ACIDIFICATION OF LACTOSE IN WASTEWATER

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ABSTRACT: Acidification of lactose in wastewater was conducted in four series of experiments in an upflow reactor to investigate individual effects of hydraulic retention time (HRT) (2–24 h), lactose concentration in wastewater (2–30 g COD/L), pH (4.0–6.5), and temperature (20°–60°C). Optimum acidification was found at pH 5.5 and 55°C. Acidification increased with HRT, but with the decrease of lactose concentration in wastewater. Degradation of lactose followed the Michaelis-Menten model with a maximum specific degradation rate of 4.39 g/g VSS·day and a half-rate concentration of 1.97 g/L. Production of volatile fatty acids, in general, favored lower lactose concentrations and higher pH, but was not sensitive to HRT and temperature. Distribution of individual volatile fatty acids/alcohols was dependent on lactose concentration, pH, and temperature, but less sensitive to HRT. Under most conditions acetate, propionate, and ethanol were the predominant products. Biogas produced under all test conditions was composed of mostly hydrogen and carbon dioxide, but no detectable methane. Sludge yield was estimated as 0.230 ± 0.021 g VSS/g COD.

INTRODUCTION

Production of 1 kg of cheese from milk, in general, produces 5.5–10 kg of whey (Chartrain and Zeikus 1986; Siso 1996), which is a wastewater comprising mostly of lactose (approximately 5%), plus residual proteins, lactate, and salts (Ghaly 1996; Kalyuzhnyi et al. 1997). The valuable protein residues are often recovered by ultrafiltration, and the final high-strength effluent is treated anaerobically (Siso 1996). Extensive studies have been conducted to evaluate the performance of anaerobic reactors, including a continuously stirred tank reactor (CSTR) (Chartrain and Zeikus 1986; Kissalita et al. 1987), an upflow anaerobic sludge blanket (UASB) (Kalyuzhnyi et al. 1997), and an anaerobic filter (Ghaly 1996), for the treatment of this type of lactose-rich wastewater.

During anaerobic degradation, lactose is first converted by acidogens to volatile fatty acids (VFA), which are further converted by acetogens to acetate and H_2/CO_2. Finally methanogens convert acetate and H_2/CO_2, respectively, into methane; however, methanogens are pH sensitive. In conventional single-stage reactors, overproduction of VFA, often due to loading shocks and/or other sudden changes of process conditions, could result in the lowering of pH. As a consequence, reactors would turn ''sour'' and cease to produce methane (Chartrain and Zeikus 1986; Kissalita et al. 1987). This operational problem has led to the development of the two-stage anaerobic process (Pohland and Ghosh 1971), in which acidification and methane production are conducted in two reactors in sequence. The two-stage process offers a number of advantages. The process is easier to control and less sensitive to shocks (Cohen et al. 1979). The overall efficiency could be enhanced by operating the reactors at optimal conditions respectively for acidification and methane production. Furthermore, pollutants that are toxic to methanogens can be degraded in the acidification reactor at the front end (Dinopoulou et al. 1988).

Studies have been conducted on acidification of wastewaters containing glucose (Cohen et al. 1979; Zoetemeyer et al. 1982a,b), sucrose (Zoetemeyer et al. 1982c), starch (Lee et al. 1999), lactose (Kissalita et al. 1987), and gelatin (Breure and van Andel 1984). Results indicated that optimal conditions of acidification are dependent on the substrate, and the effluent composition is likely to be influenced by operational parameters, such as hydraulic retention time (HRT), substrate concentration, temperature, and pH. However, the effects of individual operational parameters are not fully known. Elevated temperature was reported to enhance degradation and biomass production, probably due to reduced product inhibition (Zoetemeyer et al. 1982b; Pavlostathis and Giraldo-Gomez 1991). Increase of organic loading rate seemed to favor production of propionate over acetate, resulting from the increased accumulation of hydrogen (Eastman and Ferguson 1981; Henry et al. 1987; Dinopoulou et al. 1988). However, effects of some other operational parameters on effluent composition were more controversial. Many claimed that product distribution is sensitive to the HRT (Eastman and Ferguson 1981; Elefsiniotis and Oldham 1994), but some found otherwise (Zoetemeyer et al. 1982a; Breure and van Andel 1984). Similarly, many researchers claimed that the effluent composition was pH dependent (Henry et al. 1987; Yang et al. 1994), while others found that it was insensitive in the range of pH 5–7 (Zoetemeyer et al. 1982a).

This study was thus conducted to investigate the individual effects of HRT, substrate concentration, pH, and temperature on the acidification of wastewater containing lactose as the sole carbon source, and to analyze the kinetics of lactose acidification.

MATERIALS AND METHODS

Reactor, Wastewater, and Seed Sludge

A 2.8-L upflow reactor 84 mm in diameter was used for this study. Details of its configuration have been previously described (Fang and Chui 1993). The reactor was water jacketed and operated at temperatures as required. The pH was adjusted by using 2 N NaOH and 2N HCl solutions. Wastewater was prepared by using lactose as the sole carbon source and dosing with balanced nutrient, trace metals, and buffering chemicals (Fang and Chui 1993). Acidogenic sludge was enriched from granular methanogenic sludge treating dairy wastewater from a previous study (Fang and Chung 1999) in a 3-L CSTR. To wash out the methanogens, the CSTR was operated by feeding lactose at a concentration equivalent to 2 g/L of chemical oxygen demand (COD) at pH 5.5 and 24 h of HRT over 42 days. Near the end, VFA production became steady and no methane was detected in the biogas. The 2.8-L upflow reactor was seeded with this enriched acidogenic sludge equivalent to 34.5 g of volatile suspended solids (VSS).

