**ABSTRACT:** Effect of hydraulic retention time (HRT) on the acidogenesis of dairy wastewater was studied using an upflow reactor at pH 5.5, 37°C, and six HRTs ranging from 4 to 24 h. Results showed that the degree of acidification increased rapidly with HRT from 28.2% at 4 h to 54.1% at 12 h; further increase of HRT to 16 and 24 h only increased acidification slightly to 55.8 and 59.1%, respectively. The biodegradability of the three major constituents in dairy wastewater increased with HRT, following the order of carbohydrates > proteins > lipids. The predominant products were acetate, propionate, butyrate, lactate, and ethanol.

**INTRODUCTION**

The concept of two-phase anaerobic digestion of sludge was first proposed by Pohland and Ghosh (1971). In such a system, sludge is treated in two reactors in series, the first one being hydrolytic/acidogenic, and the second one acetogenic/methanogenic. It allows both reactors to be operated at their respective optimal conditions, thus ensuring a maximum efficiency for the overall system (Cohen et al. 1979; Ghosh 1991). Since the 1970s considerable research has been carried out for the two-phase treatment of sludge and high-strength industrial wastewater (Cohen et al. 1984; Dinopoulou et al. 1988; Ghosh 1991). The fundamentals and applications of this technology were comprehensively reviewed by Harper and Pohland (1986), and later by Fox and Pohland (1994).

The hydrolysis/acidogenesis of wastewater is greatly influenced by the chemical nature of wastewater and hydraulic retention time (HRT), among other operational parameters (Zoetemeyer et al. 1982; Henry et al. 1987). The control of HRT is critical to the successful enrichment of hydrolytic/acidogenic bacteria in the first reactor of a two-phase system. Pohland and Ghosh (1971) observed a virtual cessation of methane formation in sludge digestion when the HRT was decreased below 12.5 h. Elefsviniotis and Oldham (1994) reported a similar result that 12 h was the optimal HRT in terms of the acidification degree for the acidogenesis of primary sludge. On the other hand, there were also studies showing that the effect of HRT was of no significance. Breure and Andel (1984) claimed that HRT did not affect the volatile fatty acid (VFA) composition in the acidogenic degradation of gelatin. Similarly, Zoetemeyer et al. (1982) also reported that the VFA composition appeared to be independent of HRT in the acidogenic degradation of glucose.

This study was conducted to examine the influence of HRT on the acidification of dairy wastewater, which was chosen for its complex nature; it is composed of easily biodegradable carbohydrates, mainly lactose, as well as less biodegradable proteins and lipids. The substrate degradation, VFA production, and effects of hydrogen in biogas were investigated.

**MATERIALS AND METHODS**

A 2.8-L upflow anaerobic reactor with an 84-mm diameter was used for this study. Details of its configuration have been described previously (Fang et al. 1994). The reactor was water-jacketed and operated at a constant temperature of 37°C.

Synthetic dairy wastewater was prepared by using full-cream powdered milk supplied by the Nestle Corp. The wastewater chemical oxygen demand (COD) was kept at 4,000 mg/L, equivalent to 2,860 mg/L of powdered milk. Since the milk contained sufficient nitrogen, minerals, and vitamins for anaerobic microorganisms, only a phosphorus supplement of 20 mg-P/L was dosed as KH₂PO₄. Table 1 summarizes the composition of the simulated dairy wastewater. About 94% of the carbohydrates were identified as lactose. The corresponding COD levels were 30.9% for carbohydrates, 23.6% for proteins, and 41.9% for lipids. The remaining 3.6% of COD in wastewater could not be identified.

Granular methanogenic sludge from an anaerobic reactor treating a similar wastewater (Fang and Chung 1999) was used to seed the acidogenic reactor at an initial concentration of 9.5 g-VSS/L. The HRT was kept at 24 h in start-up. During this period, acidogenic bacteria were enriched in the reactor by controlling the pH of the mixed liquor at 5.5 ± 0.1. Start-up was completed after 63 days when the VFA production had become steady.

After the start-up, the HRT was lowered stepwise from the initial 24 h to 16 h, 12 h, 8 h, 6 h, and, finally 4 h, corresponding to an increase of a COD loading rate from the initial 4 g/L/day to 6, 8, 12, 16, and 24 g/L/day. The reactor was operated at each HRT level for 36–46 days, after the VFAs and biogas production became steady, before lowering the HRT to the next level. The solids retention time of the reactor was kept at about 15 days by wasting 1/15 of the sludge volume every day.

