High-rate anaerobic hydrolysis and acidogenesis of sewage sludge in a modified upflow reactor


* Laboratory of Clean Energy, Department of Chemistry, The University of Science and Technology of China, Hefei, Anhui, 230026 China
** School of Environmental Science and Engineering, Tongji University, Shanghai, 200092 China
1 Corresponding author

Abstract
Continuous experiments were conducted to study the hydrolysis and acidogenesis of sewage sludge in an upflow reactor with an agitator and a gas–liquid–solid separator. Results of this study showed that 34–78% of volatile suspended solids (VSS) in sewage sludge was hydrolyzed at pH in the range 4.0–6.5, 35°C and 4–24 hours of hydraulic retention time (HRT). About 31–65% of carbohydrate in sewage sludge, 20–45% of protein and 14–24% of lipid were acidified in this reactor. Hydrogen production was favored in lower pH and HRT, whereas methane production was encouraged at higher pH and HRT. Acetate, propionate, butyrate, and i-butyrate were the main aqueous acidogenic products. The distribution of these compounds in the effluent was more sensitive to pH, but was less sensitive to HRT. The maximum specific COD solubilization rate and specific volatile fatty acids production rate were 126 mg-COD/g-VSS·d and 102 mg-VFA/g-VSS·d, respectively. Compared with a CSTR, this modified upflow reactor was shown to be a more promising biosystem for the hydrolysis and acidogenesis of sewage sludge.

Keywords
Acidogenesis; hydrolysis; sewage sludge; upflow reactor; volatile fatty acids (VFA)

Introduction
In a conventional single-phase anaerobic reactor, hydrolysis, acidogenesis and methanogenesis all take place in one reactor. To maintain a favorable environment for the mixed culture of microorganisms in a single reactor, volatile fatty acids (VFA) production and utilization rates must be balanced. At shorter retention times, VFA production could exceed the utilization, leading to reactor failure (Harper and Pohland, 1986). Because the metabolic characteristics and growth rates of the methanogenic and acetogenic bacteria are different, two-phase anaerobic processes to separate VFA and methane forming phases have been proposed to optimize each phase (Pohland and Ghosh, 1971; Ghosh et al., 1995). Such a process is particularly favored for wastes containing high levels of complex organic particulates, such as sewage sludge. Hydrolysis is usually the limiting step in the degradation of complex organic particulates, whereas methanogenesis is considered rate-limiting for fermentation of soluble substrates. A large amount of research work has been conducted on the acidogenesis of sewage sludge (Eastman and Ferguson, 1981; Henry et al., 1987; Banister and Pretorius, 1998; Banerjee et al., 1999). Better understanding of the acidogenic phase can lead to improvement of reactor stability due to physical separation of acidogenesis and methanogenesis, and to an increase in the production of VFA, which can be used to optimize biological nutrient removal processes (Banister and Pretorius, 1998).

Most of studies on acidogenesis were conducted using a continuously stirred tank reactor (CSTR). Because of its intrinsic structure, the CSTR is unable to maintain high levels of fermentative biomass for hydrolysis and acidogenesis, and its specific acidogenic rate is hardly kept high. In order to keep high-concentration biomass in an acidogenic reactor and accordingly to enhance the acidogenesis efficiency of complex organic particulates, e.g.
sewage sludge, utilization of a modified reactor, i.e. a CSTR with an internal three-phase separator and an agitator, was proposed and tested in this study. On the other hand, the acidogenesis of organic wastes is greatly influenced by waste specificity, reactor configuration, operational parameters such as hydraulic retention time (HRT), solids retention time (SRT), influent organic concentration, organic loading rate, and environmental factors such as pH, temperature, oxidation-reduction potential, nutritional requirements (Eastman and Ferguson, 1981; Henry et al., 1987; Zoetemeyer et al., 1982). HRT is an important variable which can be manipulated. Zhang and Noike (1994) reported that HRT had a considerable effect on the composition of hydrolytic products. Elsfasiniotis and Oldham (1994) demonstrated that HRT had a profound effect on acid-phase anaerobic digestion of primary sludge, and that both VFA production and chemical oxygen demand (COD) solubilization increased significantly with increasing HRT. However, some contradictory results were reported for the effect of HRT. Breure and Andel (1984) claimed that HRT did not have an effect on the product composition from an acidogenic reactor fed with gelatin. Zoetemeyer et al. (1982) also observed that the percent VFA distribution appeared to be independent of HRT in a CSTR with glucose as the sole carbon source.

Studies of the pH effect on acidogenesis were mainly carried out 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 downstream. 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. However, wastes such as sewage contain high levels of not just carbohydrates, but also proteins and lipids. Hydrolysis and fermentation of complex colloidal particulates 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. Therefore, this study was conducted to investigate the effect of HRT and pH on the acidogenesis of sewage sludge in a modified reactor.

Materials and methods

The plexglass-made reactor had a working volume of 3.5 L with an internal diameter of 100 mm and a height of 560 mm. This volume, excluding the liquid in the gas–liquid–solid separator, was used to determine biomass concentration and HRT. Four evenly distributed sampling ports were installed over the height of the column. Under the cap of the reactor was a gas–liquid–solid separator with an internal diameter of 125 mm and a height of 260 mm making a filled volume of 2.9 L. A magnetic agitator was provided. The reactor was placed in a temperature-controlled wooden box and operated at a constant temperature of 35°C.