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Note. Associate Editor: Y. T. Wang. Discussion open until February 1, 2002. To extend the closing date one month, a written request must be filed with the ASCE Manager of Journals. The manuscript for this paper was submitted for review and possible publication on December 19, 2000; revised April 24, 2001. This paper is part of the ***Journal of Environmental Engineering***, Vol. 127, No. 9, September, 2001. ©ASCE, ISSN 0733-9372/01/0009-0825–0831/$8.00 + $.50 per page. Paper No. 22522.

Experimental Conditions

Four series of experiments were conducted to investigate the individual effect of four operational parameters. In Series I, the HRT was decreased stepwise from 24 to 2 h while keeping the substrate concentration, pH, and temperature constant at 4 g COD/L, pH 5.5, and 37°C, respectively. In Series II, the lactose concentration in the wastewater was increased stepwise from 2 to 30 g COD/L, keeping HRT, pH, and temperature at 12 h, 5.5, and 37°C, respectively. In Series III, the pH of the mixed liquor was lowered stepwise from 6.5 to 4.0 while keeping the lactose concentration, HRT, and temperature at 4 g COD/L, 12 h, and 37°C, respectively. Last, in Series IV, the temperature was increased stepwise from 20°C to 60°C while keeping substrate concentration, HRT, and pH at 4 g COD/L, 12 h, and 5.5, respectively. Each series consisted of 5–7 runs. Each run lasted 36–41 days to ensure reaching steady state before changing to the next condition. Effluent and biogas compositions were continuously monitored. Only those obtained under steady-state conditions are reported.

Analyses

The amount of biogas produced in the reactor was recorded daily using the water replacement method. The contents in the biogas were analyzed by a gas chromatograph (Model 5890 Series II, Hewlett-Packard, Palo Alto, Calif.) equipped with a thermal conductivity detector and a 2 × 2 mm (inside diameter) stainless steel column packed with Porapak N (80–100 mesh). Injector and detector temperatures were respectively kept at 130°C and 200°C, while column temperature was increased from 90°C to 110°C.

The effluent concentrations of VFA, including acetate, propionate, butyrate, *i*-butyrate, valerate, *i*-valerate, caproate, lactate, and alcohols, including methanol, ethanol, propanol, and butanol were analyzed by a second gas chromatograph of the same model equipped with a flame ionization detector and a 10 × 0.53 mm HP-FFAP fused-silica capillary column. Effluent samples were filtered through a 0.2 μm filter, acidified by formic acid, and measured for free acids and alcohols. The initial temperature of the column was 70°C for 4 min and then 140°C for 3 min, and finally 170°C for 4 min. The temperatures of injector and detector were both 200°C. Helium was used as the carrier gas at a flow rate of 25 mL/min. Formate, which could not be detected by gas chromatography, was measured by the colorimetric method (Lang and Lang 1972). Lactose was measured using the colorimetric ferric-cyanide method (Dubois et al. 1956). Measurements of COD, pH, and VSS were performed according to standard methods [American Public Health Association (APHA) et al. 1992].

RESULTS AND DISCUSSION

The acidogenesis of lactose produced four groups of products: (1) organic acids including C_2–C_7 VFA; (2) C_1–C_4 alcohols; (3) biogas containing H_2 and CO_2; and (4) biomass. Fig. 1 illustrates the influence of HRT on (a) lactose degradation and degree of acidification; (b) relative concentrations of key VFA; (c) relative concentrations of lactate and key alcohols; and (d) partial pressures of H_2 and CO_2. Figs. 2, 5, and 6 are the same plots illustrating, respectively, influences of lactose concentration, pH, and temperature on the performance of the acidogenic reactor.

Effect of HRT

Fig. 1(a) illustrates that lactose was readily degraded under acidogenic conditions. At pH 5.5, 37°C, and 4 g/L of influent COD, lactose degradation increased from 83% at 2 h of HRT to 93% at 12 h, and reached 97% at 24 h. Comparable results

