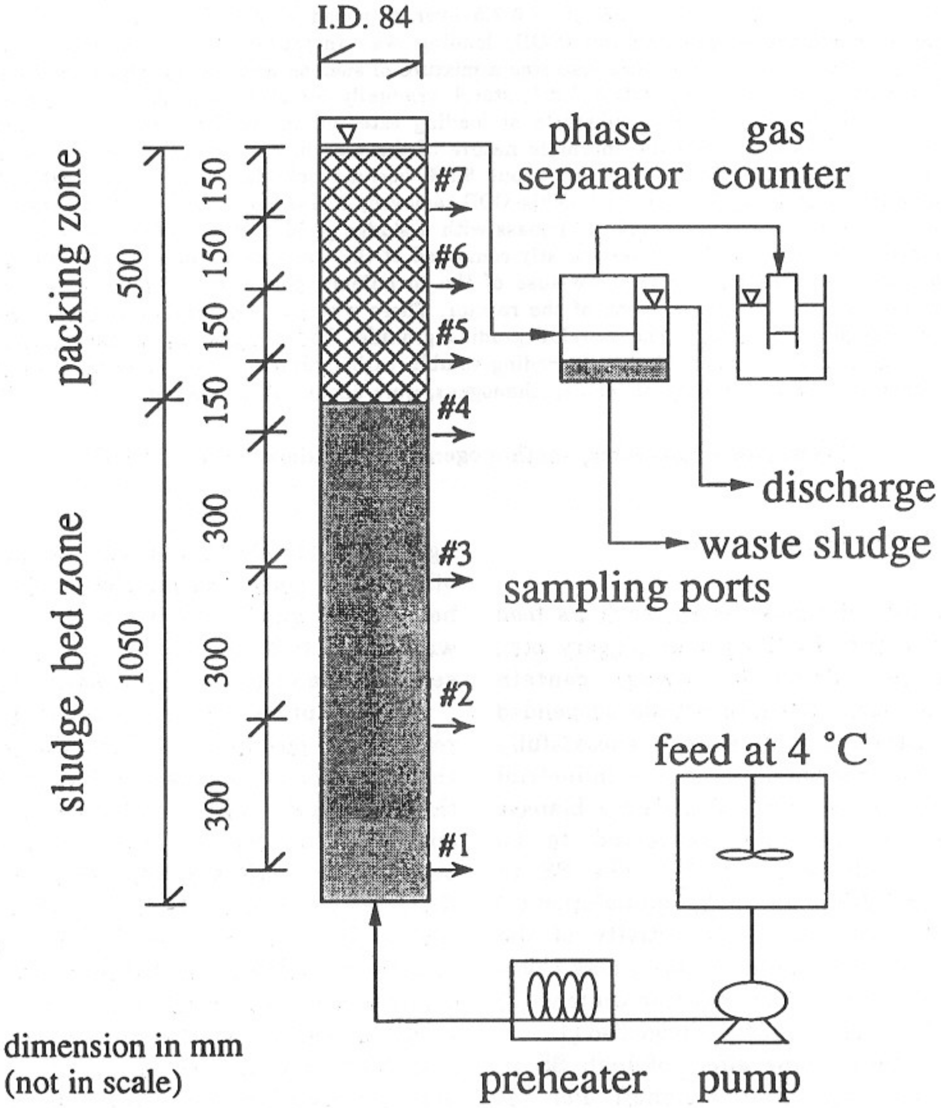

Figure 1. Process flow diagram of the UBF reactor.

