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Thermophilic acidification of dairy wastewater

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Abstract Acidification of simulated dairy wastewater was conducted in an upflow reactor at 55 °C. Results showed that the degree of acidification decreased with the increase in chemical oxygen demand (COD) loading rate, from 60.8% at 4 g l^{-1} day^{-1} to 27.1% at 24 g l^{-1} day^{-1}. Carbohydrate was readily degraded at all loading rates, but degradation of protein and lipid decreased with the increase in loading rate. Most carbohydrate degradation occurred at the reactor bottom, whereas protein was degraded mainly after the carbohydrate became depleted. The predominant acidification products were acetate, propionate, butyrate and ethanol, whereas formate, *i*-butyrate, valerate, *i*-valerate, caproate, lactate, methanol, propanol and butanol were present in lesser quantities. The increase in loading rate resulted in the increase of propionate and the decrease of acetate, but had little effect on ethanol and butyrate productions. Only 2.5–8.8% of influent COD was converted to hydrogen and methane. The biomass yield was 0.30–0.43 mg VSS mg^{-1} COD.

Introduction

The two-phase anaerobic treatment process was first proposed by Pohland and Ghosh (1971). In their system, fast-growing acidogens were predominant in the first phase, while slow-growing acetogens and methanogens were mainly concentrated in the second phase. This process has been applied to the treatment of municipal sludge (Fongsatikul et al. 1994; Ghosh 1991; Lin and Ouyang 1993; Shimizu et al. 1993) and wastewaters from cafeterias (Hanaki 1990), coffee-processing (McDougall et al. 1993), olive oil mills (Beccari et al. 1996), vegetable-processing (Viturtia et al. 1995), whey-processing (Kissalita et al. 1989), etc. Nearly all of these studies were conducted under the conventional mesophilic condition ranging over 35–40 °C.

However, many industrial effluents, such as those from food processing, are often discharged at high temperatures. Treating these effluents under conventional mesophilic conditions requires a costly pre-cooling process, and runs the risk of losing biomass activity should the cooling system break down. It is thus preferable to treat these wastewaters under thermophilic conditions. This would not only lower the operating costs, but would also be more efficient in degrading organics and killing pathogens (Wiegant et al. 1986). Ghosh (1991) reported that thermophilic acidogenesis of municipal sludge was very effective. Mitsdorffer et al. (1990) also reported significant disinfection of municipal sludge by a thermophilic acid phase digestion. *Enterobacteriaceae* and *Salmonellae* were reduced by several orders of magnitude. Dichtl (1997) reported that the performance of a thermophilic acidogenic reactor followed by a mesophilic methanogenic reactor performed considerably superior to a single-phase reactor in the degradation of organic matter. Similar results were reported by Roberts et al. (1998). However, all of these studies were limited to the digestion of municipal sludge. The objective of this study was to examine the thermophilic acidification of a complex wastewater under various organic loading conditions.

Materials and methods

Reactor

The upflow anaerobic reactor was 2.8 l in volume with an internal diameter of 84 mm and a height of 500 mm. Details of its configuration have been described previously (Fang et al. 1994a). Five evenly distributed ports were installed over the height of the column. In engineering practice, it is commonly accepted that the

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biomass in a reactor is measured by the content of volatile suspended solids (VSS). Thus the total biomass in the reactor was estimated on the basis of the VSS profile of the samples taken from the ports. On top of the reactor was a gas-liquid-solid (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 the volume of the G-L-S separator. The reactor was water-jacketed and operated at a constant temperature of 55 °C.

Wastewater and seed inoculum

The reactor was fed with simulated dairy wastewater, prepared from full-cream powdered milk supplied by Nestlé. Throughout the experiment the influent chemical oxygen demand (COD) was kept at 4,000 mg l^{-1}, equivalent to 2,860 mg l^{-1} milk. Carbohydrate, protein and lipid were the three major constituents in the wastewater, accounting for 30.9%, 23.6% and 41.9% of the total COD, respectively. Since the milk contained a sufficient amount of nitrogen, minerals and vitamins, only 20 mg P l^{-1} was added as supplement.

