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DEGRADATION OF STARCH PARTICULATES IN A HYBRID REACTOR

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ABSTRACT

The study was conducted over 265 days to study the feasibility of removing starch particulates from wastewater using an 8.5 L reactor which was a hybrid between the upflow anaerobic sludge blanket (UASB) and the anaerobic filter reactors. At pH 7.2-7.5 and 37°C, the reactor was effective for the removal of chemical oxygen demand (COD) from wastewater containing starch particulates equivalent to 5000 mg/L of COD with 12 hours of retention time, corresponding to a loading rate of 10 g-COD/L.d. Despite their insoluble nature, the starch particulates did not cause noticeable adverse effects on the granulation of biomass, probably due to its easy-to-biodegrade nature and the cautious startup strategy. About 5.8% of COD in wastewater remained in the effluent, 82.5% was converted to methane, and the remaining 11.7% was converted to granular biomass with an average sludge yield of 0.09 g-VSS/g-COD. The granules exhibited a layered microstructure. The methanogenic activity of the granular biomass was 0.86 g-methane-COD/g-VSS.d in the reactor, which was considerably lower than the 1.96 g-methane-COD/g-VSS.d measured in serum vials with an abundant supply of substrate, suggesting that further increase of loading rates was possible for the hybrid reactor.

KEYWORDS

Activity, biomass, carbohydrate, COD, granule, hybrid, loading, methane, starch, yield.

INTRODUCTION

The upflow anaerobic sludge blanket (UASB) reactor (Lettinga et al., 1980) has become popular in the past decade not only in Europe but also in East Asia for the treatment of wastewater from industries such as brewery, distillery, dairy, sugar processing, potato, etc., in which the organic pollutants are mainly composed of soluble carbohydrates (Hulshoff Pol and Lettinga, 1986; Fang, et al., 1990; Lettinga and Hulshoff Pol, 1991). However, the UASB reactor has been perceived to be unsuitable for wastewater containing high levels of suspended solids (SS), which may inhibit the sludge granulation (Lettinga et al., 1980), impair the methanogenic activity of the sludge (Sayed, 1987), or, in some extreme cases, cause a sudden washout of the sludge bed (Lettinga et al., 1980).

This study was conducted to examine the feasibility of treating wastewater containing high levels of insoluble starch in a hybrid reactor (Olthof and Oleszkiewicz, 1982), which consisted of a sludge bed at the bottom, as in a UASB reactor and a packed bed as an anaerobic filter (Young and McCarty, 1969) reactor at the top. In such a hybrid reactor, the UASB zone allows biomass to aggregate forming granules with high settleability and bioactivity, while the packed bed filled with plastic rings retains the biomass in the reactor. This kind of reactor has been applied to treating wastewaters from sugar and yeast factories and to landfill leachate (Guiot and van den Berg, 1984; Kennedy et al., 1988; van der Merwe and Britz, 1993). However, little information is available on the capability of this hybrid reactor for the treatment of wastewater containing high levels of suspended solids. A cautious startup strategy was adopted for this study. The COD removal efficiency of the reactor as well as the microstructure and microbial populations of the granules were investigated.

EXPERIMENTAL METHODS

Figure 1 illustrates the process flow diagram of the 8.5 L hybrid reactor, which had an internal diameter of 84 mm and a height of 1550 mm. The top 500 mm of the reactor was filled with plastic rings (Flexiring, Koch, Inc.), forming a packed bed. The diameter and the length of the rings were both 25mm, with a surface-to-volume ratio of 235 m^2/m^3, as illustrated in Figure 2. 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 from the concentration profiles of sludge sampled from these ports. The reactor was operated at 37°C throughout this study in a constant temperature chamber.