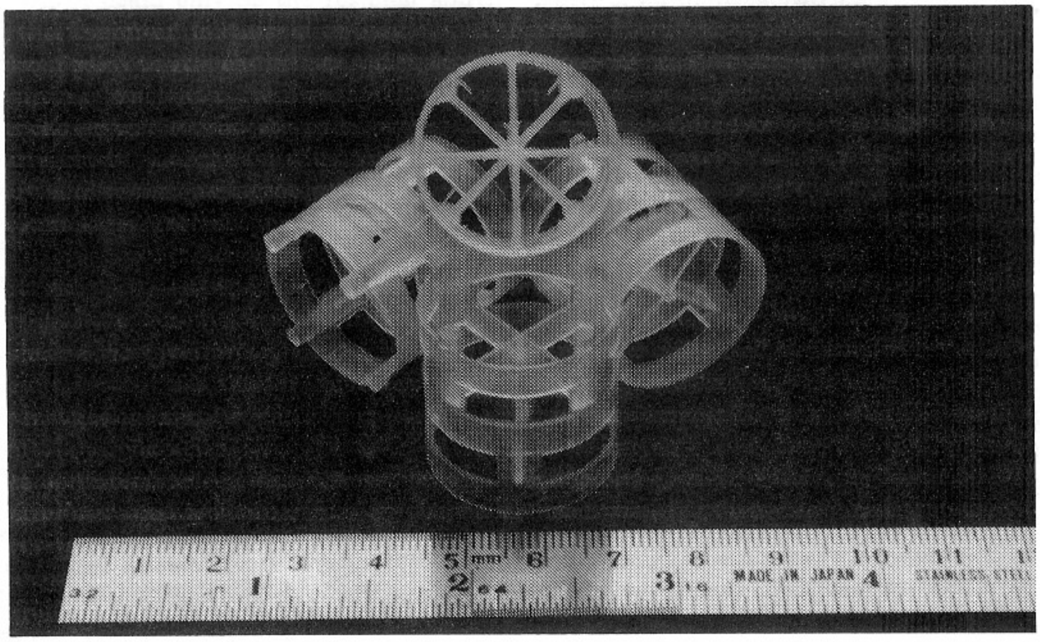

Figure 2. Flexiring (Koch Inc.).

wastewater. The biodegradable SS content in the wastewater was adjusted by the dosage of the insoluble corn starch. The ratio between the insoluble COD (COD_{ss}) and the total COD (COD_t) represents the relative amount of starch. Trace metals and balanced nutrients were supplemented to the wastewater following the formulation used in a previous study (13). Sodium bicarbonate was also added to supplement the alkalinity. For loading rates of 8 $kg.m^{-3}$ and lower, the dosage of sodium bicarbonate was equal to the COD in the wastewater; but at higher loading rates, only 8 $kg.m^{-3}$ of sodium bicarbonate was added to avoid the potential toxic effect caused by the increased concentration of 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. Detail sampling strategies and the analytical procedures also followed a previous study (13). A gas chromatograph (GC, Hewlett Packard model 5890A) was used to measure the composition VFA (from acetic to heptoic acid) in the effluent and the composition of the biogas. For VFA analyses, the GC was equipped with a 10 m x 0.53 mm HP-FFAP fused-silica capillary column and a flame ionization detector (FID), using helium as the carrier gas. Injector and detector temperatures were 200°C and 250°C, respectively. The fluid sample was filtered through a 0.45μm 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°C min^{-1} 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 m x 2 mm (inside diameter) stainless-steel column packed with Porapak N (80-100 mesh). Injector and detector temperatures were kept at 130°C while column temperature was 50°C.

The method of Lowry, *et al.* (14) was used to measure the protein contents in sludge granules and in washed-out solids. 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 UBF reactor was started with a wastewater feed containing 3000 g-$COD.m^{-3}$ and a hydraulic retention time (HRT) of 24 hours, giving an initial volumetric loading rate of 3 kg-$COD.m^{-3}.d^{-1}$. The initial COD_{ss}/COD_t ratio was kept at 0.1, while the loading rate was increased stepwise over 144 days until reaching 10 kg.m^{-3}.d^{-1}. The loading rate was kept at 10 kg.m^{-3}.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 (COD_{ss}/COD_t was increased stepwise from 0.1 to 1.0). From day 221 on, only starch was used as the sole organic substrate (COD_{ss}/COD_t = 1.0 in the wastewater), while the COD loading rate was increased stepwise. At each loading rate, the reactor was operated for at least 10 days to ensure reaching steady state, as indicated by the constant rate of biogas production. The loading rate was increased only when over 90% soluble COD was removed. The experiment was terminated on day 415 at 50 kg-COD.m^{-3}.d^{-1} because of the severe deterioration of COD removal efficiency resulting from the rapid decrease of pH in the reactor.

Additional granule samples were taken from the reactor between days 235 and 350. Granules taken on day 235, during which the reactor was operated at a loading of 10 kg-COD.m^{-3}.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 using the method of Dolfing and Bloemen (15) adapted from Owen, *et al.* (16), 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 (17, 18) using individual VFA, CO_2/H_2, sucrose, and starch as substrate. The presence of starch-degrading bacteria was considered positive on the disappearance of the starch particulates (19).