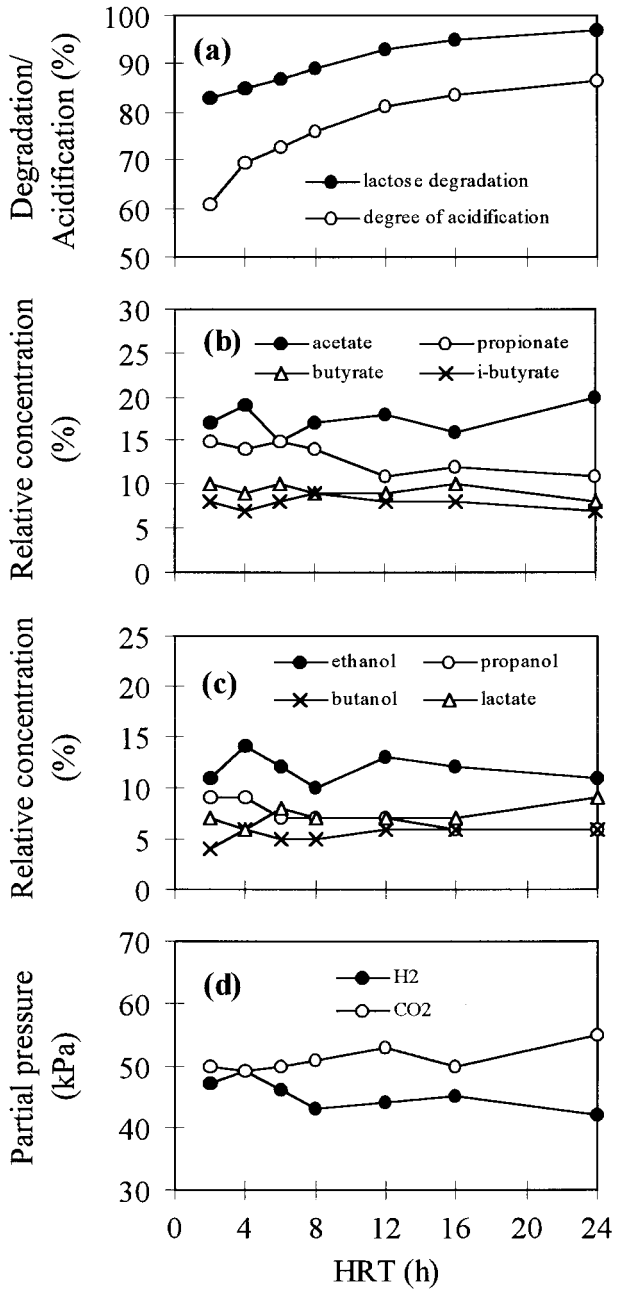

FIG. 1. Influence of HRT on: (a) Lactose Degradation and Degree of Acidification; (b) Relative Concentrations of Acetate, Propionate, Butyrate, and *i*-Butyrate; (c) Relative Concentrations of Ethanol, Propanol, Butanol, and Lactate; (d) Partial Pressures of H_2 and CO_2

were found in a previous study on the acidification of dairy wastewater, which showed that lactose degradation increased from 93% at 4 h to 98% at 24 h (Fang and Yu 2000). These results were consistent with those of a CSTR study (Kissalita et al. 1987), which showed that acidification of lactose increased only slightly for HRT above 4 h. The degree of acidification can be quantified by the ratio of the COD equivalent of acidogenic products, including VFA, alcohols, and hydrogen, to the COD of wastewater (Dinopoulou et al. 1988). Fig. 1(a) illustrates that the degree of acidification increased linearly with HRT, from 61% to 2 h to 86% at 24 h.

Acidification of lactose at pH 5.5, 37°C, and 4 g/L of influent COD produced 69–76% VFA and 24–31% alcohols. Figs. 1(b and c) illustrate that the effluent composition, in general, was not sensitive to HRT. Acetate accounted for 15–20% of the total VFA/alcohols in the effluent, whereas propionate and ethanol ranged from 10 to 15% and from 10 to 14%, respec-

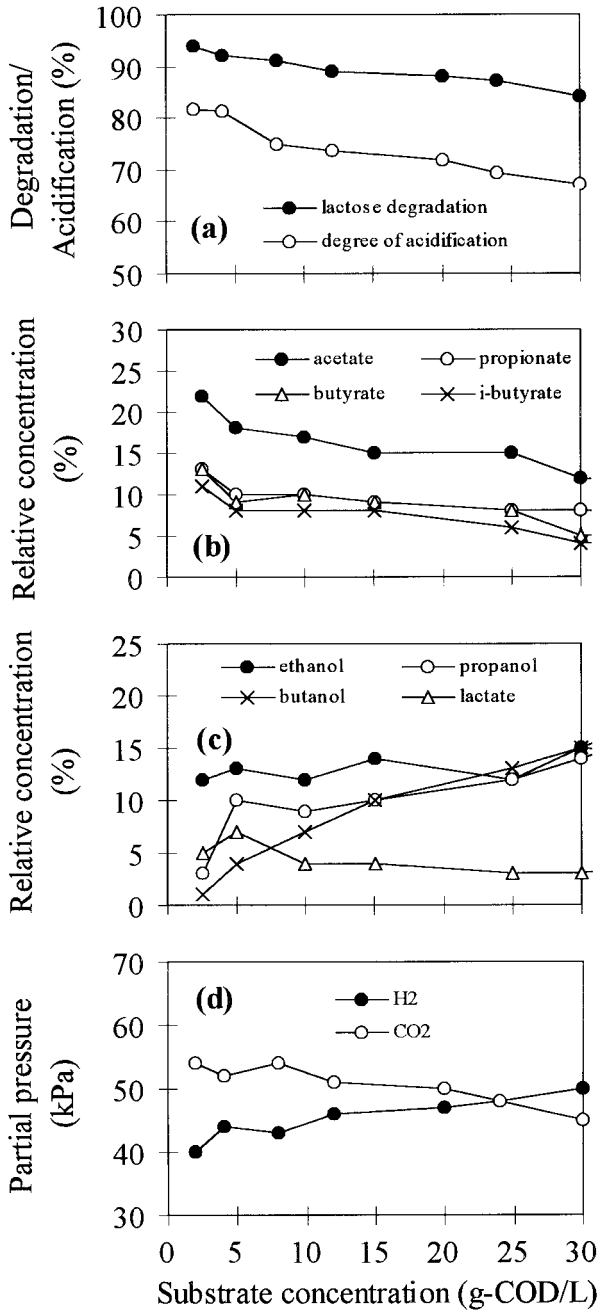

FIG. 2. Influence of Lactose Concentration in Wastewater on: (a) Lactose Degradation and Degree of Acidification; (b) Relative Concentrations of Acetate, Propionate, Butyrate, and *i*-Butyrate; (c) Relative Concentrations of Ethanol, Propanol, Butanol, and Lactate; (d) Partial Pressures of H_2 and CO_2

tively. On the average, the effluent was composed of 18% acetate, 13% propionate, and 12% ethanol. Butyrate, *i*-butyrate, lactate, propanol, and butanol ranged 6–10% each. The remaining VFA, including valerate, *i*-valerate, and caproate, ranged only 3–7% each, and methanol 2 to 3%. Formate was not detected in all runs.