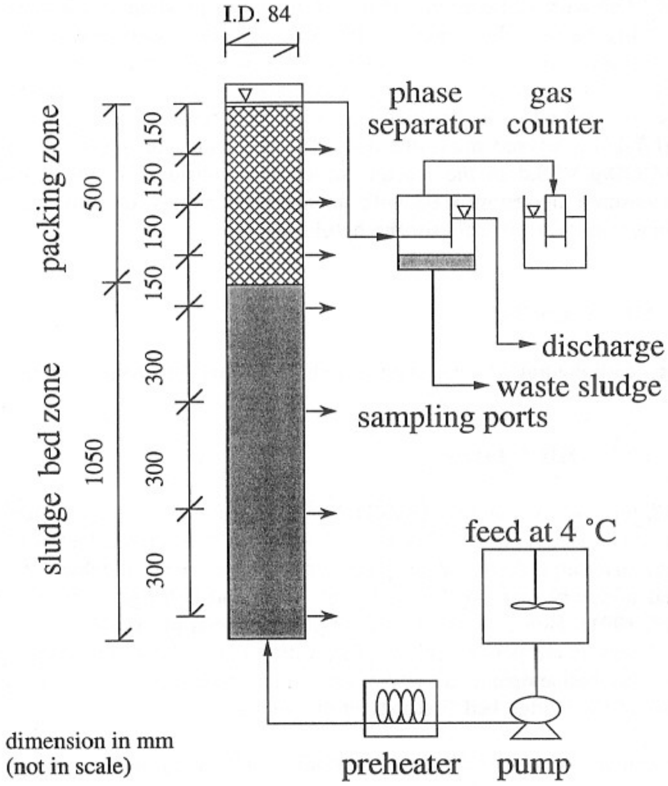

Figure 1 Process Flow Diagram

The reactor was seeded with 6.5 L of digester sludge taken from an anaerobic digester of the Shatin Wastewater Treatment Works, Hong Kong. The seeded sludge contained 6.7 g/L of total suspended solids (TSS) and 6.0 g/L of the volatile suspended solids (VSS). During the startup, the wastewater was formulated using a mixture of sucrose and corn starch to alleviate the adverse effect, if any, of the starch particulates on the granulation process. The fraction of starch in the organic substrate could be expressed by the ratio between the insoluble COD (COD_{ss}) and the total COD (COD_t) in the wastewater. Alkalinity, trace metals and balanced nutrients were supplemented to the wastewater following the formulation used in a previous study (Fang and Chui, 1993). Wastewater was made up once every two days and kept in a 4°C feed tank with constant mixing.

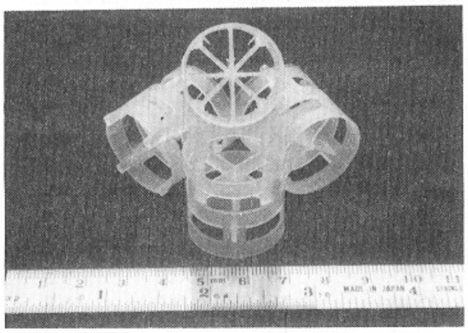

Figure 2 Plastic Packing Flexiring (Koch, Inc.)

Gas production rate, gas composition and sludge bed height were measured daily. Volatile fatty acids (VFA), pH, COD,VSS and TSS in the wastewater and/or effluent were measured twice a week. Detailed sampling strategies and the analytical procedures, such as the methane content in biogas, the VFA levels in effluent, etc., also followed those of a previous study (Fang and Chui, 1993). The protein contents in the sludge granules in the reactor and in the solids washed out in the effluent were measured at least once a week using the method developed by Lowry et al. (1951).

The reactor was started with a loading rate of 3 g-COD/L.d, which corresponded to 24 hours of hydraulic loading rate (HRT) and 3000 mg/L of COD in the wastewater. 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 g-COD/L.d. The COD loading rate was then kept at 10 g/L.d 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, which means the COD_{ss}/COD_t ratio was increased stepwise from 0.1 to 1.0. At each loading rate and COD_{ss}/COD_t ratio, the reactor was operated for at least 9 days to ensure reaching steady state, as evidenced from the constant rate of biogas production and the steady COD removal efficiency. At the end of this study on day 265, the reactor had been operated at 10 g-COD/L.d using starch as the sole organic substrate for 44 days.

Additional biomass were sampled from the reactor on day 235 for the analyses of specific methanogenic activity (SMA) and microbial populations. The SMA was measured in serum vials (Dolfing and Bloemen, 1985, and Owen et al. 1979) using various VFA, sucrose and starch as individual organic substrate. Enumeration of the microbial population was performed by the most probable number (MPN) method in triplicate (Chartrain and Zeikus, 1986, Li and Noike, 1992) 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 (Barlaz, 1988).

RESULTS AND DISCUSSION

Table 1 summarizes the operational parameters throughout this study, including the wastewater COD, hydraulic retention time (HRT), COD loading rate, and the fraction of starch in the organic substrate (COD_{ss}/COD_t). Table 1 also shows that the pH in the effluent and the methane content in the biogas were kept at the constant levels of 7.2-7.5 and 58-62%, respectively.

TABLE 1 OPERATIONAL CONDITIONS, EFFLUENT pH AND METHANE CONTENT IN BIOGAS

day	wastewater COD (mg/L)	HRT (hrs)	COD_{ss}/COD_t	COD loading (g/L.d)	pH	methane in biogas (%)
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	5.5	7.5	60
130 - 144	4500	16	0.1	6.8	7.3	58
145 - 190	5000	12	0.1	10.0	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

Granulation of Biomass

The appearance of granules was first observed on day 75 when the reactor was operated at 3 g-COD/L.d and only 10% of the organic substrate was made of starch. Granulation proceeded normally, as in a previous study (Fang and Chui, 1993) using sucrose as the sole substrate, as COD loading rate increased and as starch gradually replaced sucrose to become the sole substrate. The presence of starch particulates did not cause any noticeable adverse effect on the sludge granulation nor did it cause a sudden sludge washout. This was probably due to the easy-to-biodegrade nature of starch, and the extended acclimation period for the development of sludge granules using sucrose as the co-substrate.

