Anaerobic acidification of a synthetic wastewater in batch reactors at 55°C

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Abstract Experiments were conducted to study the acidogenesis of a dairy wastewater in batch reactors at pH 5.5 and 55°C. There was a biased fermentation sequence for carbohydrate and protein, and the protein fermentation was delayed by carbohydrate. The production of hydrogen was exclusively from the fermentation of carbohydrate. Acetate and butyrate concentrations both increased rapidly at the beginning and peaked at some points, then declined in the reactors fed with 8 g-COD (chemical oxygen demand)/l, or higher concentrations. Butanol and propanol fractions increased with the substrate concentration. The metabolism shifted from the volatile fatty acid-producing pathways to the alcohol-producing pathways when the substrate concentration increased beyond 8 g-COD/l. The acidogenic biomass yield was in the range 0.19–0.25 mg-VSS/mg-COD.

Keywords Acidogenesis; alcohol; dairy wastewater; fermentation; thermophilic; volatile fatty acids

Introduction

Many industrial effluents, such as those from food processing, are often discharged at high temperatures. For example, the effluent from the instant coffee producing process leaves the factory at between 55–65°C. Treating these effluents under conventional mesophilic conditions requires pre-cooling, which is costly, and has the risk of losing biomass activity should the cooling system break down. Therefore, thermophilic operation, if possible, would be desirable. This would lower operating costs. Furthermore, at elevated temperatures, degradation efficiency is presumably more effective (Ghosh, 1991). For the anaerobic digestion of sludge, thermophilic operation offers the potential advantages of high loading rates and therefore requires smaller treatment plants than the mesophilic system. Thermophilic acidogenesis of sludge was proposed by Ghosh (1991) and has been found to be an effective means. Dichtl (1994), using a thermophilic acidogenic reactor followed by a mesophilic methanogenic reactor, demonstrated a significant improvement in the degradation of organic matter compared with a single-phase digester. Similar results were reported by Roberts *et al.* (1998). However, very little is available for the acidogenesis of wastewaters under thermophilic conditions. The present study was conducted to investigate the thermophilic acidogenesis of a synthetic dairy wastewater at 55°C.

Materials and methods

The test was conducted in twelve 137-ml glass reactors at 55°C by treating six feed solutions containing, respectively, 2, 4, 8, 12, 20 and 30 g-COD/l. The stock solution containing KH_2PO_4 only was first prepared, as the milk had enough nitrogen, minerals and vitamins for microorganisms. The solution was purged with nitrogen gas to remove any dissolved oxygen. Each reactor was seeded with the sludge from an upflow anaerobic acidogenic reactor operated for over 260 days. About 200 ml of sludge sampled from the upflow reactor was washed with stock solution, followed by centrifugation. After decanting the

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supernatant, the sludge was then re-suspended in 1,200 ml of stock solution. About 100 ml of the mixed solution containing 60 mg of VSS was transferred to each reactor using a syringe. Powdered milk was then added to each reactor to the pre-determined concentration, and the pH was adjusted by the addition of dilute hydrochloric acid to 5.5. All reactors were submerged in a 55°C shaking water bath. The vigorous shaking motion ensured complete mixing. At given time intervals, the volume of biogas produced was measured using a syringe, and the contents of the biogas and mixed liquor were analyzed.

The contents of H_2, CH_4, CO_2 and N_2 in the biogas were analyzed by a gas chromatograph (Hewlett Packard, Model 5890 Series II) equipped with a thermal conductivity detector. The concentrations of VFA and alcohols were determined by a second gas chromatograph of same model equipped with a flame ionization detector. 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–Folin 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, 1992). Measurements of COD and pH were performed according to *Standard Methods* (APHA, 1992).

Results and discussion

Figures 1 and 2 illustrate the variations of substrate and products in the mixed liquor, using the batches degrading 4 and 20 g-COD/l, respectively, for exemplifications, throughout the experiment, including: (a) carbohydrate and protein, (b) acetate, propionate and butyrate, (c) methanol, ethanol, propanol and butanol, and (d) P_{H_2}.

Batch at 4 g-COD/l

In this dairy wastewater the COD fractions were 30.9% for carbohydrate, 23.6% for protein and 41.9% for lipid (Fang and Yu, 2000). Figure 1(a) illustrates that, after an acclimation period of 12 h, carbohydrate degraded rapidly and became nearly depleted by day 2. The protein concentration remained almost unchanged during the initial 2 days of incubation, while during this period carbohydrate was nearly consumed. Protein degradation started only after day 2, suggesting the occurrence of biased fermentation and the suppression of protein degradation by carbohydrate. This phenomenon could be attributed to the

