Acidogenesis of dairy wastewater at various pH levels

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Abstract Continuous experiments were conducted to study the influence of pH in the range 4.0–6.5 on the acidification of dairy wastewater at 37°C with 12 hours of hydraulic retention in an upflow reactor. Results showed that degradation of dairy pollutants increased with pH from pH 4.0 to 5.5. At pH 5.5, 95% of carbohydrate, 82% of protein and 41% of lipid were degraded. Based on chemical oxygen demand (COD), 48.4% of dairy pollutants were converted into volatile fatty acids and alcohols in the mixed liquor, 6.1% into hydrogen and methane in biogas, and the remaining 4.9% into biomass. The biomass yield at pH 5.5 was estimated as 0.32 mg-VSS/mg-COD. Further increase of pH, up to 6.5, increased degradation of carbohydrate, protein and lipid only slightly, but resulted in the lowering of overall acid and alcohol production due to their increased conversion into methane. Acetate, propionate, butyrate and ethanol are the main products of acidogenesis. Productions of propionate and ethanol were favored at pH 4.0–4.5, whereas productions of acetate and butyrate were favored at pH 6.0–6.5.

Keywords Acidogenesis; alcohol; dairy wastewater; pH; volatile fatty acids

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Introduction

The concept of two-stage anaerobic digestion was introduced three decades ago (Pohland and Ghosh, 1971). In a two-stage system, acidogenic and methanogenic phases can be operated separately under respective optimum conditions. Hydrolysis is usually the limiting step in the degradation of complex organic pollutants and particulates (Eastman and Ferguson, 1981). The process relies on enzymes excreted by the fermentative acidogens, and is strongly pH dependent (Gottschalk, 1986). Using a two-stage anaerobic process, hydrolysis and acidification are conducted in the first reactor at pH, temperature and hydraulic retention time (HRT) favored by the fermentative, acidogenic bacteria (Harper and Pohland, 1986); the acidic effluent is then treated in the downstream methanogenic reactor.

Most of studies of the pH effect on acidogenesis were conducted for the degradation of simple substrates, such as glucose, sucrose, and lactose. Zoetemeyer *et al.* (1982) found that acidifying glucose at pH 5.7–6.0 produced stable intermediates favored by the bacteria in the methanogenic reactor down stream. Similarly, the optimum pH for the acidification of sucrose and lactose were reported as pH 6.5 (Joubert and Britz, 1986) and pH 6.0–6.5 (Kisaalita *et al.*, 1987), respectively. The optimal pH for sulfate removal in the acidogenic treatment of distillery molasses slops was found in the range of 5.8–6.2 (Reis *et al.*, 1988); nearly all the sulfate were reduced with a maximum production of acetate. However, wastewater from many food and agricultural industries contain high levels of not just carbohydrates, but also proteins and lipids. Hydrolysis and fermentation of complex colloidal particulates, such as proteins and lipids, may prefer pH levels different from those for the acidogenesis of simple carbohydrates (Henry *et al.*, 1987), and yet little information is available on this matter. This study was conducted to investigate the effect of pH on the acidogenesis of complex and colloidal substrates in dairy wastewater, which serves as the model for wastewater from food and agricultural industries.

Materials and methods

The experiment was conducted in a 2.8-litre upflow reactor, which had an internal diameter of 84 mm and a height of 500 mm (Fang *et al.*, 1994). The reactor was water-jacketed and operated at a constant temperature of 37°C. The reactor was fed with a simulated dairy wastewater, prepared from full-cream powdered milk supplied by Nestle Corp. Throughout the experiment the influent COD (chemical oxygen demand) was kept at 4,000 mg/L, equivalent to 2,860 mg/L of milk. Concentrations of carbohydrate, protein and lipid were 1,107, 701 and 745 mg/L, respectively, correspondingly to 30.9%, 23.6% and 41.9% of the total COD. The HRT was kept at 12 h, corresponding to a loading rate of 8 g-COD/L·d. Since the milk contained sufficient amounts of nitrogen, minerals and vitamins, only 20 mg-P/L was added as nutrient supplement.