Starch Particulates in the Reactor

The VSS content in the reactor and the effluent could be contributed by both the biomass and the unhydrolyzed starch. Biomass was rich in protein, but starch contains less than 0.5% of protein. Thus, the protein content in the VSS could be used to estimate the relative amounts of biomass and unhydrolyzed starch. The protein/VSS ratio in the reactor sludge was found at a constant level of 0.68 over an extended period, from day 175 at loading rate of 6.8 g-COD/L.d to day 265 at 10 g-COD/L.d. One would expect a decrease in protein/VSS ratio should there be an accumulation of starch as COD loading increased. The constant protein/VSS ratio in the reactor indicated that there was no accumulation of starch particulates, which was consistent with visual observations. Thus, the ratio of 0.68 represented the protein/VSS ratio of the biomass in the reactor. This ratio is comparable to the 0.64-0.67 (Fukuzaki et al., 1991) for the UASB granules treating lactic acid and the 0.35-0.60 for the granules treating various wastewaters (Dolfing et al., 1985).

COD Removal Efficiency and Balance

From day 1 to day 145, the starch made up only 10% of the organic substrate while the COD loading rate was increased from 3 g/L.d up to 10 g/L.d. The reactor during this period removed on the average 95.7% of soluble COD, and 83.8% of total COD. Starting from day 145 until the end of the study, the reactor was operated at the constant loading rate of 10 g-COD/L.d. Figure 3 illustrates the performance of the reactor during this period of constant loading rate, during which the starch fraction of the substrate in wastewater increased stepwise from 10% to 100% (Figure 3c). Figure 3a illustrates that the removal efficiencies of soluble COD and total COD were consistently maintained at 96.1% and 82.7%, respectively, despite the fact that starch had gradually replaced sucrose to become the sole substrate. 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%

The insoluble fraction of COD in the effluent could be contributed by either the unhydrolyzed starch or the washed out biomass. Figure 3b illustrates that 80-90% of the insoluble fraction of COD in the effluent was contributed by the washed out biomass; only 1.9% of the starch in wastewater remained unhydrolyzed and were discharged along with the effluent. Since the reactor relied on the packed bed to retain the biomass, the extent of biomass washout could likely be reduced by increasing the depth of the packed bed.

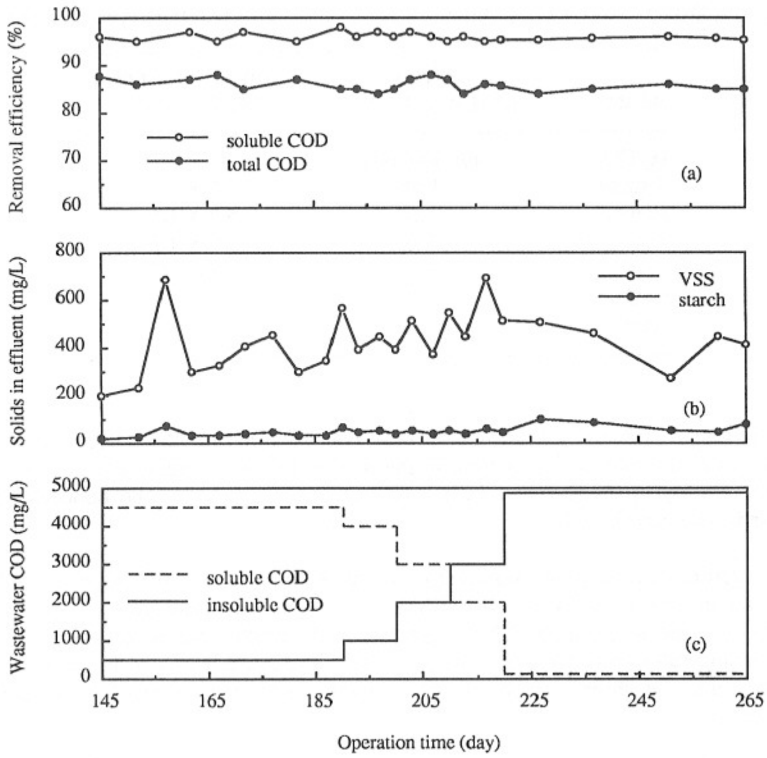

Figure 3 Reactor Performance at 10 g-COD/L.d : (a) COD Removal Efficiencies, (b) VSS and Unhydrolyzed Starch Content in Effluent, and (c) Fractions of Starch and Sucrose in Wastewater.