Results of previous studies showed that product composition in acidification of biological sludge (Henry et al. 1987), primary sludge (Elefsiniotis and Oldham 1994), and dairy wastewater (Yu and Fang 2000) was strongly affected by HRT. However, results of this study indicate that lactose was readily degraded at HRT as low as 2 h, and the product composition was not significantly influenced by HRT, similar to observations in the acidification of other readily biodegradable substrates such as glucose (Cohen et al. 1979), sucrose (Zoetemeyer et al. 1982c), and gelatin (Breure and van Andel 1984). It seems that HRT has more influence on the product composition in acidification of substrates that are more recalcitrant to biodegradation.

Acidification of lactose at pH 5.5 produced a biogas containing mostly hydrogen and carbon dioxide, plus a small fraction of nitrogen but without detectable methane, for HRT ranging 2–24 h. Fig. 1(d) illustrates that partial pressures of hydrogen and carbon dioxide both fluctuated with the narrow range of 42–49 kPa. The biogas production rate decreased with the increase of HRT, from 43.2 L/day at 2 h to 7.3 L/day at 24 h.

Effect of Lactose Concentration

Fig. 2 illustrates the effect of lactose concentration in wastewater or acidification for Series II experiments conducted at pH 5.5, 37°C, and 12 h of HRT. Fig. 2(a) illustrates that degradation of lactose decreased slightly with the increase of wastewater COD, from 94% at 2 g COD/L to 84% at 30 g COD/L. Likewise, the degree of acidification was lowered from 82% at 2 g COD/L to 67% at 30 g COD/L. The degree of acidification increased almost linearly with the increase of lactose concentration, similar to those observed in the acidification of beef extract-based wastewater (Dinopoulou et al. 1988), pharmaceutical wastewater (Penaud et al. 1997), and solid food waste (Argelier et al. 1998). Comparison between Figs. 1(a) and 2(a) seem to indicate that the degree of acidification was more sensitive to HRT than to the lactose concentration in wastewater.

Acidification at pH 5.5, 37°C, and 12 h of HRT, and lactose equivalent to 2–30 g COD/L produced 46–84% VFA and 16–54% alcohols. Lower lactose concentrations favored the production of VFA. Treating wastewater containing 2 g COD/L of lactose, 84% of products in the effluent were VFA and only 16% alcohols. However, treating 30 g COD/L of lactose produced effluent containing 46% VFA and 54% alcohols. The VFA were mostly composed of acetate and propionate, plus smaller quantities of butyrate, *i*-butyrate, valerate, *i*-valerate, caproate, and lactate. Formate was not detected in the effluent. Fig. 2(b) illustrates that acetate, propionate, butyrate, and *i*-butyrate concentrations in the effluent decreased with the increase of wastewater COD. At 2 g COD/L, acetate accounted for 22% of total VFA/alcohols in the effluent, propionate 13%, butyrate 12%, and *i*-butyrate 11%. At 30 g COD/L, acetate represented only 11% of total VFA/alcohols, whereas the other three VFA were lowered to 7, 4, and 4%, respectively. Relative concentrations of valerate, *i*-valerate, caproate, and lactate varied slightly within 3–7%.

Fig. 2(c) illustrates that the ethanol concentration relative to total VFA/alcohols varied within the narrow range of 12–15%. Both propanol- and butanol-relative concentrations increased sharply with lactose concentration in the wastewater. Treating wastewater containing 2 g COD/L lactose, the effluent contained 4% of propanol and 1% of butanol. The corresponding concentrations increased to 17 and 19%, respectively, when treating 30 g COD/L of lactose. The sharp increase of propanol and butanol, along with the decrease of acetate and butyrate, indicates that the metabolic pathways were significantly influenced by the lactose concentration. A similar observation was reported by Jones and Woods (1986) that VFA were the main acidogenic products for low-strength wastewaters, but propanol and butanol were the main products for high-strength wastewaters.

It has been reported by some researchers that volumetric organic loading rate (OLR) is an operational parameter that is often critical to the reactor performance (Fang and Chui 1993). Volumetric OLR is calculated by dividing the substrate concentration in wastewater by the HRT. Since relative concen-

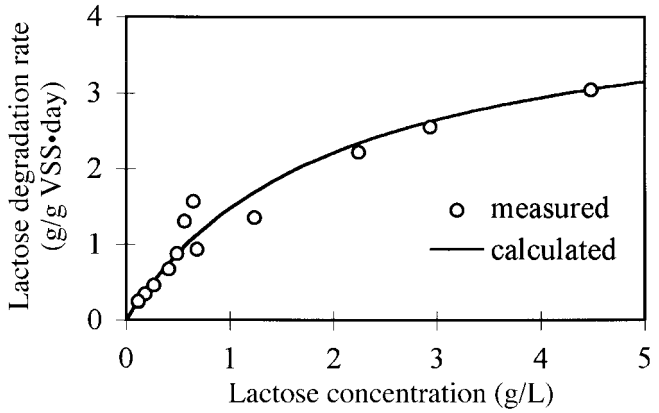

FIG. 3. Relation of Degradation Rate of Lactose and Its Concentration in Effluent

trations of VFA and alcohols were not sensitive to HRT, the product composition was equally sensitive to OLR and lactose concentration.

