ACIDIFICATION OF MID- AND HIGH-STRENGTH DAIRY WASTEWATERS

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Abstract—Batch and continuous experiments were conducted to study the influence of dairy wastewater strength (2–30 g-COD/L) on acidification at pH 5.5 and 37 °C. Results of batch experiments showed that carbohydrate was preferentially acidified as compared to protein and lipid. Production of VFAs (mainly acetate, propionate and butyrate) and hydrogen corresponded to carbohydrate acidification, whereas production of alcohols (mainly ethanol, propanol and butanol), plus i-butyrate and higher molecular-weight VFAs, corresponded to protein acidification. In treating high-strength wastewaters (8–30 g-COD/L), acetate, butyrate and P[H2 decreased after reaching their peak levels before leveling off. Results of continuous experiments with 12 h of hydraulic retention showed that acidification decreased with the increase of wastewater COD, from 57.1% at 2 g-COD/L to 28.8% at 30 g-COD/L; among the constituents in dairy wastewater, 92–99% of carbohydrates, 59–85% of protein and 12–42% of lipid were acidified. High-strength wastewater favored production of hydrogen and alcohols, especially propanol and butanol. The biomass yield was 0.258 g-VSS/g-COD.

Keywords—acidification, alcohol, dairy wastewater, volatile fatty acid (VFA)

INTRODUCTION

In anaerobic degradation, complex organics, such as polysaccharides, proteins, and lipids, are first hydrolyzed by enzymes, forming sugars, amino acids, and fatty acids. These intermediate products are then degraded by acidogens, forming volatile fatty acids (VFAs), which are further degraded by acetogens, forming acetate, carbon dioxide, and hydrogen. Lastly, both acetate and H2/CO2 are converted by methanogens to methane (Harper and Pohland, 1986). Acidogens grow relatively faster and are less sensitive to pH variation than acetogens/methanogens (Cohen et al., 1980). This usually results in the accumulation of organic acids and lowering of pH, leading to the suppression of methanogenic activities and, in some cases, even process failure (Zoetemeyer et al., 1982). Instability or failure of single-phase methanogenic digesters has been widely reported for a variety of wastewaters, especially under high loading conditions (Fox and Pohland, 1994; Ghosh et al., 1995).

In order to improve the process stability and efficiency, the concept of two-phase reactor was thus proposed. In a two-phase reactor system, acidification is conducted in the first reactor followed by acetogenesis and methanogenesis in the second (Massey and Pohland, 1978; Pohland and Ghosh, 1971). Each reactor can be operated at its optimal condition (Pipyn and Verstraete, 1981). Although the proper operational conditions for the acetogenic/methanogenic phase have been extensively studied, little information is available for the acidogenic phase (Elwfsiniotis and Oldham, 1994; Fox and Pohland, 1994; Henry et al., 1987). The lack of such knowledge is one of the major barriers for the widespread of the two-phase process (Ghosh et al., 1995). The present study was conducted to investigate the influence of substrate concentration on the acidification of dairy wastewater, which contained complex organics, such as protein, carbohydrate and lipid.

MATERIALS AND METHODS

Continuous experiment

The continuous experiment was conducted in a 2.8-L 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. A synthetic dairy wastewater was prepared by using full-cream powder milk supplied by Nestle Corp. Since the milk contained enough nitrogen, minerals and vitamins, only phosphorus as KH2PO4 was dosed to keep the ratio of chemical oxygen demand (COD) to P at 200:1.

Throughout the experiment, the hydraulic retention time (HRT) was kept at 12 h. The acidogenic condition was controlled by keeping the pH at 5.5 ± 0.1 so that
methanogenesis was suppressed. The reactor was seeded with the sludge taken from a conventional single-phase upflow sludge blanket reactor treating the same synthetic wastewater for another study (Fang and Chung, 1999). The seed sludge contained 26.2 g volatile suspended solids (VSS), resulting in an initial VSS concentration of 9.5 g/L. After seeding, the wastewater COD was increased stepwise from the initial 2 g/L to 4, 8, 12, 20, and lastly 30 g/L, respectively. The reactor was operated at each COD level for 31–40 days to ensure reaching steady state before increasing the COD to the next level. The sludge retention was controlled at 15 days, by wasting about 1/15 of the sludge in the reactor daily (or every 2 days during weekends). The amount of biogas produced was recorded daily using water displacement method.