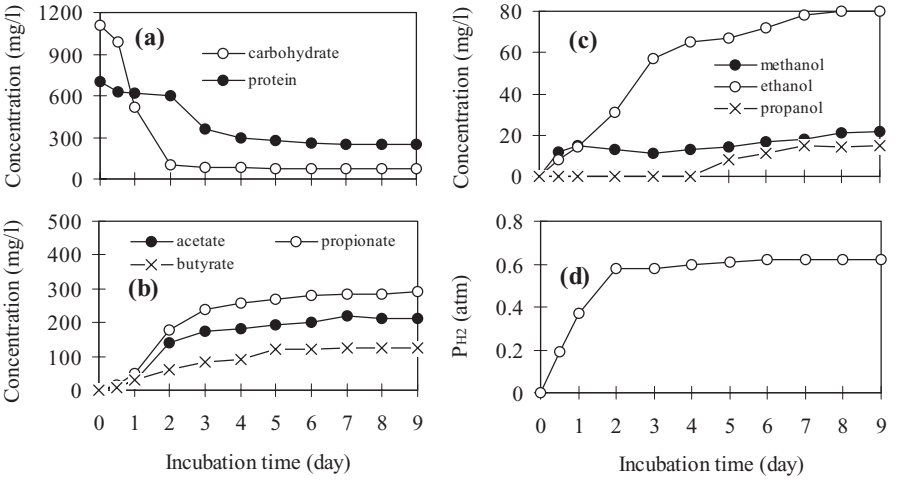

Figure 1 Changes of reactor conditions during the acidogenesis of wastewater at 4 g-COD/l

repression of carbohydrates on the synthesis of the enzymes involved in protein hydrolysis (Russell and Martin, 1984).

Acetate, propionate and butyrate production was in accord with the degradation of carbohydrate, as shown in Figure 1(b). The acetate, propionate and butyrate concentrations initially increased rapidly, reaching 140, 176 and 62 mg/l, respectively, by day 2. After the degradation of carbohydrate ceased and the degradation of protein started, these three VFA still kept increasing, and peaked at 218, 285, and 123 mg/l respectively on day 7. Afterwards, all of them leveled off. As illustrated in Figure 1(c), ethanol was the main alcohol produced, reaching 72 mg/l on day 6. Methanol was also produced but in much lower concentrations. Propanol could not be detected until day 5, but its peaking concentration was only 15 mg/l at the conclusion of the test. No butanol was produced during the 9-day incubation. Compared to VFA, alcohols were minor products.

The degradation of carbohydrate resulted in a rapid increase in P_{H_2} in the initial stages, as shown in Figure 1(d), from the initial 0 atm to 0.19 atm on day 0.5. It reached 0.58 atm when carbohydrate became depleted by day 2. The P_{H_2} was slightly increased to 0.62 atm on day 4, and thereafter was leveled off. The hydrogen production in correspondence with the depletion of carbohydrate [Figure 1(a)] indicates that hydrogen exclusively came from the carbohydrate degradation and that the protein degradation did not produce hydrogen.

Batch at 20 g-COD/l

Figure 2(a) shows that the 20 g-COD/l batch had a similar degradation pattern of carbohydrate and protein to the observed for the batch at 4 g/l. This result once more suggests the occurrence of biased fermentation and the suppression of protein degradation by carbohydrate when both of them were present in the substrate.

As illustrated in Figure 2(b), acetate, propionate and butyrate production immediately began when carbohydrate degradation started. The acetate and butyrate concentrations initially increased rapidly, reaching 420 and 305 mg/l, respectively, by day 2. Before the degradation of carbohydrate stopped, the acetate and butyrate concentrations kept increasing, and peaked at 584 and 394 mg/l on day 4. Afterwards, both of them gradually declined, reaching 451 and 183 mg/l, respectively, by day 7. However, propionate had a similar profile to that observed for the 4 g-COD/l batch.

The P_{H_2} increased rapidly in the initial stages, as is shown in Figure 2(d). It peaked at

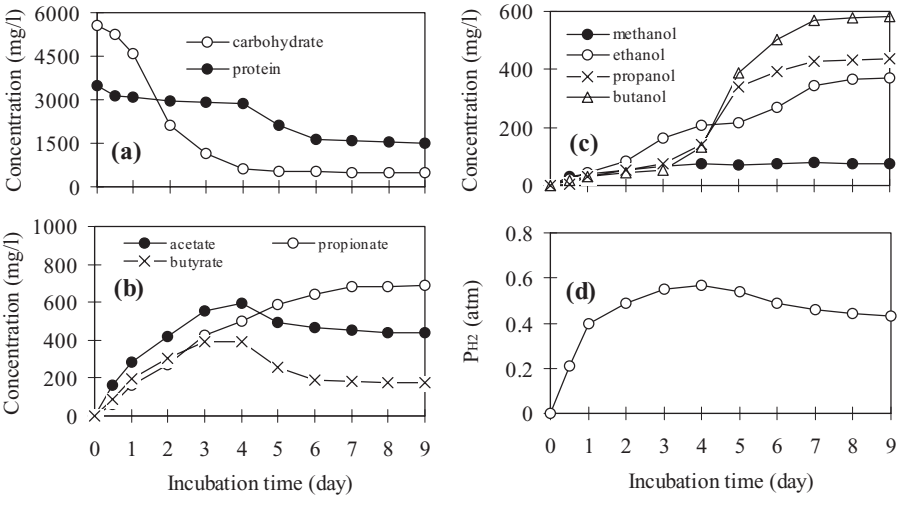

Figure 2 Changes of reactor conditions during the acidogenesis of wastewater at 20 g-COD/l