The reactor was seeded with sludge taken from a conventional single-stage upflow sludge blanket reactor treating the same synthetic wastewater for another study (Fang and Chung, 1999). The initial seed sludge contained 26.2 g volatile suspended solids (VSS), resulting in an initial VSS concentration of 9.5 g/L. Six runs at various pH were conducted. The pH of the mixed-liquor was first operated at pH 6.5, and was lowered stepwise by 0.5 increments until reaching pH 4.0. The sludge retention was controlled at 15 days, by wasting 1/15 of the sludge from the reactor daily. At each pH level, the reactor reached steady state within 20–28 days, judging from the volatile fatty acids (VFA), alcohol and biogas productions. The reactor was operated at each pH level for 36–45 days before lowering to the next level. The amount of biogas produced was recorded daily using the water replacement method. During each run, pH, gas production and composition were recorded daily, and all other parameters were measured three times weekly.

The contents in biogas and effluent were analyzed following previously established procedures (Fang and Chung, 1999). The detectable levels were 1 mg/L for individual VFA and 3 mg/L for individual alcohols. 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). This method for lipid measurement also accounted for long-chain fatty acids. Measurements of COD, pH, NH_3-N, and VSS were performed according to the *Standard Methods* (APHA, 1992).

Results and discussion

Substrate degradation

Figure 1 illustrates the degradations of the three main constituents in dairy wastewater, i.e. carbohydrate, protein and lipid, increased with pH. At pH 4.0, 69%, 34% and 16% of carbohydrate, protein and lipid were degraded. Degradation of carbohydrate increased to 95% at pH 5.5, reaching 98% at pH 6.5. Degradation efficiency of carbohydrate was pH sensitive at pH less than 5.5; this is consistent with the previous finding that fermentation of lactose, the key constituent of carbohydrate in dairy wastewater, was mainly regulated by pH, and was independent of HRT and lactose concentration (Kisaalita *et al.*, 1987). Degradation of protein reached a maximum at pH 6.5, similar to those reported by others. The low conversion of protein under acidic conditions might be attributed to the poor solubility of protein and the decrease of enzymatic activity. Compared with carbohydrate and protein, lipid had much lower degrees of degradation at any given pH, but the variation with pH had a trend similar to those of carbohydrate and protein. Figure 1 illustrates that only 16–50% of lipid was degraded at pH 4.0–6.5, which is consistent to the reported 17–23% for the acidification of olive oil wastewater (Beccari *et al.*, 1998), 20–25% for palm oil wastewater (Tsonis and Grigoropoulos, 1988), and <10% for the lipid in primary sludge (Eastman and

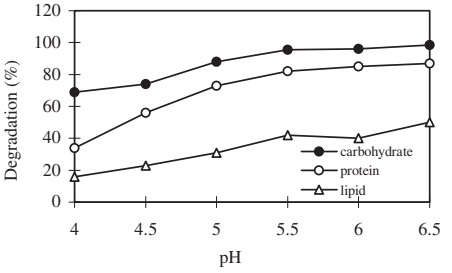

Figure 1 Degradation of carbohydrate, protein, and lipid

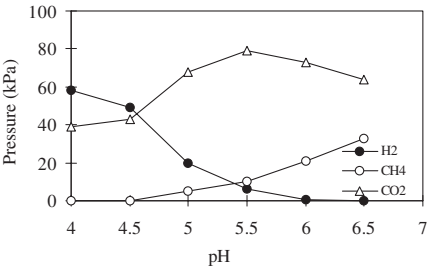

Figure 2 Partial pressures of H_2, CH_4 and CO_2

Ferguson, 1981). In degradation, lipid is first hydrolyzed to long-chain fatty acids, which are further degraded via β-oxidation producing hydrogen as a byproduct. That means lipid degradation is suppressed by the increase of hydrogen pressure.

Biogas production

Figure 2 illustrates that the gas composition was strongly influenced by pH. At pH 4.0, the gas was composed of 38% carbon dioxide, 58% hydrogen, 4% nitrogen and free of methane. As pH increased, partial pressure of hydrogen decreased accompanied by the increase of methane production. At pH 6.5, the biogas contained 31% methane and became free of hydrogen. This indicates that most of the hydrogen produced was consumed by the hydrogenotrophic methanogens at this pH. In order to effectively separate the acidogenic phase from the methanogenic phase, it is important to keep the pH low, say at 5.5 or less.