The biogas production rate increased with the wastewater COD, from 6.0 L/day at 2 g COD/L to 33.2 L/day at 30 g COD/L. Fig. 2(d) illustrates that the partial pressure of hydrogen increased with the lactose concentration, from 40 kPa to 2 g COD/L to 50 kPa at 30 g COD/L, whereas carbon dioxide correspondingly decreased from 54 to 45 kPa.

Kinetics of Lactose Degradation

Under steady-state conditions, the mass balance of lactose in the acidogenic reactor can be expressed as follows:

$$QS_i = QS + RM \tag{1}$$

where Q = flow rate (L/day); S_i and S = lactose concentrations (g/L) in the influent and effluent, respectively; R = specific lactose degradation rate (g/g VSS · day); and M = total biomass (g VSS). From the following equation R can be accordingly calculated:

$$R = Q(S_i - S)/M \tag{2}$$

in which all parameters on the right side can be accurately measured. Results calculated from data of Series II experiments showed that the lactose degradation rate was dependent on the lactose concentration in the effluent, following the commonly used Michaelis-Menten model as

$$R = R_{max}S/(K + S) \tag{3}$$

where R_{max} = maximum lactose degradation rate; and K = parameter representing the half-rate concentration. Based on regression analysis, R_{max} and K were found as 4.39 g/g VSS · day and 1.97 g/L (with a correlation coefficient of 0.987), respectively, to best fit the experimental data. Comparisons between experimental results and those calculated from (3) using these two best-fit parameters are illustrated in Fig. 3. Table 1 lists R_{max} and K values (all converted to COD equivalent) in literature on the acidification of lactose, glucose, sucrose, cellulose, as well as gelatin and secondary sludge from an activated sludge plant for comparison. It shows that the R_{max} value obtained in this study is substantially higher than those in literature. This could be due to the differences in microbial population and/or reactor configuration in the acidification systems.

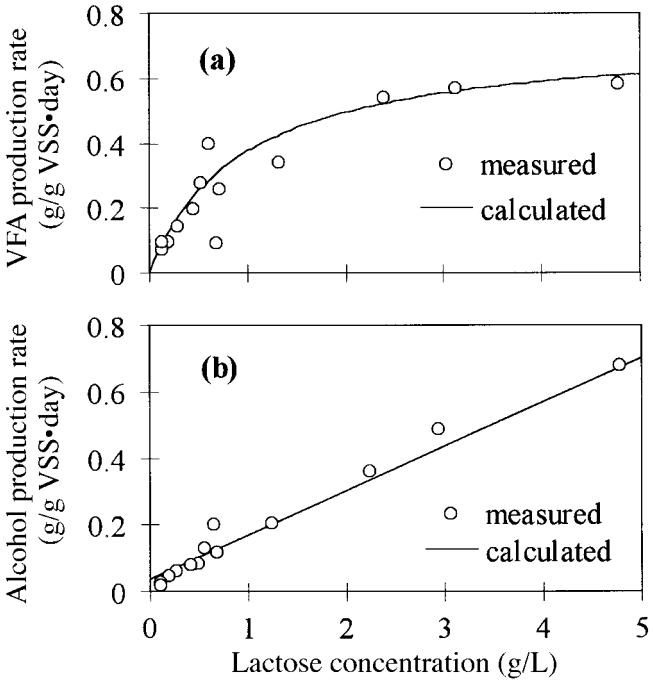

FIG. 4. Relationship Concentration of Lactose in Effluent and Specific Production Rates of: (a) VFA; (b) Alcohols

Kinetics of VFA and Alcohol Productions

Degradation of lactose produces VFA and alcohols. Thus, specific productions rates of VFA and alcohols should also be dependent on the lactose concentration S similar to lactose degradation. Regression analysis of experimental results showed that the specific production rate of VFA R_V can be expressed as

$$R_V = R_{V_{max}}S/(K_V + S) \tag{4}$$

where the maximum specific VFA production rate $R_{V_{max}}$ was found as 0.73 g/g VSS · day; and the half-rate lactose concentration K_V, 0.94 g/L with a correlation coefficient of 0.957. Fig. 4(a) illustrates the dependence of specific VFA production rate on lactose concentration, as compared to the curve calculated from (4) using the two best-fit parameters.