Table 1 Final pH, peak P_{H_2}, and maximum concentrations of main products

Influent COD (g/l)	Final pH	Peak P_{H_2} (atm)	Peak acetate (mg/l)	Peak propionate (mg/l)	Peak butyrate (mg/l)	Peak ethanol (mg/l)	Peak propanol (mg/l)	Peak butanol (mg/l)
2	6.05	0.65	203	154	95	62	0	0
4	6.17	0.61	218	290	126	80	15	0
8	6.29	0.62	338	437	231	214	145	58
12	6.33	0.58	579	650	328	278	217	204
20	6.21	0.62	584	686	394	372	435	561
30	6.24	0.60	594	721	421	634	623	658

0.57 atm, when carbohydrate became nearly depleted by day 4. After day 4, the P_{H_2} slightly dropped, even the degradation of protein started. This once more suggests that the fermentation of carbohydrate, rather than protein, produced hydrogen. The change of P_{H_2} was markedly different from that observed for the batch at 4 g-COD/l [Figure 1(d)]. The P_{H_2} had leveled off to 0.44 atm by day 7 in this batch.

Effect of substrate concentration

Batch experiments treating milk at 2, 4, 8, 20 and 30 g/l of COD produced some similar results. Hydrogen and carbon dioxide were the main gas products; acetate, propionate, and butyrate were the main VFAs; ethanol was always present; lactate, formate, i-butyrate, valerate, i-valerate, caproate, and methanol were found in lower quantities; butanol and propanol were produced in higher levels in the reactors with higher substrate concentrations. Table 1 summarizes some results of all six milk-fed reactors.

In general, the propionate and ethanol concentrations and their time to reach the plateau increased with the substrate concentration. On the other hand, the acetate and butyrate concentrations both increased rapidly at the beginning and peaked at sometime during the experiment, then declined in the reactors with equal to or more than 8 g-COD/l. Butanol and propanol were produced in very small quantities, or even could not be detected in the reactors with less than 8 g-COD/l, but increased with the substrate concentration. The increase of propanol and butanol, along with the decrease of acetate and butyrate, indicates that the metabolic pathways were significantly influenced by the substrate concentration. It is known that at low substrate concentrations VFA are the main products, while at high substrate concentrations propanol and butanol become the main products (Jones and Woods, 1986).

The P_{H_2} in all the batches increased initially, and kept almost unchanged after peaking in the reactors with less than 8 g-COD/l. However, in the reactors with 8, 12, 20 and 30 g-COD/l, the P_{H_2} dropped after peaking.

Table 2 summarizes the overall performance of the six reactors. At 2 to 30 g-COD/l, the carbohydrate conversion ranged from 96% to 90%, showing that carbohydrate could be acidified readily and that substrate concentration had a small effect on fermentation of carbohydrate. The protein conversion was substantially influenced by the substrate concentration. The low protein conversions at high substrate levels could partly be due to the high residual content of carbohydrate in the mixed liquor. Lipid conversion was the lowest among the three components, decreasing from 31% at 2 g-COD/l to only 12% at 30 g-COD/l.

The performance of an acidogenic reactor can be evaluated using a term "degree of acidification" (Fang and Yu, 2000). In this study the degree of acidification decreased from 49.6% at 2 g-COD/l to 30.4% at 30 g-COD/l. Table 2 also shows the COD fraction converted to gas at various substrate concentrations. For instance, at 8 g-COD/l, about 68.3%

Table 2 Overall performance of the six reactors

Influent COD (g/l)	Conversion (%)			Degree of acidification	$COD_{gas}/COD_{removed}$	Biomass yield (mg-VSS/mg-COD)
	carbohydrate	protein	lipid	(%)	(%)	
2	96	88	31	49.6	72.5	0.19
4	95	84	29	47.5	69.0	0.22
8	93	81	27	43.2	68.3	0.23
12	92	78	25	40.8	66.2	0.24
20	92	72	18	37.3	66.9	0.23
30	90	63	12	30.4	64.1	0.25

of COD removed was converted to hydrogen and methane; the remaining 31.7% was presumably converted to biomass. Assuming a chemical formula of $C_5H_7O_2N$, biomass has a COD-equivalent of 1.42 mg-COD/mg-VSS. Hence, the biomass yield at 8 g-COD/l was estimated as 0.23 mg-VSS/mg-COD.

Conclusions

The experiment demonstrated that the substrate concentration had a substantial influence on the acidogenesis of the dairy wastewater. There was a biased fermentation sequence for carbohydrate and protein, and the protein fermentation was delayed by carbohydrate. The production of hydrogen was exclusively from the fermentation of carbohydrate, rather than protein. Acetate and butyrate concentrations both increased rapidly at the beginning and peaked at some time, then declined in the reactors with 8 g-COD/l or higher concentrations. Butanol and propanol fractions increased with the substrate level. These results suggest that the metabolism shifted from the VFA-producing pathways to the alcohol-producing pathways when the substrate concentration increased beyond 8 g-COD/l. The acidogenic biomass yield was in the range 0.19–0.25 mg-VSS/mg-COD.

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