Overall bioactivity

Since acidogenesis produces not only acids and alcohols in the effluent but also hydrogen and methane in the biogas, the overall bioactivity should take products in both effluent and biogas into account. Figure 3 illustrates the overall bioactivity, as expressed by the total COD equivalents of VFA, alcohols, hydrogen and methane, at various pH. It shows that overall bioactivity increased from 146 mg-COD/g-VSS·d at pH 4.0 to the maximum rate of 320 mg-COD/g-VSS·d at pH 5.5. Further increase of pH slightly decreased the bioactivities to 308 mg-COD/g-VSS·d at pH 6.0, and 299 mg-COD/g-VSS·d at pH 6.5. The bacterial activities may be controlled by the overall enzymatic activity. Since enzymes are made of amino acids, their activities are thus pH dependent, as shown in the following:

$$E^+ \leftrightarrow E + H^+ \tag{1}$$

$$E \leftrightarrow E^- + H^+ \tag{2}$$

where E represents the active enzyme, and E^+ and E^- are the less active forms of charge-carrying enzyme (Bailey and Ollis, 1986). Assuming K_1 and K_2 are the respective equilibrium constants of reactions (1) and (2), the enzymatic activity can be expressed as:

$$R = \frac{R_{max}}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} \tag{3}$$

where R is the specific enzymatic reaction rate, and R_{max} is the maximum value of R assuming all enzymes are present in the most active charge-neutral form of E. Figure 3 illustrates

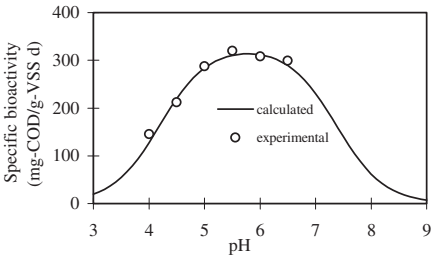

Figure 3 Specific enzymatic activity at various pH

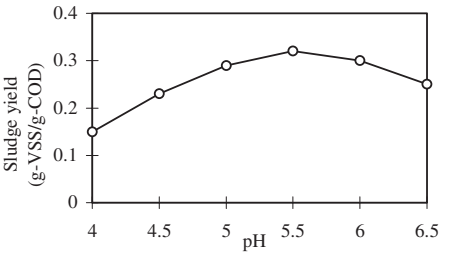

Figure 4 Biomass yield at various pH levels

that the measured specific overall bioactivities at various pH matched the best-fit curve of Eq. (3). The best-fit curve was plotted based on the following parameters obtained from regression analysis: R_{max} of 312 mg-COD/mg-VSS·d, K_1 of 2.36×10^{-5} M and K_2 of 9.33×10^{-8} M. Based on Eq. (3), the maximum enzymatic activity occurred at $(pK_1 + pK_2)/2$, i.e. pH 5.8. These calculated results match satisfactorily with the observed results, i.e. maximum bioactivity of 320 mg-COD/mg-VSS·d at pH 5.5.

VFA and alcohol distribution

VFA and alcohols are the main products of the acidogenesis of organic matters. Table 1 summarizes the percentages of individual VFA and alcohols at various pH. It shows that acetate, propionate and butyrate were the three main products. At pH 4.0, the effluent products were composed of 18% acetate, 38% propionate, 6% butyrate. These three products represented 64% of the total VFA and alcohols at pH 5.0, and 79% at pH 6.5. Other VFA and alcohols included i-butyrate, valerate, i-valerate, caproate, ethanol and methanol, plus trace amounts of propanol at pH 5.5 or lower. Formate and butanol were not detected in all the effluent. Acetate, propionate, butyrate and i-butyrate could be formed directly from the fermentation of carbohydrates, proteins and lipids. The higher molecular-weight VFA, including valerate, i-valerate and caproate are largely associated with the fermentation of proteins (Gottschalk, 1986; McInerney, 1988); acidogenesis of non-proteinaceous substrates produced little of these three VFA (Zoetmeyer *et al*., 1982). These acids could be formed via reductive deamination of single amino acids or by an oxidation–reduction between pairs of amino acids via Stickland reaction (McInerney, 1988). Their presence in this study may be attributed to the acidogenesis of protein, mostly casein, in the dairy wastewater. Ethanol was another key acidogenic product, representing 10–12% of the effluent products at pH 4.0–5.5, and 4–5% at pH 6.0–6.5. This concurred with a previous finding that pH 5.0 or less favored the production of ethanol (Ren *et al*., 1995).