On the other hand, regression analysis of alcohols data showed that specific production rate of alcohols R_{alc} increased linearly with lactose concentration S as follows:

$$R_{alc} = k_{alc}S \tag{5}$$

TABLE 1. Kinetic Constants of Lactose Degradation

Substrate	Reactor	pH	Temperature (°C)	R_{max} (g COD/g VSS · day)	K_s (g COD/L)	Reference
Lactose	Upflow	5.5	37	4.39	1.97	This study
Lactose	CSTR	6.0	35	1.52	0.08	Kissalita et al. (1989)
Glucose	CSTR	5.5	35	2.63	0.24	Cohen et al. (1979)
Sucrose	Upflow	6.0	25	1.46	0.65	Zoetemeyer et al. (1982c)
Cellulose	CSTR	6.6–7.3	35	0.545	30.9	Ghosh et al. (1995)
Gelatin	Upflow	6.5–7.0	30	0.286	3.432	Breure and van Andel (1984)
Second sludge	CSTR	5.8	36	0.395	37	Ghosh et al. (1995)

where k_{alc} was 0.13 L/g VSS · day with a correlation coefficient of 0.973. Fig. 4(b) illustrates such a linear relationship between R_{alc} and S. In the Michaelis-Menten model, as shown in (3), the rate becomes linear to substrate concentration when $K >> S$. Thus, (5) means that the half-rate constant for alcohol production is substantially >4.79 g/L, the highest residual lactose concentration measured in the effluent.

Effect of pH

Series III experiments were conducted at 37°C, 12 h of HRT, 4 g COD/L of lactose in wastewater, and pH ranging 4.0–6.5. Fig. 5(a) illustrates that 89–93% of lactose was degraded at pH 5.0–6.5; the degradation efficiencies were 84% at pH 4.5 and only 78% at pH 4.0. This indicates that maximum lactose degradation occurred at pH near the range of 5.0–6.5, similar to the lactose degradation in the CSTR (Kissalita et al. 1987). The efficiency of lactose degradation decreased sharply at lower pH, which also concurs with the observations in the degradation of gelatin, in which the maximum degradation occurred at pH 7.0 (Breure and van Andel 1984).

Fig. 5(a) also illustrates that the degree of acidification increased with pH from 57% at pH 4.0 to the maximum of 81% at pH 5.5. However, the degree of acidification was reduced to 78% at pH 6.0 and further reduced to 75% at pH 6.5. The sensitive response of acidification at lower pH could be due to the reducing enzymatic activities for lactose degradation. The optimum pH for acidification can be affected by the wastewater characteristics and operating conditions. In the acidification of complex substrate, the optimum pH was found as pH 6.8 for beef extract (Dinopoulou et al. 1988) and pH 7.0 for gelatin (Breure and van Andel 1984). The optimum pH of 5.5 for acidification found in this study is similar to the optimum pH of 5.0–5.5 reported for wastewater containing concentrated carbohydrates such as glucose (Cohen et al. 1979), sucrose (Zoetemeyer et al. 1982c), and starch (Lee et al. 1999).

The effluent composition was strongly dependent on the pH. In general, a lower pH favored the production of alcohols, whereas higher pH favored VFA. VFA represented 67% of acidification products in the effluent at pH 4.0, and 78% at pH 6.5 (the balance being alcohols). Fig. 5(b) illustrates that acetate, butyrate, and *i*-butyrate concentrations relative to the concentration of total VFA/alcohols increased with pH from 12, 7, and 7%, respectively, at pH 4.0 to 20, 12, and 13% at pH 6.5; but propionate decreased correspondingly from 18 to 8%. Fig. 5(c) illustrates that the relative concentrations of lactate and ethanol, on the other hand, decreased with the increase of pH, from 12% and 18%, respectively, at pH 4.0 to 6% and 9% at pH 6.5. The relative concentrations of valerate, *i*-valerate, caproate, methanol, propanol, and butanol were, in general, not sensitive to the change of pH.

The predominant products were acetate, butyrate, and *i*-butyrate at pH 6.0–6.5, but propionate and ethanol at pH 4.5. The change of product distribution was probably due to the shift of microbial population in the reactor. Product distribution was also found sensitive to pH for the acidification of glucose (Zoetemeyer et al. 1982a). Since the composition of the acidification effluent would affect the performance of the methanogenic reactor downstream, pH control in the acidification reactor could be crucial to the overall performance of the two-stage treatment process.

Biogas production rate increased from 5.1 L/day at pH 4.0 to 13.3 L/day at pH 6.5. Fig. 5(d) illustrates that the biogas composition was strongly influenced by pH. The biogas was composed of 43% carbon dioxide and 55% hydrogen at pH 4.0. At pH 6.5, the corresponding compositions became 36 and