Throughout the study, only a trace amount of VFA (below 31.9 mg/L) was found in the effluent. This indicates that the intermediate VFA produced from acidogenesis and acetogenesis were readily converted to methane. The yield of sludge can be estimated from the COD balance. On average, 5.8% of the COD in wastewater remained in the effluent, of which 1.9% was the unhydrolyzed starch particulates and the other 3.9% was in soluble form. Of the 94.2% COD removed, 82.7% was converted to methane (each gram of methane is equivalent to 4.0 grams of COD) and the remaining 11.7% was presumably converted to biomass. Each gram of biomass was found to have 1.40 grams of COD. The average sludge yield, as a result, was estimated to be 0.09 g-VSS/g-COD, which is comparable to the 0.08-0.23 g-VSS/g-COD for the anaerobic sludge treating wastewater of carbohydrates estimated by Henze and Harremoes (1983).

Specific Methanogenic Activity and Microbial Population

Table 2 summarizes the result of SMA and the microbial counts (MPN) of granules taken out from the reactor on day 235. Using either formate or sucrose as the sole substrate, the SMA of the granules of this study were comparable to those of granules grown on sucrose and sugar (Fang et al., 1994). However, using either acetate or butyrate as sole substrate, the SMA of the granules of this study was substantially higher than those of granules degrading soluble carbohydrates. The reason behind the SMA differences between starch-degrading and sucrose-degrading granules are not clear. The result of MPN counts on microbial populations showed that the microbial populations in the granules of this study are in general comparable to those in the literature (Fang et al., 1994; Dolfing et al., 1985) for UASB granules treating wastewaters containing carbohydrates.

TABLE 2 SMA AND MPN OF STARCH-DEGRADING GRANULES

substrate	SMA ($g-CH_4-COD/g-VSS.d$)	microbial count (MPN/g-VSS)
H_2/CO_2	not measured	1.1×10^7
formate	1.98	5.8×10^6
acetate	2.26	5.8×10^6
propionate	0.19	2.2×10^6
butyrate	2.33	5.8×10^6
sucrose	0.99	2.2×10^7
starch	1.96	2.2×10^7

Sayed (1987) suggested that the adsorption of colloidal materials on the surface of granules would hamper the supply of substrate to the bacteria in the interior of the granules, and thus would deteriorate the methanogenic activity of the granules. In this study, however, there was no noticeable adverse effect by the starch particulates on the SMA.

The maximum specific methane production rate of granules observed in the reactor was 0.86 g-methane-COD/g-VSS.d, which was only 44% of the SMA of the granules measured in serum vials with an abundant supply of starch as substrate. This suggests that the reactor was potentially capable of treating wastewater at loading rates considerably higher than 10 g-COD/L.d. The consistent and effective COD removal efficiency and the absence of starch accumulation in the reactor provided supporting evidence to such a speculation.

Microstructure of Granular Sludge

A three-layered microstructure has been reported for UASB granules treating carbohydrates as substrates (Fang et al., 1994). Although the starch-degrading granules in this study also illustrated the layered microstructure when observed under SEM, the outer layer was different from those reported previously. The outer layer of a typical starch-degrading granule was predominantly composed of streptococci (0.7μm in diameter). Presumably these streptococci were responsible for carrying out the initial hydrolysis of starch particulates and the subsequent acidogenesis. The middle layer was composed of long thin filaments, small rods and cocci in syntrophic microcolonies along with the scattered streptococci. The central core was predominantly composed of acetoclastic *Methanothrix.*

CONCLUSIONS

At pH 7.2-7.5 and 37°C, the hybrid reactor was effective for the removal of starch particulates from wastewater containing 5000 mg/L of COD with 12 hours of retention time, corresponding to a loading rate of 10 g-COD/L.d. About 5.8% of COD in wastewater remained in the effluent, 82.5% was converted to methane, and the remaining 11.7% was converted to granular biomass with an average sludge yield of 0.09 g-VSS/g-COD. Despite its insoluble nature, the starch particulates in wastewater did not have noticeable adverse effect on the granulation of biomass. This was probably due to the easy-to-biodegrade nature of starch and the extended acclimation period for the development of sludge granules using sucrose as the co-substrate. The methanogenic activity of the biomass was 0.86 g-methane-COD/g-VSS.d in the reactor, which was considerably lower than the 1.96 g-methane-COD/g-VSS.d measured in serum vials with an abundant supply of substrate, suggesting that further increase of loading rates was possible for the hybrid reactor. The granules exhibited a layered microstructure with a large amount of streptococci in the outer layer for the starch hydrolysis and acetoclastic *Methanothrix* in the interior core.

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