**Batch experiment**

Batch tests were conducted to investigate the degradation patterns at various substrate conditions. Six sets of tests were conducted in duplicate in 157 mL glass serum vials at 37 °C using feed solutions containing, respectively, 2, 4, 8, 12, 20 and 30 g-COD/L. The feed solutions were first purged with nitrogen to remove any dissolved oxygen. Each vial was seeded with the sludge from the continuous reactor on day 260 of the operation when the influent COD was 12 g/L and organic loading rate was 24 g-COD/L day. About 200 mL of sludge sampled from the reactor was washed with stock solution, followed by centrifugation. After decanting the supernatant, the sludge was re-suspended in 1200 mL of stock solution. About 100 mL of the mixed solution containing 60 mg of VSS was transferred to each vial using a syringe, and the pH was adjusted to 5.5.

All vials were submerged in a 37 °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.

**Analyses**

The contents of H2, CH4, CO2 and N2 in the biogas were analyzed by a gas chromatograph (Hewlett-Packard, Model 5890 Series II) 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, respectively, kept at 130 °C and 200 °C, while column temperature was increased from 90 °C to 110 °C. The concentrations of VFAs, including acetate, propionate, butyrate, i-butyrate, valerate, i-valerate, caprate and lactate, and alcohols, including methanol, ethanol, propanol and butanol, were determined by a second gas chromatograph–graph of same model equipped with a flame ionization detector and a 10 m × 0.53 mm HP-FFAP fused-silica capillary. Samples were filtered through a 0.2 μm membrane, acidified by formic acid, and measured for free acids. The temperature of the column was initially 70 °C for 4 min, followed by 140 °C for 3 min, and lastly 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. The detectable levels were 1 mg/L for individual VFAs (from C2 to C7) and 3 mg/L for individual alcohols. The formate concentration was measured by the colorimetric method (Lang and Lang, 1972).

Carbohydrate and protein were measured by the phenol–sulfuric acid 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.

**RESULTS AND DISCUSSION**

**Batch experiments**

Concentrations of carbohydrate and protein as well as degradation products in the mixed liquor were monitored in all batches at various time intervals. Lipid concentration was only measured at the end. In all batches, the biogas was mainly composed of hydrogen and carbon dioxide, and the mixed liquor was composed of VFAs and alcohols. The VFAs were mostly acetate, propionate, and butyrate, plus smaller quantities of lactate, formate, i-butyrate, valerate, i-valerate and caproate; whereas the alcohols were mostly ethanol, propanol and butanol, plus trace amount of methanol.

The final pH in all the vials fell in a narrow range of pH 6.1–6.4. The production of ammonium from protein degradation counter balanced the effect of VFA production on pH. Figure 1 illustrates the concentration changes of (a) carbohydrate and protein, (b) acetate, propionate and butyrate, (c) i-butyrate, valerate, i-valerate and caproate, (d) methanol, ethanol, propanol and butanol, and (e) P_H, in batches treating mid-strength wastewaters, using the one of 4 g-COD/L as an example. Figure 2 illustrates the corresponding results treating high-strength wastewaters, using the one of 20 g-COD/L as an example, for comparison.

**Treatting mid-strength wastewater.** Figure 1(a) illustrates that, after a brief acclimation period, carbohydrate was degraded rapidly and became nearly depleted within two days. Similar observation was reported (Kissalita et al., 1989) for the methanogenic and non-methanogenic degradations of lactose, the sugar being 94% of the total carbohydrate in the wastewater. On the other hand, protein was degraded at a much slower rate. The protein concentration remained almost unchanged during the first 2 days, and began to decrease only after carbohydrate became depleted. This suggests that protein degradation was suppressed by the presence of carbohydrate. This could be attributed to the suppression of carbohydrates on the synthesis of the enzymes involved in protein hydrolysis (Russell and Martin, 1984). A similar phenomenon was observed in a continuous-flow acidogenic reactor (Breure et al., 1986), where the degradation of gelatin was inhibited by the presence of glucose in the feed. Concentration of lipid was not monitored during the experiment except at the end. Only 19.5% of lipid was degraded after 9 days when the experiment was terminated.