In this study, the seed inoculum was taken from a mesophilic reactor treating the same synthetic wastewater (Fang and Chung 1999). The reactor was seeded with 26.8 g VSS inoculum, resulting in an initial mixed liquor VSS concentration of 9.6 g l^{-1}.

Start-up

During the start-up, wastewater was fed to the acidification reactor at the organic loading rate (OLR) of 4 g COD l^{-1} day^{-1}. The operating temperature was increased from 37 °C to 55 °C over a 9-day period and was then kept at 55 °C throughout the rest of study. Acidogenic bacteria were enriched in the reactor by controlling the pH at 5.5 ± 0.1.

The effluent VFA (volatile fatty acid) concentration increased initially during the start-up, but gradually leveled off. Start-up was completed after 67 days, when the VFA concentration in the effluent became steady. The OLR was then increased stepwise from the initial 4 g COD l^{-1} day^{-1} to 6, 8, 12, 16 and then 24 g COD l^{-1} day^{-1}. At each OLR level, the reactor was operated for 34–43 days to ensure the reactor reached steady state before the OLR was increased to the next level.

Analyses

The amount of biogas produced in the reactor was recorded daily using the water replacement method. The level of hydrogen, methane, carbon dioxide and nitrogen in the biogas were analyzed by a gas chromatograph (GC, model 5890; Hewlett Packard), equipped with a thermal conductivity detector and a 2 m × 2 mm (inside diameter) stainless-steel column packed with Porapak N (80–100 mesh). Injector and detector temperatures were kept at 130 °C and 200 °C, respectively, while the column temperature was increased from 90 °C to 110 °C.

The concentrations of VFA, including acetate, propionate, butyrate, *i*-butyrate, valerate, *i*-valerate, caproate and lactate, and alcohols, including methanol, ethanol, propanol and butanol, were determined by a second GC of same model, equipped with a flame ionization detector and a 10 m × 0.53 mm HP-FFAP fused-silica capillary column. Samples were filtered through a 0.2-μm filter, acidified by formic acid, and measured for free acids. The initial temperature of the column was 70 °C for 4 min, then 140 °C for 3 min and finally 170 °C for 4 min. The injector and detector temperatures were both 200 °C. Helium was used as the carrier gas at a flow rate of 25 ml min^{-1}. The formate concentration was measured by the colorimetric method (Lang and Lang 1972).

Carbohydrate and protein were measured by the phenol-sulfuric method (Herbert et al. 1971) and the Lowry method (Lowry et al. 1951), respectively. Lipid was extracted by the Bligh-Dyer method from the acidified sample and was then measured gravimetrically after the solvent was evaporated at 80 °C (APHA et al. 1992). Measurements of COD, pH, and VSS were performed according to the Standard Methods of APHA et al. (1992).

Results

Substrate degradation patterns

As illustrated in Fig. 1a, the degradation of carbohydrate, protein and lipid increased individually with the decrease in OLR, following the order: carbohydrate > protein > lipid. Carbohydrate was readily degraded at all loading rates, from 92.1% at 24 g COD l^{-1} day^{-1} to 98.7% at 4 g COD l^{-1} day^{-1}. However, protein degradation was significantly influenced by the OLR. The degradation of protein decreased from 89.2% at 4 g COD l^{-1} day^{-1} to 67.1% at 24 g COD l^{-1} day^{-1}. Similarly, the lipid degradation also decreased with the increase in OLR, from 42.9% at 4 g COD l^{-1} day^{-1} to only 18.0% at 24 g COD l^{-1} day^{-1}.

Degree of acidification

The main acidification products were VFA and alcohols in the mixed liquor, and hydrogen and methane in the biogas. The degree of acidification can be quantified by comparing the COD-equivalent of these acidification products with the wastewater COD. Fig. 1b illustrates that the degree of acidification decreased only slightly