Measurements of COD, pH, and volatile suspended solids (VSS) were performed according to the Standard Methods (APHA 1992). The amount of biogas produced in the reactor was recorded daily using the water replacement method. The concentrations of VFAs and alcohols in the effluent, including acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, caproate, lactate, methanol, ethanol, propanol, and butanol, were determined by a second gas chromatograph of the same model. Details for the operating conditions of the two gas chromatographs can be found elsewhere (Fang et al. 1994). Formate was measured by the colorimetric method (Lang and Lang 1972).

Lactose was measured using the colorimetric ferric-cyanide method (Dubois et al. 1956), while total carbohydrate was measured by the phenol-sulfuric method (Herbert et al. 1971), and proteins by the Lowry-Folin method (Lowry et al. 1951). Lipids were extracted by trichlorotrifluoroethane with the Bligh-Dyer method from the acidified sample, and were then measured gravimetrically after the solvent was evaporated at 80°C (APHA 1992). The lipids thus measured also accounted for the long-chain fatty acids (LCFA).
RESULTS AND DISCUSSION

Degradation of Carbohydrates, Proteins, and Lipids

Fig. 1(a) illustrates that degradations of carbohydrates, proteins, and lipids all increased with HRTs. Among them, carbohydrates were most easily degradable, over 93% were degraded at HRTs as low as 4 h. This indicates that the effect of HRT was insignificant on the degradation of carbohydrates in dairy wastewater. This is consistent with previous results that acidogenesis of lactose was mainly regulated by pH, not by HRT (Kissaalita et al. 1989).

Fig. 1(a) also illustrates that degradations of proteins and lipids increased, respectively, from 57 and 20% at 4 h of HRT to 86 and 46% at 24 h. The poor protein conversion at low HRTs could partly be due to the higher residual content of carbohydrates in the mixed liquor. Breure et al. (1986) reported that the acidification of gelatin was retarded by the presence of carbohydrates, and McInerney (1988) reported that carbohydrates could suppress the synthesis of exopeptidases, a group of enzymes facilitating protein hydrolysis.

During anaerobic degradation, lipid is first hydrolyzed to glycerol and three LCFAs, followed by β-oxidation producing acetate and hydrogen (McInerney 1988)

\[ n \text{LCFA} \rightarrow (n-2)\text{LCFA} + \text{CH}_3\text{COOH} + 2\text{H}_2 \]

where \( n \) represents the number of carbon in the acid. The change of standard Gibbs free energy at pH 7 (\( \Delta G^{\circ} \)) for this reaction averages +48 kJ/mol (Fox and Pohland 1994), which means that the β-oxidation of LCFA is thermodynamically unfavorable, especially at high levels of hydrogen. Thus, the poor lipid degradation at low HRTs found in this study is likely due to the higher hydrogen partial pressure in the reactor.

Production of VFAs and Alcohols

Table 2 summarizes the concentrations of VFAs and alcohols in the effluent at each HRT. In the previous studies regarding acidogenesis of simple substrates using glucose (Cohen et al. 1984), gelatin (Breure et al. 1984), and lactose (Kissaalita et al. 1989), the effluent products were much less complicated with very little valerate and alcohols in the effluent. The complex product distribution of this study is likely attributed to the complex nature of the simulated dairy wastewater.

Table 2 shows that the three main acidogenic products (acetate, propionate, and butyrate) accounted for 61–65% of total VFAs and alcohols. Acetate and propionate in the effluent products were highly influenced by the variation of HRT. Acetate accounted for 19% of the total VFAs/alcohols in the effluent at 4 h of HRT, and 40% at 24 h. Propionate decreased from 32% at 4 h of HRT to 10% at 24 h, suggesting that shorter HRT favored the production of propionate. These results show that the HRT had a significant effect on the distribution of effluent products, as reported by Elefsiniotis and Oldham (1994) and Henry et al. (1987). On the other hand, butyrate was rather steady in the effluent, from 13% at 4 h of HRT to 16% at 24 h.

Table 2 also shows that ethanol and lactate were important products of acidogenesis as well, each accounting for about 9% of the total effluent VFAs/alcohols. It is well known that lactate is the main acidogenic product of lactose (Kissaalita et al. 1989). Results of this study show that variation of HRT had little effect on the effluent lactate concentration. There are two common types of acidogenesis: one produces not only butyrate and acetate, but also carbon dioxide and hydrogen, whereas the other produces propionate, acetate, and some valerate, with no significant gas production (Cohen et al. 1984). Neither of these two types produces ethanol. However, a third type of acidogenesis was recently suggested by Ren et al. (1995). The main products of this type of acidogenesis, which requires a pH of <4.5, are ethanol, acetate, hydrogen, and carbon dioxide. Based on the VFA and alcohol distributions (Table 2) and gas production [Fig. 1(b)], it appears that all three types of acidogenesis were in coexistence even at pH 5.5.