Figure 1(b) illustrates that productions of acetate, propionate and butyrate were in accord with the degradation of carbohydrate. The acetate,
Propionate and butyrate concentrations increased rapidly, reaching 140, 167 and 51 mg/L, respectively, by day 2. Thereafter, they continued to increase, even though carbohydrate had become depleted, in response to the degradation of protein. Acetate concentration peaked at 184 mg/L on day 5, propionate at 251 mg/L on day 6, butyrate at 103 mg/L on day 6.

On the other hand, Fig. 1(c) illustrates that the productions of $i$-butyrate, valerate, $i$-valerate and caproate were largely associated with the acidification of protein. The concentrations of these acids were negligible in the first 2 days. They began to increase thereafter with the decrease of protein concentration, reaching 56, 22, 18 and 26 mg/L, respectively, by day 4, and to 72, 29, 23, and 28 mg/L, respectively, by day 6. The production of these acids could be either via reductive de-amination of protein. The concentrations of these acids were negligible in the first 2 days. They began to increase thereafter with the decrease of protein concentration, reaching 56, 22, 18 and 26 mg/L, respectively, by day 4, and to 72, 29, 23, and 28 mg/L, respectively, by day 6. The production of these acids could be either via reductive de-amination of
individual amino acids or by an oxidation–reduction reaction between amino acid pairs, known as the Stickland reaction (McInerney, 1988). Nevertheless, the production of these four VFAs was not as significant as those of acetate, propionate and butyrate.

Figure 1(d) illustrates that production of alcohols was much lower than that of VFAs. Ethanol was the main alcohol produced, reaching 67 mg/L by day 6. Methanol and propanol were produced at much lower concentrations, each reaching about 10 mg/L by day 9. Butanol was not detected throughout the 9-day experiment.

Figure 1(e) illustrates that hydrogen was produced in direct response to the degradation of carbohydrate. \( P_{H_2} \) reached 0.17 atm in 12 h, and 0.55 atm when carbohydrate became depleted by day 2. It then remained nearly unchanged afterwards. Although it is well known that acidification of carbohydrate, including lactose (Kissalita et al., 1989), produces hydrogen as a by-product, Figure 1(e) illustrates that protein acidification had little effect on hydrogen production.

Treating high-strength wastewater. Results of treating high-strength wastewater, using the one of 20 g-COD/L as example, are illustrated in Fig. 2. Most of results in treating dairy wastewater were independent of the wastewater strength, but there were a few exceptions. Figure 2(a) illustrates that carbohydrate was preferentially degraded over protein, as in Fig. 1(a). Figure 2(b) illustrates that the productions of acetate, propionate and butyrate were directly associated with the degradation of carbohydrate; however, unlike in mid-strength wastewater, acetate and butyrate decreased after reaching peak concentrations on day 4 when protein degradation began. On the other hand, Fig. 2(c) illustrates that, like in mid-strength wastewaters, \( i \)-butyrate, valerate, \( i \)-valerate and caproate were produced mainly in association with the protein acidification, and the total amount was not significant as compared to the three lower acids.

In all batches, productions of alcohols increased with the increased acidification of protein. However, Fig. 2(d) illustrates that, propanol and butanol were produced at much higher amounts, as compared to batches treating mid-strength wastewaters (butanol was not even detected in Fig. 1(d)). The \( P_{H_2} \) in all batches increased rapidly initially, due to the acidification of carbohydrate. However, the \( P_{H_2} \) in batches treating 2–8 g-COD/L remained unchanged after reaching the peaks, but the \( P_{H_2} \) in batches treating high-strength wastewater decreased after reaching a peak pressure (0.53 atm in Fig. 2(e)) before leveling off. This was coinciding with the sharp increases of propanol and butanol and the sharp decreases of acetate and butyrate. The sharp increases of propanol and butanol correspond to the decreases of acetate, butyrate and \( P_{H_2} \). Acetate, butyrate and hydrogen were likely to partially converted to propanol and butanol by Clostridia (Jones and Woods, 1986).

Alcohols, except methanol, cannot be directly utilized by methanogens. However, they can be readily converted into acetate, hydrogen, carbon dioxide, which can be subsequently utilized by methanogens. Thus, high alcohol production probably has no negative effect on methanogenesis.