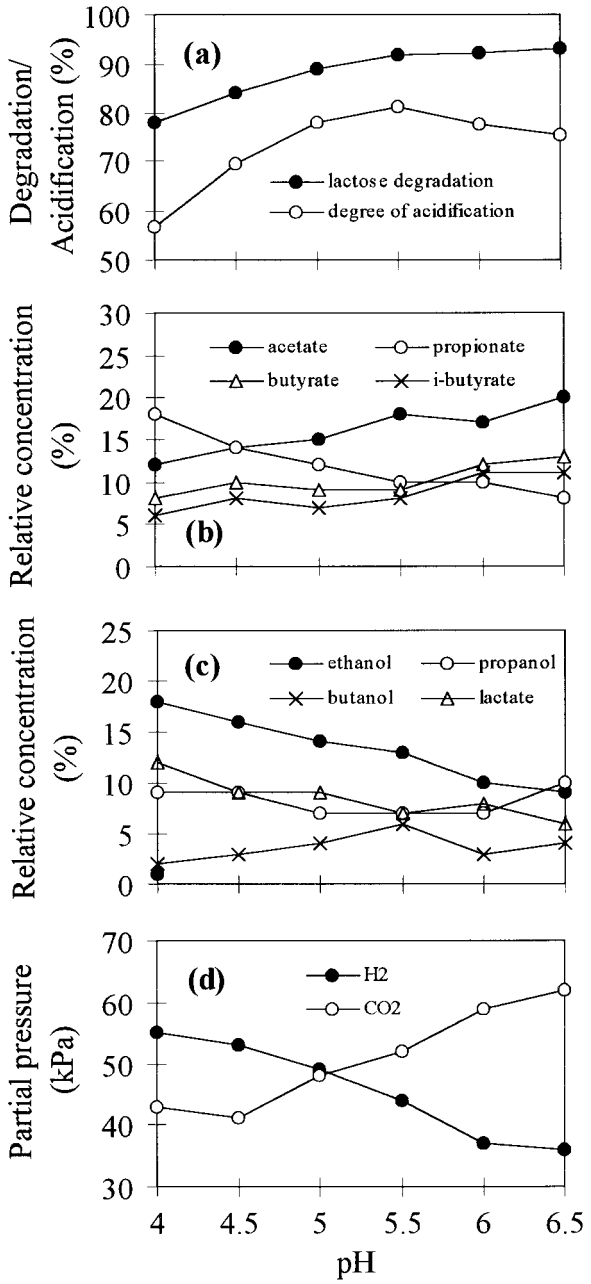

FIG. 5. Influence of pH on: (a) Lactose Degradation and Degree of Acidification; (b) Relative Concentrations of Acetate, Propionate, Butyrate, and *i*-Butyrate; (c) Relative Concentrations of Ethanol, Propanol, Butanol, and Lactate; (d) Partial Pressures of H_2 and CO_2

62%. The biogas contained 2% nitrogen and no detectable methane at all pHs.

Effect of Temperature

Series IV experiments were conducted at 4 g COD/L, 12 h of HRT, pH 5.5, and temperature ranging 20–60°C. Fig. 6(a) illustrates that degradation of lactose increased linearly with temperature from 85% at 20°C to 95% at 55°C. Further increase of temperature to 60°C however, lowered the degradation efficiency to 90%. Fig. 6(a) also illustrates that the degree of acidification increased with temperature reaching the maximum of 86% at 55°C, and was lowered to 80% at 60°C. The optimal temperature for the acidification of lactose appeared to be 55°C.

Conventionally, single-stage methanogenic reactors are operated either at the mesophilic temperature of 35°C–40°C or the thermophilic temperature of 55°C–60°C (Pavlostathis and

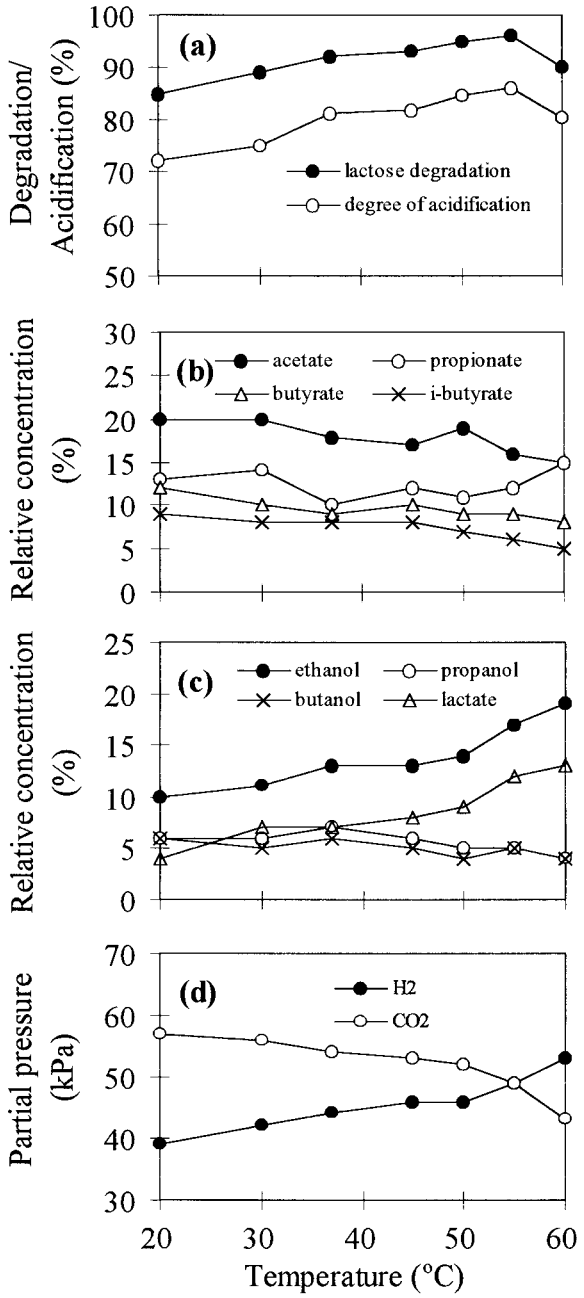

FIG. 6. Influence of Temperature on: (a) Lactose Degradation and Degree of Acidification; (b) Relative Concentrations of Acetate, Propionate, Butyrate, and *i*-Butyrate; (c) Relative Concentrations of Ethanol, Propanol, Butanol, and Lactate; (d) Partial Pressures of H_2 and CO_2

Giraldo-Gomez 1991). Substrate degradation efficiency is believed to decrease sharply at temperatures outside of these two ranges (Zoetemeyer et al. 1982b). However, results of this study showed that acidification efficiency increased with temperature until reaching maximum at 60°C. The engineering implication is that although acidification of lactose is preferred to be operated at the optimum temperature of 55°C, temperature control may not be as critical as in the single-stage methanogenic reactors.