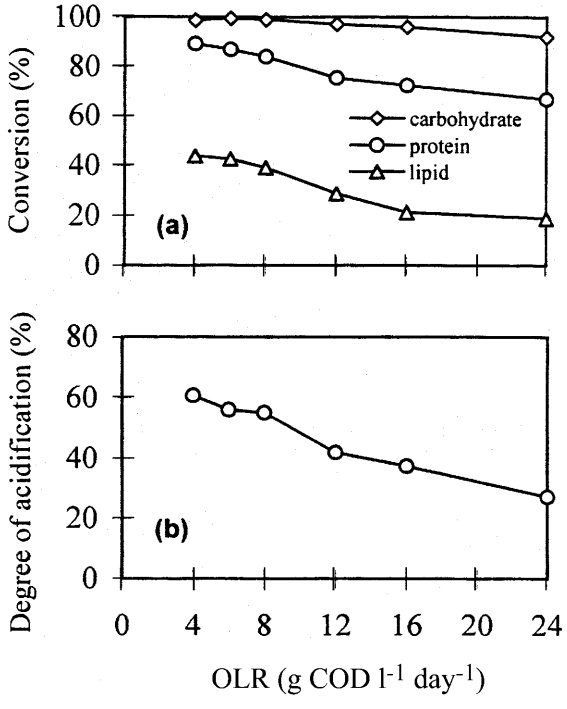

Fig. 1 Performance of acidification reactor: **a** % conversion of *carbohydrate*, *protein* and *lipid* and **b** degree of acidification. *OLR* Organic loading rate

from 60.8% to 54.9% when the loading rate was increased from 4 to 8 g COD l^{-1} day^{-1}. But the degree of acidification decreased drastically at higher loading rates. Only 27.1% of organic matters were acidified at 24 g COD l^{-1} day^{-1}.

Effluent VFA and alcohol distribution

Table 1 summarizes the concentrations of 13 VFAs and alcohols in the effluent at each OLR. It suggests that the OLR was critical to the distribution of VFA/alcohol in the effluent. Acetate, propionate, butyrate and ethanol were the main products. Formate, *i*-butyrate, lactate, valerate, *i*-valerate, caproate and methanol were present in smaller quantities. Propanol and butanol were found only in certain runs. At 4 g COD l^{-1} day^{-1}, the acidification products were composed of 34% acetate, 16% propionate, 10% butyrate and 12% ethanol, plus 28% of other metabolites. The percentage of acetate decreased with the increase in OLR, down to 17% at 24 g COD l^{-1} day^{-1}, whereas that of propionate increased with OLR up to 32% at 24 g COD l^{-1} day^{-1}. The percentages of butyrate and ethanol were not sensitive to the OLR.

Profiles in reactor

Samples taken from points at 0, 100, 200, 300, 400 and 500 mm from the reactor bottom provided an insight into organic degradation in the reactor. Figures 2a and 2b illustrate the concentration profiles of acetate, propionate, butyrate and ethanol at 6 g COD l^{-1} day^{-1} in the reactor. The product composition varied substantially at different reactor levels. This indicates that the mixed liquor in this acidification upflow reactor was not in complete-mix mode, unlike in a single-phase methanogenic upflow reactor (Fang and Chui 1993; Fox and Pohland 1994). This was due to the low biogas production in the acidogenic reactor.

Figure 2c illustrates the degradation patterns of carbohydrate and protein in the reactor operated at 6 g COD l^{-1} day^{-1}. Most of the carbohydrate (about 85%) was degraded near the reactor bottom (0–100 mm). But little protein was converted in this zone. Instead, most of the protein was degraded between 100–200 mm, where carbohydrate was less than 100 mg l^{-1}. This seems to indicate that acidification in the reactor took place in two steps. Carbohydrate was first degraded near the reactor bottom; and degradation of protein took place at higher levels only when the carbohydrate became depleted.

Gas production

Throughout the experiment, the production of hydrogen and methane accounted for only 2.5–8.8% of the

Table 1 Concentration and percentage of individual volatile fatty acids and alcohols in effluent. *Bol* Butanol, *COD* chemical oxygen demand, *Eol* ethanol, *HAc* acetate, *HBu* butyrate, *HCa* caproate, *HFr* formate, *HLa* lactate, *HPr* propionate, *HVa* valerate, *i-HBu* *i*-butyrate, *i-HVa* *i*-valerate, *Mol* methanol, *OLR* organic loading rate, *Pol* propanol, *VFA* volatile fatty acid