Continuous experiment

Acidification of carbohydrate, protein and lipid. In the continuous experiment, dairy wastewater was treated in the upflow reactor at 37°C with 12 h of hydraulic retention. The COD of this wastewater constituted 30.9% of carbohydrate, 23.6% of protein and 41.9% of lipid. Figure 3(a) illustrates degradations of carbohydrate, protein and lipid in general all decreased with the increase of wastewater COD level and loading rate. Among the three major constituents, carbohydrate was acidified most effectively from 99.0% at 2 g-COD/L to 91.5% at 30 g-COD/L. It appears that the presence of protein and lipid did not have noticeable adverse effect on the acidification of carbohydrate.

Figure 3(a) illustrates that protein was acidified much less effectively than carbohydrate. About 85% of protein was acidified for the wastewater of 2 g-COD/L, but only 59% for that of 30 g-COD/L. Ammonium was produced during the acidification of protein. Although nitrogen is an essential nutrient, ammonium over 5000 mg/L is generally toxic to anaerobic bacteria (Angelidaki and Ahring, 1993; Henry et al., 1987), including acidogens (Breure and van Andel, 1984; Russell and Martin, 1984). However, the effluent ammonium concentration, ranging...
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from 46 mg/L at 2 g-COD/L to 496 mg/L at 30 g-COD/L, was substantially lower than the inhibition concentration of 5000 mg/L, suggesting that the influence of ammonium was of no significance to the acidification of protein.

Lipid was the least acidified, only 12–42%, as illustrated in Fig. 3(a). This is consistent to the reported 17–23% for the acidification of olive oil wastewater (Beccari et al., 1998), 20–25% for palm oil wastewater (Tsousis and Grigoropoulos, 1988), and <10% for the lipid in municipal sludge (Eastman and Ferguson, 1981).

Figure 3(b) illustrates that the specific conversion rates for carbohydrate, protein and lipid increased with concentration. The degradation rates of carbohydrate and protein substantially increased with the substrate concentration, from 0.065 g/g-VSS day at 2 g-COD/L to 0.591 g/g-VSS day at 30 g-COD/L for carbohydrate, and from 0.035 g/g-VSS day at 2 g-COD/L to 0.241 g/g-VSS day at 30 g-COD/L for protein. The specific lipid degradation rate averaged 0.046 g/g-VSS day in treating 4–30 g-COD/L of wastewater.

Acidification products. Acidification produces VFAs and alcohols in the effluent, plus hydrogen and methane in the biogas. Table 1 summarizes the concentrations of individual VFAs and alcohols in the effluent. It shows that acetate, propionate and butyrate were the main VFA products, whereas formate, \textit{i}-butyrate, valerate, \textit{i}-valerate, caproate and lactate were also present, but in substantially lower quantities. On the other hand, ethanol, propanol and butanol were the main alcohol products, whereas methanol was found in relatively low concentrations.

The overall efficiency of a reactor can be evaluated by the “degree of acidification”, which is the ratio of COD-equivalent of all the acidogenic products (in both effluent and biogas) and the wastewater COD (Dinopoulou et al., 1988). Figure 4(a) illustrates that the degree of acidification decreased with the increase of wastewater COD, from 57.1% at 2 g-COD/L to 33.2% at 20 g-COD/L, and to 28.8% at 30 g-COD/L. The overall activity of the biomass in the reactor, as expressed by the specific acidification rate, increased with wastewater COD, from 0.121 g-COD/g-VSS day at 2 g-COD/L to 0.582 g-COD/g-VSS day at 30 g-COD/L, as illustrated in Fig. 4(b).

As illustrated in Figs 4(a) and (b), the degree of acidification had an opposite tendency to the VFA/alcohol formation rate when influent COD was varied. Therefore, the optimum conditions for this acidogenic reactor have to be established as a compromise between the two objectives. When influent COD increased from 12 to 20 g/L, the VFA/alcohol formation rate only slightly increased from 0.259 to 0.261 g/g-VSS day, whereas the degree of acidification markedly dropped from 44.5% to 33.1%. Therefore, 12 g-COD/L could be regarded as the optimum influent concentration for this study.