About 70–77% of acidification products were VFA at temperature ranging 20°C–60°C. Distributions of key acidogenic products are illustrated in Figs. 6(b and c). Acetate, propionate, and ethanol ranged 15–20%, 10–15%, and 10–19%, respectively. The average concentrations were 18, 14, and 15%, respectively. Butyrate, *i*-butyrate, and lactate averaged 9, 9, and 10%, respectively, whereas valerate, *i*-valerate, caproate, propanol, and butanol were present at lower levels. Methanol was found only in some runs. Figs. 6(b and c) show that considerably more VFA were produced than alcohols. Productions of lactate and ethanol increased with temperature, from 4 and 10% at 20°C, respectively, to 13 and 19% at 60°C. This concurs with the findings of Zoetemeyer et al. (1982b), in which lactate and ethanol became predominated products in the acidification of glucose at 55–65° C.

Biogas production rate increased slightly from 8.6 L/day at 20°C to 9.6 L/day at 60°C. Fig. 6(d) illustrates that hydrogen partial pressure increased with temperature, from 39 kPa at 20°C to 53 kPa at 55°C, whereas that of carbon dioxide changed correspondingly from 43 to 66 kPa. No methane was detected in all runs.

Sludge Yield

COD is a wastewater parameter indirectly measuring the amount of electrons in substrates available for oxidation. In a strict anaerobic process, no electron acceptor is added to the system. In such a case, although the COD in the influent can be transformed into VFA, alcohols, hydrogen, and biomass, the overall COD should remain unchanged. As a result, in a strict anaerobic process, the amount of COD removed should be equal to the influent COD minus COD in the biogas, i.e., hydrogen and methane, if any, and COD in the biomass. Consequently, COD in the biomass can be estimated from the other three terms of COD, all of which can be accurately measured. The sludge yield can be estimated, as a result, by assuming that each gram of biomass is equivalent to 1.42 g of COD based on the chemical formula of $C_5H_7NO_2$. Sludge yields of many anaerobic treatment systems were estimated accordingly (Fang et al. 1995).

TABLE 2. Comparison of Sludge Yield in Acidification and Methane Production

Process	Substrate	Reactor	pH	Temperature (°C)	Yield (g VSS/g COD)	Reference
Acidification	Lactose	Upflow	4.0–6.5	20–60	0.230	This study
Acidification	Glucose	CSTR	5.5	35	0.272	Cohen et al. (1979)
Acidification	Glucose	CSTR	4.5–7.9	30	0.257	Zoetemeyer et al. (1982a)
Acidification	Sucrose	Upflow	6.0	25	0.244	Zoetemeyer et al. (1982c)
Acidification	Gelatin	Upflow	6.5–7.0	30	0.301	Breure and van Andel (1984)
Acidification	Dairy	Upflow	5.5	55	0.324	Yu and Fang (2000)
Methane production	Glucose	Fluidized bed	6.6–7.3	35	0.080	Shieh et al. (1985)
Methane production	Brewery	UASB	7.0	35	0.080	Borja et al. (1994)
Methane production	Starch	UASB	7.0–7.2	37	0.101	Kwong and Fang (1996)
Methane production	Mixed VFA	UASB	6.0–6.5	37	0.054	Fang et al. (1995)
Methane production	Phenol	UASB	6.9–7.5	37	0.038	Fang et al. (1996)
Methane production	Whey	UASB	6.9–7.4	35	0.076	Kalyuzhnyi et al. (1997)
Methane production	Peptone	UASB	7.2–7.5	37	0.066	Fang and Chung (1999)

The yield of lactose-acidifying sludge, based on all the experimental data in this study, was estimated accordingly as 0.230 ± 0.021 g VSS/g COD. Table 2 summarizes yield values reported in literature of both acidogenic and methanogenic sludges for comparison. It shows that sludge yield obtained in this study is consistent with those of acidogenic sludge reported in literature ranging 0.230–0.324 g VSS/g COD.

CONCLUSIONS

Optimum acidification of lactose was found at pH 5.5 and 55°C. The percentage degradation of lactose increased with increasing HRT, but with the decrease of lactose concentration in wastewater. Degradation of lactose followed the Michaelis-Menten model. Regression analysis of kinetic data showed that a maximum specific lactose degradation rate of 4.39 g/g VSS·day and a half-rate concentration of 1.97 g/L. The same model also described the kinetics of VFA production with the kinetic parameters of 0.73 g/g VSS·day and 0.94 g/L. Specific production rate of alcohols increased linearly with lactose concentration with a slope of 0.13 L/g VSS·day. Production of VFA, in general, favored lower lactose concentrations and higher pH, but was not sensitive to HRT and temperature. Distribution of individual VFA/alcohols was more sensitive to lactose concentration, pH, and temperature, but less sensitive of HRT. Acetate, propionate, and ethanol were the predominant products under most conditions. Biogas produced under all test conditions was composed of hydrogen and carbon dioxide plus a small fraction of nitrogen, but no detectable methane. Sludge yield was estimated as 0.230 ± 0.021 g VSS/g COD.

ACKNOWLEDGMENTS

The writers wish to thank the Hong Kong Research Grants Council for the support of this study (HKU 7004/98E), and the first writer wishes to thank the Croucher Foundation for the Senior Research Fellowship.

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