Table 1 also shows that propionate production increased with the decrease of pH, as reported by many others, from 12% at pH 6.5 to 38% at pH 4.0. In the acidogenesis of primary sludge, propionate increased steadily with the decrease of pH from 7.0 to 5.0 (Eastman and Ferguson, 1981). On the other hand, in the acidogenesis of glucose over the pH range of 4.5 to 8.0, propionate production increased at lower pH (Zoetemeyer *et al*., 1982). Another study (Hsu and Yang, 1991) showed that the propionate production increased substantially with decreasing pH from 6.0 to 4.5, despite the optimum pH for the growth of propionate-producing bacteria being above pH 6.0. In contrast, the fractions of acetate and butyrate in effluent products both decreased with pH, from 34% both at pH 6.5, to 18% and 6%, respectively, at pH 4.0. These results clearly show that pH has a significant effect of the distribution of effluent products. Acetate and butyrate were the dominant products at pH > 5.5, whereas propionate was dominant at pH<5.5. Since methanogenesis of propionate is slower than that of acetate and butyrate (Harper and Pohland, 1986), it is

thus preferably to control the acidogenic effluent at pH 5.5 according to the results in Table 1.

Biomass yield

The net sludge yield in a reactor can be estimated from the COD balance (Fang *et al*., 1994). Under strict anaerobic conditions, there is no external electron acceptor available and thus the COD in the reactor remains unchanged throughout the process. Substrate COD are transformed into VFA, alcohols, hydrogen, methane and biomass. Thus, the overall COD reduction in wastewater should equal the sum of COD equivalents of hydrogen, methane and biomass produced. The total COD reduction, as well as hydrogen and methane production, can be accurately measured. Based on these measurements, the sludge yield can be estimated.

Table 2 shows the COD fraction converted to gas at various pH values. For instance, at pH 5.5, about 55% of COD removed was converted to hydrogen and methane; the remaining 45% was presumably converted to biomass. Assuming a chemical formula of $C_5H_7O_2N$, biomass has a COD-equivalent of 1.42 mg/mg-VSS. Hence, the biomass yield at pH 5.5 was estimated as 0.32 mg-VSS/mg-COD-removed. Figure 4 illustrates that biomass yield was pH dependent, and the maximum yield occurred at pH 5.5. For comparison, the acidogenic sludge yields were reported as 0.26 g-VSS/g-COD for degrading glucose (Zoetemeyer *et al*., 1982). Fang and Chung (1999), using the same reactor as a single-stage system to treat the same dairy wastewater, found that 90.6% of COD removed was converted to methane; the biomass yield for the methanogenic reactor was only 0.066 g-VSS/g-COD.

Conclusions

Results of this study showed that 69–99% of carbohydrate in dairy wastewater, 34–87% of protein and 16–50% of lipid were acidified at pH ranging 4.0–6.5, 37°C and 12 hours of

Table 1 Percentage of VFA and alcohols in effluent

pH	HAc (%)	HPr (%)	HBu (%)	i-HBu (%)	HVa (%)	i-HVa (%)	HCa (%)	Mol (%)	Eol (%)	Pol (%)
4.0	18	38	10	6	6	5	4	3	12	2
4.5	20	34	10	8	6	6	2	3	11	1
5.0	26	28	14	9	4	4	5	4	10	0
5.5	28	18	13	8	5	4	5	5	10	1
6.0	33	13	14	7	6	4	6	3	5	0
6.5	34	12	14	6	3	2	3	2	4	0

Note: HAc = acetate, HPr = propionate, HBu = butyrate, i-HBu = i-butyrate, HVa = valerate, i-HVa = i-valerate, HCa = caproate, HLa = lactate, Mol = methanol, Eol = ethanol, Pol = propanol

Table 2 Performance of the reactor

pH	MLVSS (g/L)	Carbohydrate (mg/L)	Effluent Protein (mg/L)	Lipid (mg/L)	Biogas production (L/L·d)	$COD_{gas}/COD_{removal}$ (%)
4.0	11.80	343	463	626	0.62	78
4.5	12.39	288	308	574	1.00	67
5.0	13.92	133	189	514	1.43	59
5.5	13.60	48	126	563	1.46	55
6.0	15.38	44	105	462	1.18	57
6.5	16.04	17	91	373	1.17	64

hydraulic retention. Degradation of dairy pollutants increased with pH from pH 4.0 to 5.5. Further increase of pH increased degradation of carbohydrate, protein and lipid only slightly, but resulted in the lowering of overall acid and alcohol production due to their increased conversion into methane. Based on COD, 48.4% of dairy pollutants were converted into volatile fatty acids and alcohols, 6.1% into hydrogen and methane, and the remaining 4.9% into biomass. The biomass yield at pH 5.5 was estimated as 0.32 mg-VSS/mg-COD.

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