Results of the present study show that wastewater COD has a considerable effect on the distribution of the acidification products, as illustrated in Fig. 5. Ethanol in the effluent remained in the narrow range of 10–13%. Acetate and butyrate decreased with the increase of wastewater COD, and leveled off at high concentrations; at 20–30 g-COD/L, they represented 14% and 4% of the acidification products in the effluent, respectively. Propionate, on the other hand, increased with wastewater COD until reaching the maximum of 24% and then gradually reduced to

\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline
Influent COD (g/L) & HFr & HAc & HPr & HBu & \textit{i}-HBu & Hva & \textit{i}-Hva & Hca & Hla & Mol & Eol & Pol & Bol \\
\hline
2 & 23 & 266 & 82 & 68 & 41 & 20 & 27 & 26 & 28 & 40 & 68 & 0 & 0 \\
4 & 12 & 344 & 221 & 112 & 89 & 57 & 42 & 52 & 53 & 61 & 123 & 17 & 0 \\
8 & 21 & 504 & 502 & 132 & 110 & 66 & 65 & 43 & 64 & 67 & 263 & 88 & 65 \\
12 & 88 & 584 & 701 & 146 & 175 & 145 & 117 & 115 & 146 & 58 & 292 & 176 & 174 \\
20 & 32 & 450 & 643 & 161 & 129 & 96 & 95 & 94 & 65 & 97 & 386 & 388 & 515 \\
30 & 40 & 522 & 723 & 160 & 162 & 120 & 81 & 122 & 82 & 119 & 602 & 602 & 803 \\
\hline
\end{tabular}
\caption{Effluent concentration of individual VFAs and alcohols (in mg/L)\textsuperscript{a}}
\end{table}

\textsuperscript{a}HFr = formate, HBu = butyrate, \textit{i}-HVa = \textit{i}-valerate, Mol = methanol, Bol = butanol, HAc = acetate, \textit{i}-HBu = \textit{i}-butyrate, HCa = caproate, Eol = ethanol, HPr = propionate, HVa = valerate, HLa = lactate, Pol = propanol.
18% in treating wastewater of 30 g-COD/L. The most prominent effect of wastewater COD was on the production of propanol and butanol. They were undetectable in the effluent treating 2 g-COD/L of wastewater, but reached 15% and 20%, respectively, in treating 30 g-COD/L of wastewater. The sharp increase of propanol and butanol, along with the decrease of acetate and butyrate (Fig. 5(a)), indicates that the metabolic pathways were significantly influenced by the substrate concentration. This is similar to the general observation reported by Jones and Woods (1986) that VFAs were the main acidification products for the acidification of low-strength wastewaters, but propanol and butanol were for high-strength wastewaters. Many acidogens, such as Clostridium acetobutylicum (Bahl et al., 1982), Clostridium butyricum (Andel et al., 1985), Clostridium cellobioparum (Chung, 1976), Clostridium fallax (Ueki et al., 1991), and Clostridium pasteurianum (Dabrock et al., 1992), produce hydrogen, acetate, butyrate at low substrate concentrations and shift the metabolic pathways when the substrate concentration exceed certain thresholds to produce propanol and butanol, and in some cases, acetone.

The high molecular-weight VFA and alcohols could also be produced from the reduction of low molecular-weight VFA when $P_{H_2}$ is high and production of these compounds are energetically feasible (Smith and McCarty, 1989). For instance, butanol could be produced from the utilization of butyrate when the free energy of the reaction is negative. However, minor production of alcohols from mid-strength wastewater did not agree with the above assumption as $P_{H_2}$ was in the same range for both mid- and high-strength wastewaters. In acidogenesis, when the concentration of the low molecular-weight VFA becomes sufficiently high, they result in a collapse of the pH gradient across the membrane and the total inhibition of all metabolic functions in the cell (Gottschalk, 1986). The shift from VFA production to alcohol production in Clostridium acetobutylicum and related species is an adaptive response of the cell to inhibitory effects produced by low molecular-weight VFA (Gottschal and Morris, 1981; Jones and Woods, 1986). The shift appears to be able to act as a detoxification mechanism which allows the cell to avoid the inhibitory effects that would occur when low molecular-weight VFA reach toxic levels. The addition of 600 mg/L acetate or butyrate to batch cultures of Clostridium acetobutylicum resulted in a rapid production of alcohols (Gottschal and Morris, 1981). Similar results have been obtained for Clostridium acetobutylicum and related species (Bahl et al., 1982; Andel et al., 1985; Jones and Woods, 1986). In continuous reactors, addition of butyrate also resulted in a shift from VFA production to alcohol production (Bahl et al., 1982; Martin et al., 1983). It has been reported that alcohol production did not initiate until acetate or butyrate reached a level of 0.4–0.6 g/L (Jones and Woods, 1986). These threshold values are comparable with those shown in Figs. 2(b) and (d). This provides the explanation for the phenomenon that the high production of alcohols was found at the high-strength wastewater and not at the mid-strength wastewater.