OLR (g COD l^{-1} day^{-1})	HFr (mg l^{-1})	HAc (mg l^{-1})	HAc (mg l^{-1})	HBu (mg l^{-1})	*i*-HBu (mg l^{-1})	HVa (mg l^{-1})	*i*-HVa (mg l^{-1})	HCa (mg l^{-1})	HLa (mg l^{-1})	Mol (mg l^{-1})	Eol (mg l^{-1})	Pol (mg l^{-1})	Bol (mg l^{-1})
4	23 (2%)	430 (34%)	202 (16%)	127 (10%)	40 (3%)	60 (5%)	41 (3%)	50 (4%)	76 (6%)	63 (5%)	152 (12%)	0 (0%)	0 (0%)
6	26 (2%)	410 (32%)	205 (16%)	149 (12%)	51 (4%)	52 (4%)	62 (5%)	77 (6%)	38 (3%)	52 (4%)	142 (11%)	26 (2%)	0 (0%)
8	32 (3%)	344 (28%)	221 (18%)	108 (9%)	50 (4%)	41 (3%)	45 (4%)	74 (6%)	86 (7%)	61 (5%)	123 (10%)	37 (3%)	0 (0%)
12	21 (2%)	206 (23%)	179 (20%)	83 (9%)	39 (4%)	32 (4%)	22 (2%)	63 (7%)	54 (6%)	72 (8%)	90 (10%)	36 (4%)	9 (1%)
16	23 (3%)	203 (24%)	220 (26%)	79 (9%)	28 (3%)	20 (2%)	14 (2%)	42 (5%)	42 (5%)	69 (8%)	99 (12%)	0 (0%)	17 (2%)
24	12 (2%)	103 (17%)	188 (32%)	51 (9%)	22 (4%)	11 (2%)	6 (1%)	46 (8%)	40 (7%)	6 (1%)	68 (11%)	17 (3%)	23 (4%)

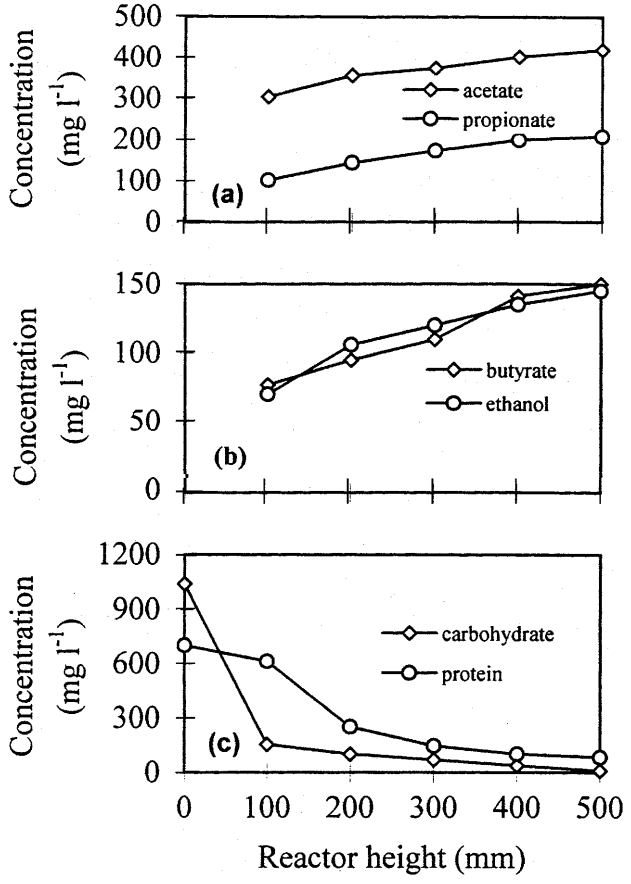

Fig. 2 Concentration of acidification products and carbohydrate/protein at various reactor heights at loading rates of 6 g (chemical oxygen demand influent) l^{-1} day^{-1}: **a** acetate and propionate, **b** butyrate and ethanol, and **c** carbohydrate and protein