RESULTS AND DISCUSSION

Table 1 summarizes the major operational parameters, the pH in the effluent and the methane content in the biogas throughout the study. Key results and related discussion are as follows:

Starch Particulates in the Reactor and the Effluent

Biomass and residual starch particulates could both contribute to the VSS content in the reactor and the effluent. Their individual contents could be estimated from the protein levels of the VSS. The corn starch contained only trace amount of protein, while the biomass in the reactor was found to have a protein/VSS ratio of 0.68, comparable to previously reported values of 0.64-0.67 for UASB granules treating lactic acid (20) and 0.35-0.60 for granules treating various wastewaters (21). The protein/VSS ratio in the reactor remained at a constant level of 0.68 from day 175 to day 410, indicating that there was no starch accumulation in the reactor. On the other hand, the protein/VSS ratio in the washed-out

Table 1. Operational conditions, effluent pH and methane content in the biogas.

Day	Wastewater COD ($g\ m^{-3}$)	HRT (hr)	COD_{ss}/COD_t	COD loading rate ($kg\ m^{-3} d^{-1}$)	Effluent pH	Methane content (%)
0-105	3000	24	0.1	3.0	7.3	59
106-120	3000	18	0.1	4.0	7.3	62
121-129	4125	18	0.1	4.5	7.5	60
130-140	4500	16	0.1	5.5	7.3	58
141-190	5000	12	0.1	6.3	7.2	60
191-200	5000	12	0.2	10.0	7.3	61
201-210	5000	12	0.4	10.0	7.4	62
211-220	5000	12	0.6	10.0	7.2	61
221-265	5000	12	1.0	10.0	7.2	61
266-280	5000	9.6	1.0	12.5	7.1	59
251-298	6000	9.6	1.0	15.0	7.1	54
299-326	7000	9.6	1.0	17.5	7.2	53
327-353	10000	12	1.0	20.0	7.1	60
354-375	12500	12	1.0	25.0	7.2	60
376-359	15000	12	1.0	30.0	7.3	59
390-409	20000	12	1.0	40.0	7.0	55
410-415	25000	12	1.0	50.0	5.8	0

solids was slightly less than 0.68, indicating that there were non-proteinaceous solids in the effluent, which could only be the residual starch particulates.

Granulation and COD Removal Efficiency

The appearance of granules was first observed on day 75 when the COD_{ss}/COD_t ratio was 0.1 at 3 $kg\text{-}COD.m^{-3}.d^{-1}$. Granulation proceeded normally, as in the previous study using sucrose as the sole substrate (13), even when the starch became the sole substrate and COD loading rate increased. There was no noticeable adverse effect of starch particulates on the sludge granulation. This was probably due to the readily biodegradable nature of the starch, and partly due to the extended acclimation period for the development of sludge granules using sucrose as the co-substrate.

Figures 3a-3c illustrate the overall performance of the reactor throughout this study, including the removal efficiencies of total and soluble COD (Figure 3a), rate of biogas production (Figure 3b), and COD loading rates and COD_{ss}/COD_t ratios (Figure 3c). The soluble and total COD removal efficiencies were defined as follows:

soluble COD removal efficiency = (1- soluble COD in the effluent/wastewater COD) x 100%

total COD removal efficiency = (1- total COD in the effluent/wastewater COD) x 100%