No effort was made to identify the microorganisms responsible for the production of high concentration of alcohols. However, the product distribution suggests that the microorganisms, classified as Clostridium acetobutylicum and Clostridium butylicum, might be involved in the alcohol production. A number of species of clostridia have been found for alcohol production (Jones and Woods, 1986). Clostridium butylicum produces alcohols in approximately the same ratio as Clostridium acetobutylicum, but propanol is produced by Clostridium butylicum in place of acetone. Since high concentration of propanol, instead of acetone, was produced in the present study, Clostridium butylicum appeared to be responsible for the production of high concentration of alcohols from the high-strength wastewater.

Table 2 summarizes the specific production rates of acetate, propionate, butyrate, ethanol, propanol and butanol at various levels of wastewater COD. It shows that specific production rates of acetate and butyrate were nearly constant, averaging 0.044 ± 0.005 and 0.013 ± 0.002 g/g-VSS day, respectively. On the other hand, that of propionate was highly dependent on substrate concentration, increasing from 0.010 g/g-VSS day at 2 g-COD/L to 0.056 g/g-VSS day at 30 g-COD/L. The specific production rates of ethanol, propanol and butanol all substantially increased with the substrate concentration.
Gas production. The biogas production rate increased with the wastewater COD. Figure 6 illustrates the partial pressure of the three constituents in biogas, i.e. methane, hydrogen and carbon dioxide, at various levels of wastewater COD. It shows that methane production was about 15% of the biogas at 2 g-COD/L, and became negligible at 8 g-COD/L, or higher. On the other hand, hydrogen production was low at 2-4 g-COD/L, but the $P_{H_2}$ increased to 0.41–0.52 atm in treating wastewater of 12–30 g-COD/L. The increase of $P_{H_2}$, coupled with the decrease of acetate and butyrate (Fig. 5(a)), as well as the increase of propanol and butanol (Fig. 5(b)), suggests the metabolism shift from the hydrogen- and VFA-producing pathways to the alcohol-producing pathways with the increase of substrate concentration. Similar phenomenon was also observed by Bahl et al. (1982), Dabrock et al. (1992), and Ueki et al. (1991).

Sludge yield. Under strict anaerobic conditions, there are no external electron acceptors available to remove the COD from the system, and thus the overall electrons available for reduction remains unchanged. The wastewater COD is transformed to VFAs, alcohols, hydrogen, methane and sludge. Thus, the net sludge yield can be estimated from the COD balance of the system. The COD in yielded sludge should equal to the wastewater COD minus those in VFAs, alcohols, hydrogen and methane, all of which can be accurately measured. This method has been applied to the estimation of sludge yield in many single-phase methanogenic systems (Fang and Chung, 1999; Fang et al., 1994; Kwong and Fang, 1996).

<table>
<thead>
<tr>
<th>Wastewater (g-COD/L)</th>
<th>Acetate (g/g-VSS day)</th>
<th>Propionate (g/g-VSS day)</th>
<th>Butyrate (g/g-VSS day)</th>
<th>Ethanol (g/g-VSS day)</th>
<th>Propanol (g/g-VSS day)</th>
<th>Butanol (g/g-VSS day)</th>
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CONCLUSIONS

Results of batch experiments showed that degradation of protein began only after carbohydrate became depleted. The acidification products were VFAs (mainly acetate, propionate and butyrate), alcohols (mainly ethanol, propanol and butanol) and hydrogen. Productions of hydrogen and the three VFAs corresponded to carbohydrate acidification. The three alcohols, plus $i$-butyrate and higher molecular-weight VFAs, corresponded to protein acidification. In treating high-strength wastewaters...
(8–30 g-COD/L), acetate, butyrate and \( P_{\text{H}_{2}} \) decreased after reaching their peak levels before leveling off. Results of continuous experiments showed that the overall degree of acidification decreased with the increase of wastewater COD, from 57.1% at 2 g-COD/L to 28.8% at 30 g-COD/L; among the constituents in dairy wastewater, 92–99% of carbohydrate, 59–85% of protein and 12–42% of lipid were acidified. High-strength wastewater favored production of hydrogen and alcohols, especially propanol and butanol. The biomass yield was estimated to be 0.258 g-VSS/g-COD.

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