influent COD. Biogas from an acidogenic reactor differed considerably in composition from that of a methanogenic reactor (Ghosh 1991). Table 2 shows that the biogas composition was markedly influenced by the OLR. At 4 g COD l^{-1} day^{-1}, the partial pressures of hydrogen, methane and carbon dioxide were 0.05, 0.34 and 0.53 atm, respectively. At 24 g COD l^{-1} day^{-1}, the corresponding partial pressures changed to 0.40, 0.0 and 0.56 atm. Operating at low loading rates, say 4 g COD l^{-1} day^{-1}, significantly encouraged the activity of the methanogenic bacteria. The decrease of methane in biogas was accompanied by the increase of hydrogen. This implies that most of the methane produced at low OLRs resulted from the utilization of hydrogen by the fast-growing methanogenic bacteria (Fang et al. 1995).

COD reduction and biomass yield

Since the main acidogenic products were VFA and alcohols, only a small amount of wastewater COD was removed by an acidogenic reactor. Table 3 shows that the COD reductions ranged from only from 6.5% at 24 g COD l^{-1} day^{-1} to 15.2% at 4 g COD l^{-1} day^{-1}. For comparison, in a thermophilic single-phase methanogenic UASB reactor treating a similar dairy wastewater, the production of methane accounted for the removal of 86% influent COD at 32 g COD l^{-1} day^{-1} (Fang and Chung 1999).

The net sludge yield in a reactor is usually estimated by monitoring the COD reduction as well as the VSS contents in both the reactor and the effluent. The amount of VSS accumulated inside the reactor, plus that washed out, divided by the total COD removed during the same period equals the net sludge yield. However, the accuracy of this method is strongly dependent on the reliability of the VSS data. In reality, it is difficult to obtain accurate data for VSS in the reactor. Another means for estimating the net sludge yield was proposed by Fang et al. (1994a) and has been applied to the single-phase anaerobic digestion of various wastewaters (Fang and Chung 1999; Fang et al. 1994b; Kwong and Fang 1996). In a strict anaerobic degradation process, there is no external supply of electron acceptors. Thus, the overall electrons in the anaerobic reactor available for oxidation, as measured by COD, remains unchanged throughout the process. The COD in substrates was transformed to the COD in biomass and in products, including VFA, alcohols, hydrogen and methane. Thus, the net sludge yield can be estimated from the total COD removed, the COD-equivalent of the biomass, and the COD-equivalent of hydrogen and methane produced, all of which can be accurately measured.

The COD fractions converted to hydrogen and methane at various OLRs are listed in Table 3. At 4 g COD l^{-1} day^{-1}, 57.9% of COD removed was converted to hydrogen and methane; and the remaining 42.1% was presumably converted to biomass. Assuming a chemical formula of $C_5H_7O_2N$, the biomass has a COD-equivalent of 1.42 mg COD mg^{-1} VSS. Hence, the biomass yield was estimated as 0.43 mg VSS mg^{-1} COD.

Table 2 Biogas production at various OLRs

OLR	Partial pressure (atm)			COD_{gas}	$COD_{gas}/COD_{influent}$
(g COD l^{-1} day^{-1})	H_2	CH_4	CO_2	(mg day^{-1})	(%)
4	0.05	0.34	0.54	986	8.8
6	0.19	0.14	0.60	890	5.3
8	0.20	0.07	0.70	1080	4.8
12	0.24	0.02	0.68	1510	4.5
16	0.34	0	0.61	1330	3.0
24	0.40	0	0.56	1720	2.5

Table 3 COD reductions and conversions to biomass

OLR (g COD l^{-1} day^{-1})	COD reduction (%)	COD reduced (mg day^{-1})	$COD_{biomass}/COD_{reduced}$ (%)	Biomass yield (mg VSS mg^{-1} COD)
4	15.2	1702	42.1	0.30
6	13.1	2200	59.5	0.42
8	9.9	2218	51.5	0.36
12	9.0	3024	50.0	0.35
16	7.1	3180	57.8	0.41
24	6.5	4368	61.5	0.43

Table 4 COD constituents in the effluent

OLR (g COD l^{-1} day^{-1})	Total (mg l^{-1})	VFA (mg l^{-1})	Alcohols (mg l^{-1})	Residual substrate (mg l^{-1})	Unidentified products (mg l^{-1})
4	3392	1589	413	1055	335
6	3476	1631	410	1115	320
8	3604	1570	438	1235	361
12	3640	1091	405	1536	608
16	3716	998	363	1712	643
24	3740	731	247	1863	899

Table 3 shows that the biomass yield in this acidification reactor was estimated as 0.30–0.43 mg VSS mg^{-1} COD.