Total VFA and Alcohol Production

Fig. 2(a) illustrates the total VFA/alcohol production at various HRTs. It shows that production of total VFA/alcohol nearly doubled when the HRT increased from 4 to 12 h. However, further increase of HRT to 16–24 h only slightly increased the total VFA/alcohol production by 4–5%. The degree of acidification can also be quantified by comparing the COD equivalent of the acidogenic products (i.e., VFAs and alcohols, plus hydrogen and methane in the biogas) to the wastewater COD

\[
\text{degree of acidification } = \frac{\sum \text{COD}_{\text{VFA}} + \sum \text{COD}_{\text{alcohols}} + \text{COD}_{\text{H}_2} + \text{COD}_{\text{CH}_4}}{\text{COD}_{\text{inf}}} \times 100\%
\]

Fig. 2(b) illustrates that the degree of acidification increased with HRT, from 28.2% at 4 h to 54.3% at 12 h. Doubling the HRT from 12 to 24 h only further increased acidification degree slightly to 59.1%. Results in Figs. 2(a and b) suggest that
TABLE 2. Effluent Concentration of Individual VFAs and Alcohols (in mg/L)

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>HFR (mg/L)</th>
<th>HAc (mg/L)</th>
<th>HPr (mg/L)</th>
<th>HBu (mg/L)</th>
<th>i-HBu (mg/L)</th>
<th>HVa (mg/L)</th>
<th>i-Va (mg/L)</th>
<th>HCa (mg/L)</th>
<th>HLa (mg/L)</th>
<th>Mol (mg/L)</th>
<th>Eol (mg/L)</th>
<th>Pol (mg/L)</th>
<th>Bol (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>122</td>
<td>204</td>
<td>86</td>
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<td>10</td>
<td>10</td>
</tr>
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<td>26</td>
<td>77</td>
<td>7</td>
<td>63</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
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<td>209</td>
<td>246</td>
<td>152</td>
<td>15</td>
<td>32</td>
<td>10</td>
<td>27</td>
<td>76</td>
<td>10</td>
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<tr>
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<td>387</td>
<td>223</td>
<td>200</td>
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<td>17</td>
<td>43</td>
<td>113</td>
<td>27</td>
<td>100</td>
<td>13</td>
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<td>16</td>
<td>26</td>
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<td>78</td>
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<td>104</td>
<td>52</td>
<td>105</td>
<td>39</td>
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</tr>
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Note: HFr = formate; HAc = acetate; HPr = propionate; HBu = butyrate; HVa = valerate; i-HBu = isobutyrate; i-Va = isovalerate; HCa = caproate; HLa = lactate; Mol = methanol; Eol = ethanol; Pol = propanol; and Bol = butanol.

*Below the detection limit of 3 mg/L.

FIG. 2. Performance of Acidogenic Reactor at Various HRTs: (a) Effluent VFA/Alcohol Concentrations; (b) Degree of Acidification

the most effective HRT for acidogenesis of the simulated dairy wastewater was 12 h.

The degree of acidification is also strongly dependent on the complexity of the pollutants in wastewater. For comparison, over 70% of glucose, starch, and other easily degradable carbohydrates could be acidified at <12 h of HRT (Cohen et al. 1979; Zoetemeyer et al. 1982); on the other hand, only 30–60% of beef extract could be acidified at 6–17 h of HRT (Dinopoulou et al. 1988) and 40% of gelatin at 5 h (Breure et al. 1984).

Gas Production

In the acidogenic reactor, the biogas is mostly composed of the acidogenic by-products, carbon dioxide and hydrogen. Fig. 1(b) illustrates that at 4 h of HRT, the hydrogen partial pressure was 0.33 atm and there was no detectable methane in the biogas. Fig. 1(b) also illustrates that methanogenic activity increased with further increase of HRT. Hydrogen was consumed by the methanogens as electron donors for the formation of methane. The hydrogen partial pressure decreased, along with the increase of methane, as HRT increased. At 24 h of HRT, there were only 0.5 kPa of hydrogen, whereas methane was increased to 31 kPa.