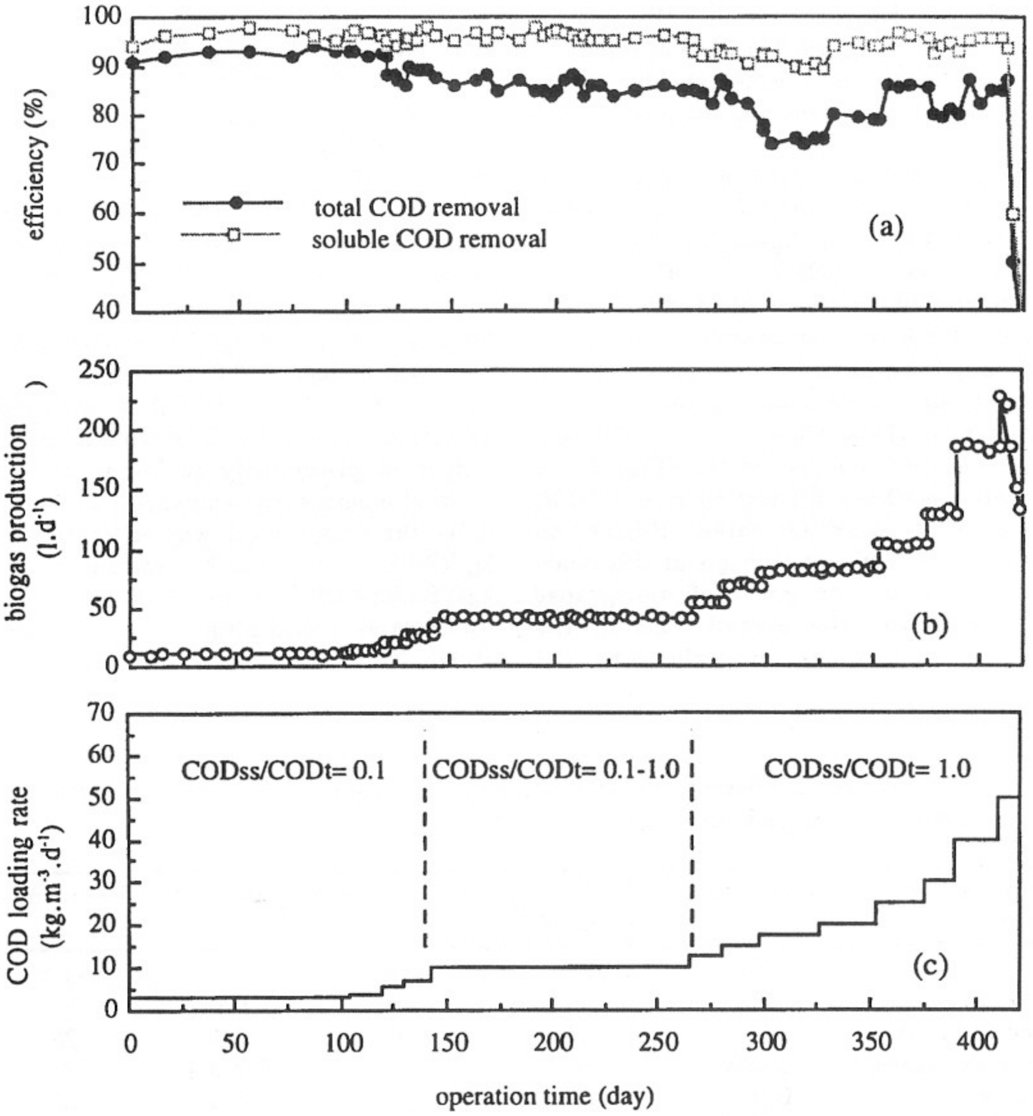

Figure 3. Performance of the UBF reactor: (a) COD removal efficiencies, (b) rate of biogas production, and (c) COD loading rate.

From day 1 to day 190, when the wastewater COD was composed of 10% starch and 90% sucrose, the reactor effectively removed on average 95.7% of soluble COD, for loading rates ranging from 3 kg-COD.m^{-3}.d^{-1} up to 10 kg-COD.m^{-3}.d^{-1}. From day 191 to day 265, the reactor removed consistently 96.1% of soluble COD at 10 kg-COD.m^{-3}.d^{-1}, even though all the sucrose in the wastewater was gradually replaced by starch during this period. From day 266 until the end of the study, the reactor was fed solely with starch particulates. As illustrated in Figure 3, the reactor during this period removed 94.1% soluble COD for loading rates up to 40 kg-COD m^{-3}.d^{-1}. The average COD removal efficiency of this study is compared, as shown in Table 2, with those in literature treating wastewater containing insoluble solids in various types of reactors, including UASB, membrane bioreactor system (MBS) and batch reactor. The UBF reactor was capable of removing soluble COD at a loading rate (40 kg-COD.m^{-3}.d^{-1}) considerably higher than those applied in previous studies (2-16.5 kg-COD.m^{-3}.d^{-1}).