Effluent COD balance

By mass balance, the effluent COD equals the sum of effluent COD in the forms of: (1) VFA, (2) alcohols, (3) residual carbohydrate, protein, lipid and (4) unidentified metabolites. The first three can be calculated by summing the COD values of individual chemical species. The remaining COD is thus attributed to the unidentified metabolites. Table 4 summarizes the COD contributions from each of the four groups in the effluent at various OLRs. At OLRs less than 12 g COD l^{-1} day^{-1}, VFA and alcohols contributed the majority of the effluent COD. However, the fraction of the unidentified acidification products in the effluent increased with OLR, from 9.9% at 4 g COD l^{-1} day^{-1} to 24.0% at 24 g COD l^{-1} day^{-1}.

Discussion

Results of this study show that the OLR had a significant impact on the acidification of dairy wastewater. It affected the degradation of carbohydrate, protein and lipid, the degree of acidification, the distribution of VFA/alcohols and the biogas composition.

The carbohydrate and protein degradation pattern inside the reactor suggests that the low degrees of protein degradation at higher OLRs could partly be due to the higher residual content of carohydrates in the mixed liquor. The high carbohydrate content in the wastewater reduced the amount of proteolytic enzymes synthesized, resulting in low levels of protein degradation. McInerney (1988) reported that carbohydrates could suppress the synthesis of exopeptidases, a group of enzymes facilitating protein hydrolysis. Breure et al. (1986) also reported that the degradation of gelatin was suppressed by the presence of carbohydrates.

There are three acidogenic fermentation pathways through butyrate, propionate (Cohen et al. 1984) and ethanol (Ren et al. 1995), respectively. The butyrate fermentation is characterized by the production of butyrate and acetate, plus carbon dioxide and hydrogen, whereas the propionate fermentation produces propionate, acetate and some valerate, with no significant gas production (Cohen et al. 1984). Ethanol fermentation, in contrast, occurs only at the low pH of 4.5, producing ethanol, acetate, hydrogen and carbon dioxide (Ren et al. 1995). In this study, the production of propionate was always higher than butyrate production, and hydrogen was always present in the biogas. This suggests that neither butyrate fermentation nor propionate fermentation was predominant in the reactor. However, although ethanol was present in significant quantities in all runs, it was never a primary end-product. Results of this study seem to suggest that the three types of fermentation co-existed in the acidification reactor, probably due to the complex nature of dairy wastewater, and the predominance of a fermentation pathway could be affected by the OLR. Cohen et al. (1984) and Ren et al. (1995) found a perdominant fermentation pathway in their studies, probably because, unlike in this study, their wastewaters contained only carbohydrates, a simple substrate.

The biomass yield in the acidification reactor was estimated as 0.38 ± 0.04 g VSS g^{-1} COD, which is consistent with the yields reported in the literature, such as 0.42 g VSS g^{-1} COD for acidifying starch (Speece and McCarty 1964), 0.54 g VSS g^{-1} COD for acidifying a mixture of glucose and peptone (Andrews and Pearson 1965) and 0.62 g VSS g^{-1} COD for acidifying glucose (Cohen et al. 1982). The yield of acidification sludge was considerably higher than the yield of 0.066 g VSS g^{-1} COD for methanogenic sludge treating the same dairy wastewater (Fang and Chung 1999). This is because acidification produces substantially more energy than methanogenesis.

Table 4 shows that a substantial amount of unidentified metabolites were present in the effluent, especially at high OLR. Glycerol, ketones, aldehydes and amino acids are likely to be among these unidentified metabolites. Increased hydrogen partial pressure in the reactor would suppress the further degradation of many amino acids (Orlygsson et al. 1993; Russell and Martin 1984). Since the partial pressure of hydrogen consistently exceeded 0.05 atm in this study, accumulation of amino acids in the effluent is to be expected.

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