However, the overall conversion of hydrogen and methane from the substrates was insignificant. Table 3 shows that only 1.7–7.9% of the COD in wastewater was converted to either hydrogen or methane. The presence of hydrogen had a significant effect on the distribution of acidogenic products, especially propionate and acetate (McInerney 1988). Fig. 3 illustrates that increasing partial pressure of hydrogen resulted in the decrease of acetate and the increase of propionate in the effluent.

Unidentified Metabolites

In a strict anaerobic reactor, when there is no external electron acceptor introduced to the system, the overall COD remains unchanged. Therefore, the wastewater COD should equal the sum of COD in the effluent, biomass, and biogas (in the form of hydrogen and methane). The COD in the effluent

FIG. 3. Percentages in Effluent VFA/Alcohol at Various Hydrogen Partial Pressures: (a) Acetate; (b) Propionate

TABLE 3. COD Conversions to Biogas and Biomass

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>COD&lt;sub&gt;influent&lt;/sub&gt; (mg/d)</th>
<th>COD&lt;sub&gt;gas&lt;/sub&gt; (mg/d)</th>
<th>H&lt;sub&gt;2&lt;/sub&gt; (atm)</th>
<th>CH&lt;sub&gt;4&lt;/sub&gt; (atm)</th>
<th>Total (atm)</th>
<th>COD&lt;sub&gt;gas/COD&lt;sub&gt;influent&lt;/sub&gt;&lt;/sub&gt; (%)</th>
</tr>
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<td>4</td>
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<td>0</td>
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<td>1.19</td>
<td>1.7</td>
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<tr>
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<td>0</td>
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<td>0.992</td>
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<tr>
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<td>735</td>
<td>429</td>
<td>1,164</td>
<td>1.598</td>
<td>3.5</td>
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<tr>
<td>12</td>
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<td>114</td>
<td>772</td>
<td>886</td>
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<tr>
<td>16</td>
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<td>93</td>
<td>646</td>
<td>739</td>
<td>0.87</td>
<td>4.5</td>
</tr>
<tr>
<td>24</td>
<td>11,200</td>
<td>4</td>
<td>883</td>
<td>887</td>
<td>0.995</td>
<td>7.9</td>
</tr>
</tbody>
</table>
TABLE 4. COD Constituents in 1 L of Effluent

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>COD&lt;sub&gt;eff&lt;/sub&gt; (mg)</th>
<th>VFAs (mg)</th>
<th>Alcohols (mg)</th>
<th>Carbohydrates (mg)</th>
<th>Proteins (mg)</th>
<th>Lipids (mg)</th>
<th>Unknown metabolites (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3,720</td>
<td>962</td>
<td>163</td>
<td>73</td>
<td>508</td>
<td>1,325</td>
<td>689</td>
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<tr>
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<td>3,690</td>
<td>1,140</td>
<td>240</td>
<td>53</td>
<td>437</td>
<td>1,304</td>
<td>516</td>
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<td>3,600</td>
<td>1,351</td>
<td>237</td>
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<td>391</td>
<td>11</td>
<td>150</td>
<td>891</td>
<td>245</td>
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</table>

was contributed by the residual carbohydrates, proteins, and lipids, plus VFA/alcohol and other unidentified metabolites. All of these could be calculated from the measured concentrations of individual species, except those from the unidentified metabolites, which could be glycerol, ketones, aldehydes, etc. The COD difference between the effluent and the sum of individual contributions is the COD from the unidentified metabolites. Table 4 summarizes the COD contributions by the individual identifiable species in the effluent. It shows that 18.5% of the effluent COD was contributed by the unidentified metabolites at 4 h of HRT. However, the contributions by these unidentified metabolites decreased with HRT, reaching only 8.3% at 12 h and 7.3% at 24 h.

CONCLUSION

Results showed that the degree of acidification of dairy wastewater increased rapidly with HRT from 28.2% at 4 h to 54.1% at 12 h; further increase of HRT to 16 and 24 h only increased acidification slightly up to 55.8 and 59.1%, respectively. The biodegradability of the major constituents in wastewater increased with HRT, following the order of carbohydrates > proteins > lipids. The predominant products were acetate, propionate, butyrate, lactate, and ethanol, plus lesser quantities of formate, valerate, caproate, methanol, propanol, and butanol. HRT had a significant effect on the distribution of effluent products except butyrate. Only 1.7–7.9% of COD in wastewater was converted to either hydrogen or methane.

ACKNOWLEDGMENT

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APPENDIX. REFERENCES