However, the UBF reactor only removed on average 80.9% of total COD for loading rates ranging from 10 to 40 kg-COD.m^{-3}.d^{-1}. The insoluble fraction of COD in the effluent was mostly contributed by the washed-out biomass; only about 1.2% of the starch particulates remained unhydrolyzed and were washed out with the effluent, based on estimations from the protein content in the washed-out solids. Figure 4 illustrates the characteristics of the effluent and the volumetric methane production rate (VMPR) at various COD loading rates. Figure 4a illustrates that the effluent throughout this study contained less than 148 g.m^{-3} of undegraded starch particulates. The overall VSS in the effluent increased almost linearly with the increase of COD loading rate. The increased biomass washout at high COD loadings resulted from the vigorous mixing action caused by the gas produced at increased loading rate and the insufficient depth of the reactor to retain the biomass. The biomass in the reactor was highly active in converting the intermediate VFA into methane. Figure 4b illustrates that the effluent in general contained less than 120 g.m^{-3} of VFA, except between days 281-326. During that period the reactor was operated at 15.0-17.5 kg-COD m^{-3}.d^{-1} and the VFA concentrations in the effluent were 195-220 g.m^{-3} VFA. The increase of VFA in the effluent was due to the decrease of HRT during this period from the normal 12 hours to 9.6 hours. The VFA concentrations were lowered to 112 g.m^{-3}, after the HRT was returned to 12 hours, and remained below this level until day 410.

COD Balance and Sludge Yield

The methane content in the biogas was 53-62% throughout this study. Figure 4c illustrates that the VMPR, expressed as kg-methane-COD m^{-3}.d^{-1} (each gram of methane is equivalent to 4 grams of COD), increased linearly with the COD loading rate with a slope of 0.837. Figure 5, on the other hand, illustrates that the VMPR increased linearly with the volumetric substrate utilization rate with a slope of 0.861. The slope in Figure 5 indicates that of all the COD removed, 86.1% was converted to methane. The remaining 13.9% was converted presumably to biomass. Since each gram of biomass was equivalent to 1.40 grams of COD, the sludge yield was estimated to be 0.10 kg-VSS.kg-COD^{-1}, which is comparable to the 0.13 kg-VSS.kg-COD^{-1} of the UASB granules treating wastewater containing sucrose estimated by Hulshoff Pol (27), and the 0.08-0.23 kg-VSS

Table 2. Performance of different reactor designs in the treatment of wastewaters containing high levels of biodegradable suspended solids.

Wastewater Source	Reactor type	COD loading (kg.m^{-3}d^{-1})	COD removal (%)	COD^{ss}/COD^{t}	Reference
corn starch	UBF	40.0	94	1.0	this study
shochu	MBS	7	98	0.6	22
food industry	UASB	10	95	0.4	23
pharmaceutical	UASB	8	74	0.2-0.4	24
maize	UASB	2	95	0.2	25
distillery	UASB	16.5	-	-	6
piggery	batch	6.5	85	-	26

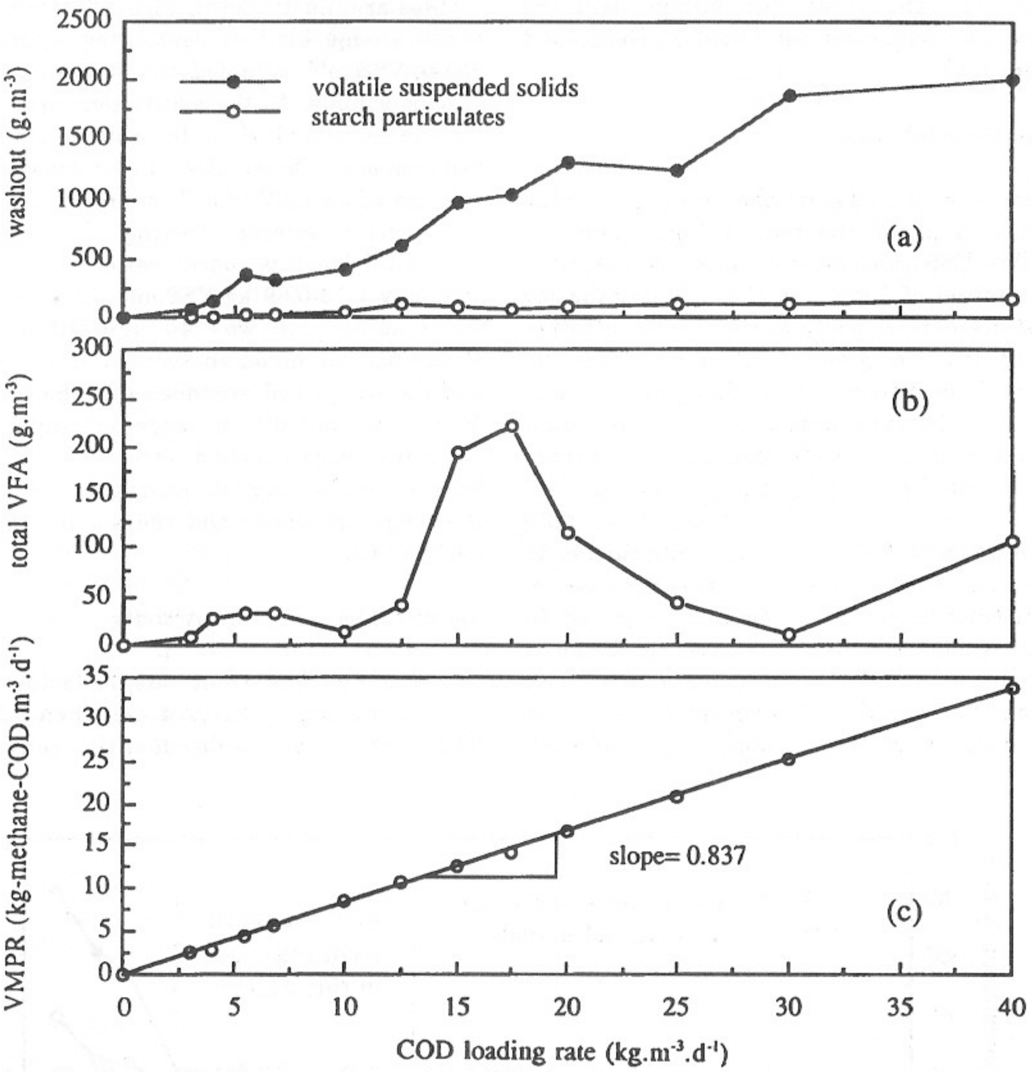

Figure 4. Performance of reactor at various COD loading rates: (a) VSS and unhydrolyzed starch particulates in the effluent, (b) VFA in the effluent, and (c) volumetric methane production rate (VMPR).

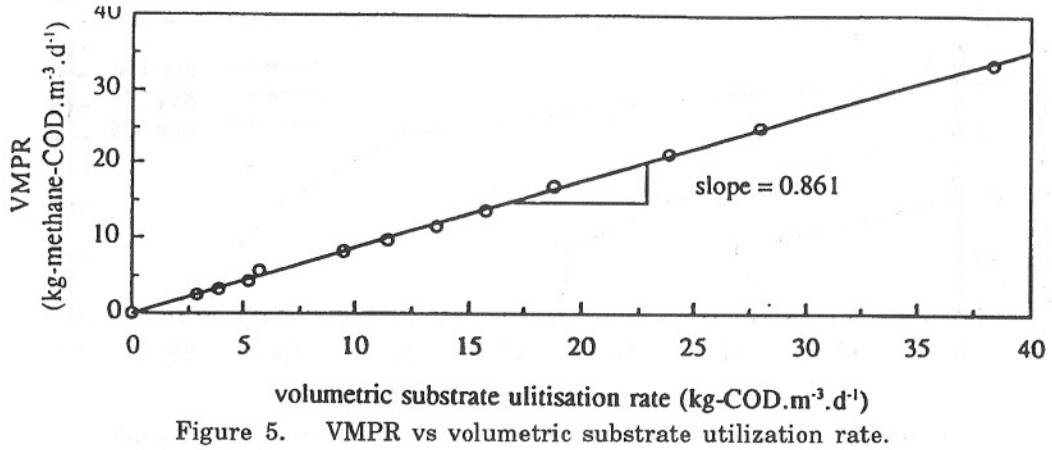

Figure 5. VMPR vs volumetric substrate utilization rate.

kg-COD^{-1} for the anaerobic sludge treating carbohydrate wastewater estimated by Henze and Harremoes (28).

Biomass Concentrations

Figure 6 illustrates the average sludge concentration inside the reactor throughout the study. The VSS concentration increased steadily from the initial of 5 g.m^{-3} to 41 g.m^{-3} near the end of the study period, while the TSS concentration increased from 8 g.m^{-3} to 83 g.m^{-3}. The SS concentrations remained unchanged between days 220-350 because sludge were removed from the reactor during that period for various analyses and for seeding other reactors. The steady decrease of VSS/TSS ratio from 0.89 during start-up to 0.60 on day 116, and further to 0.50 on day 405, was due to the increase of mineral content in the sludge, as observed in previous studies (13, 29). The average biomass concentrations in the reactor were estimated based on the profile of biomass obtained at various heights of the reactor. Three of such profiles are illustrated in Figure 7. On day 116, a dense sludge blanket containing approximately 20 kg-VSS.m^{-3} occupied the bottom 30% of the reactor volume. As the experiment proceeded, the total biomass and, thus, the volume of the sludge bed increased. On day 275, the sludge bed, with an average of 38 kg-VSS.m^{-3}, occupied about 50% of the reactor volume. During this period, the suspended biomass concentration in the AF zone was only 0.13-0.69 kg-VSS.m^{-3}. By day 405, when the loading rate was 40 kg-COD.m^{-3}.d^{-1}, the sludge bed contained an average of 55 kg-VSS.m^{-3} and the sludge bed expanded into the AF zone. At 50 kg-COD.m^{-3}.d^{-1}, a large amount of sludge granules were washed out from the reactor because of the vigorous mixing action caused by the biogas produced and the small depth of the packed bed.

Specific Methanogenic Activity

During anaerobic degradation, starch particulates are hydrolyzed and then acidified to VFA, which are subsequently converted to

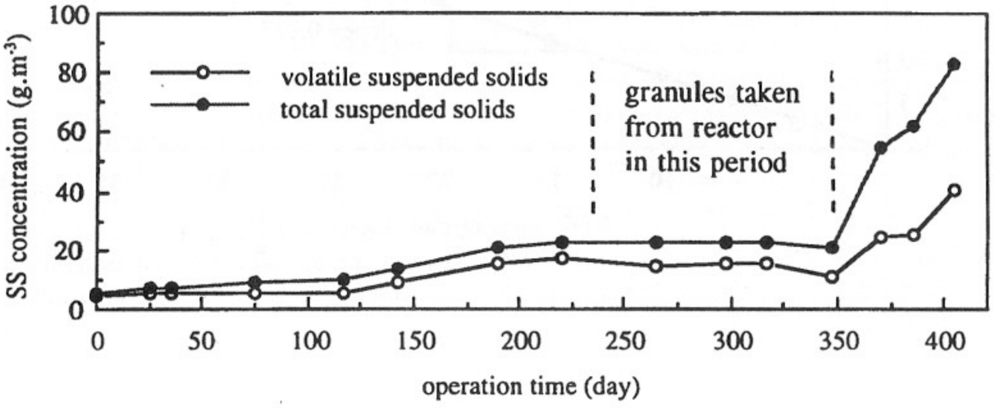

Figure 6. Average biomass concentration in the reactor throughout the study.

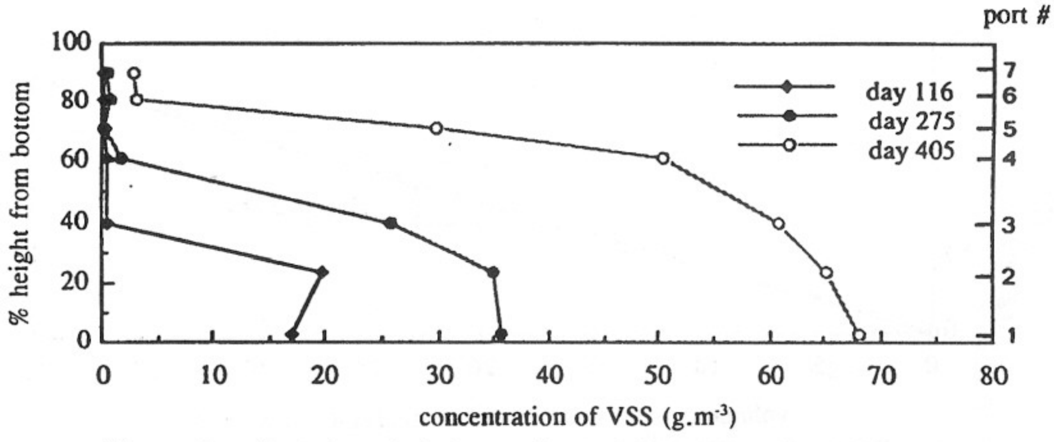

Figure 7. Variation of sludge profile at different periods of the study.

methane. Formate, acetate, propionate and butyrate are among the key intermediates (30). Table 3 summarizes the SMA of the granules using 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 either formate or sucrose as the sole substrate, the SMA of the starch-degrading granules in this study were slightly higher than those of sucrose- and sugar-degrading granules (21, 31). However, using acetate or butyrate as sole substrate, the SMA of starch-degrading granules in this study were substantially higher than those of granules degrading soluble carbohydrates. Using propionate as sole substrate, the SMA of starch-degrading granules was comparable to that of carbohydrate-degrading granules.

Sayed (2) suggested that the adsorption of colloidal materials on the surface of granules will hamper the supply of substrate to the bacteria in the interior of the granules, which may reduce the methanogenic activity of the granules. In his study, the SMA of granular sludge decreased from 0.3 to 0.1 kg-methane-COD.kg-VSS^{-1}.d^{-1} after treating slaughterhouse wastewater with coarse suspended solids. In this 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 starch.

The maximum specific methane production rate of granules observed in the reactor was 1.23 kg-methane-COD.kg-VSS^{-1}.d^{-1}, which was only 63% of the SMA of the granules measured in serum vials using starch as substrate. This suggests that the granules in the UBF reactor had the potential to degrade starch at loading rates higher than 40 kg-COD.m^{-3}.d^{-1}. The consistent and effective COD removal efficiency and the absence of starch accumulation in the reactor provide supporting evidence to such a speculation.

Table 3. Specific methanogenic activity (SMA) of granules using VFA and carbohydrates as substrates.

Sludge origin	SMA (kg-CH_4-COD.kg-VSS^{-1}.d^{-1}) using substrate						Reference
	formate	acetate	propionate	butyrate	sucrose	starch	
digester sludge	0.11	0.16	0.07	0.2	0.16	0.19	this study
starch	1.98	2.26	0.19	2.33	0.99	1.96	this study
sucrose	1.22	1.20	0.52	0.61	0.85	-	31
brewery	1.26	0.40	0.13	0.12	0.32	-	31
sugar	1.01	0.90	0.41	-	-	-	21
maize starch	0.74	0.09	0.12	-	-	-	32
starch		0.18-0.88					33

Table 4. Bacterial counts on UASB granules of different origins.

Metabolic group	Substrate	Number of organisms per ml of granular sludge			
		this study	A	B	C
Acidogens	starch particulates	$4.5x10^8$	-	-	-
	sucrose	$4.5x10^8$	$1.1x10^9$	10^{10}	$9.5x10^9$
methanogens	H_2/CO_2	$4.5x10^8$	$2.5x10^8$	10^9	$4.5x10^9$
	acetate	$4.5x10^8$	$2.5x10^8$	10^8	$4.5x10^9$
	formate	$9.5x10^8$	-	-	-
syntrophs	propionate	$9.5x10^7$	$2.5x10^8$	10^7	$4.5x10^9$
	butyrate	$1.5x10^8$	$1.1x10^8$	10^7	$2.0x10^{10}$

Microbial Populations

Table 4 shows that the microbial populations in the granules in this study were in general comparable to those in literature for UASB granules treating wastewaters containing carbohydrates. In the starch-degrading granules of this study, the three distinct groups of bacteria, i.e. acidogens, syntrophic acetogens, and methanogens, were about 10^8-10^9 per ml (MPN).

CONCLUSIONS

The following conclusions were drawn regarding the anaerobic digestion of starch particulates in a UBF reactor at 37°C and pH 7.0-7.5:

1. The UBF reactor effectively removed 94.1% of soluble COD from the wastewater containing starch as the sole organic substrate at loading rates up to 40 kg-COD.m^{-3}.d^{-1}. Despite its insoluble nature, the particulate starch in wastewater did not have any noticeable adverse effect on the sludge granulation and on the COD removal efficiency. There was no accumulation of starch particulates in the reactor throughout this study. About 1.2% of starch particulates remained unhydrolyzed and were washed out with the effluent.
2. The achievement of effective COD removal at high loading rates was probably due to the easy-to-biodegrade nature of the starch, the balanced nutrients, and the systematic acclimation strategies during the start-up. The amount of biomass washed out increased with loading rate, which could be partly attributed to the vigorous mixing caused by the biogas, and partly attributed to the insufficient depth of the reactor. The reactor failed at 50 kg-COD.m^{-3}.d^{-1} because of the rapid decrease in pH.
3. Of the COD removed, about 86.1% was converted to methane, and the rest was converted to biomass with a sludge yield of 0.10 kg-VSS.kg-COD^{-1}.
4. The granules exhibited high methanogenic activities. The SMA using either individual VFA or sucrose as substrate were in general higher than the corresponding literature data for those of granules treating soluble carbohydrates. The bacterial populations of acidogens, syntrophic acetogens, and methanogens, were about 10^8-10^9 per ml (MPN).

ACKNOWLEDGEMENT

The authors wish to thank the Hong Kong Research Grants Council for the financial support of this study.

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