The inoculum was obtained from the secondary settling tank in a local sewage treatment plant. The inoculum was acclimated with glucose (5 g-COD/L) in a 4-L CSTR. In order to wash out the methanogens, the CSTR was operated by feeding glucose at 20–25°C, pH 5.5 and 12 h of HRT over 40 days. Near the end, VFA production became steady and no methane was detected in the biogas. The upflow reactor was then seeded with this enriched acidogenic inoculum equivalent to 32.4 g of volatile suspended solids (VSS).

Six series of experimental runs were conducted, each one showing the effect of HRT and pH. The sludge obtained from a local sewage treatment plant was screened through an 0.8-cm screen and adjusted to a value of 5,000 mg/L total solids (TS), either by diluting with distilled water or by settling and decanting to ensure uniform feed characteristics for the entire experimental program. The pH of the mixed liquor in the reactor was adjusted by using 2N NaOH and 2N HCl solutions. In Series I, the HRT was decreased stepwise from
24 h to 4 h while keeping substrate concentration and pH constant at around 5,000 mg-VSS/L and pH 5.5, respectively; in Series II, the pH of the mixed liquor was lowered step-wise from 6.5 to 4.0 while keeping substrate concentration and HRT at 5,000 mg-VSS/L and 12 h, respectively. Each run lasted over five to six weeks to ensure the reactor reaching steady-state before changing to the next condition. The amount of biogas produced was recorded daily using the water replacement method. During each run, gas production and composition were recorded daily, and all other parameters were measured three times weekly. Only those obtained under steady-state conditions are reported.

The contents in biogas and effluent were analyzed following previously established procedures (Yu and Fang, 2001). The detectable level was 1 mg/L for individual VFA. Total organic carbon (TOC) was measured by using a TOC analyzer (Shimadzu TOC-5000). 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, NH₃-N, and VSS were performed according to the Standard Methods (APHA, 1992).

Results and discussion

Effect of HRT

Figure 1 illustrates the effect of HRT on: (a) VSS reduction efficiency; (b) net TOC concentration; and (c) net VFA concentration, whereas Figure 2 illustrates: (a) degradations of carbohydrate, protein and lipid; (b) concentration of acetate, propionate, butyrate and i-butyrate; and (c) partial pressures of H₂, CH₄ and CO₂ at various HRTs.

Figure 1a shows that the VSS reduction increased from 42% at 4 h to 75% at 16 h; a further increase of HRT resulted in a slight increase of VSS reduction to 78% at 24 h. The high
percent VSS reduction demonstrates that the particulate substrate in sewage sludge was readily solubilized in this acidogenic reactor. Since the particulates were converted to soluble products, the amount of particulate organic carbon solubilization could be estimated from the net filtered TOC concentration (effluent TOC minus influent TOC). Results in Figure 1b illustrate that the net TOC concentration increased with the increasing HRT, from 280 mg/L at 4 h to 406 mg/L at 16 h; a further increase of HRT to 24 h resulted in a slight increase of net TOC to 417 mg/L. A similar effect on the net VFA concentration was found, as illustrated in Figure 1c. Results in Figure 1 suggest that 16 h of HRT should be chosen for the acidogenesis of sewage sludge in this acidogenic reactor, since increase in HRT from 16 h to 24 h had no significant effect on the acidogenesis.

Figure 2a illustrates that degradations of carbohydrate, protein and lipid all increased with HRT. Among them, carbohydrate was most easily degradable, over 32% was degraded at an HRT as low as 4 h. At 24 h, about 63% carbohydrate was degraded. Figure 2a also illustrates that degradations of protein and lipid increased, respectively, from 27% and 15% at 4 h of HRT to 40% and 24% 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. McNerney (1988) reported that carbohydrates could suppress the synthesis of exopeptidases, a group of enzymes facilitating protein hydrolysis. In degradation, lipid is first hydrolyzed to long-chain fatty acids, which are further degraded via \( \beta \)-oxidation producing hydrogen as a byproduct. That means lipid degradation is suppressed by the increase of hydrogen pressure.

Acetate, propionate, butyrate and i-butyrate were the main products from the acidogenesis of sewage sludge. As shown in Figure 2b, the distribution of main VFA in the effluent was not significantly influenced by the variation of HRT. At 4 h, the acidification products were composed of 32% acetate, 25% propionate, 18% butyrate, 9% i-butyrate, plus 16% of other metabolites. At 24 h, acetate, propionate, butyrate and i-butyrate were 34%, 29%, 19% and 10%, and 8% of other metabolites, respectively, of the total VFA. Formate, lactate, valerate, i-valerate, and caproate were present in the effluent from this acidogenic reactor, but in smaller quantities.

In the acidogenic reactor, the biogas is mostly composed of the acidogenic by-products, carbon dioxide, hydrogen and methane. Figure 2c illustrates that at 4 h, the partial pressures of hydrogen and methane were 32 and 15 kPa, respectively. Figure 2c 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, there were only 8 kPa of hydrogen whereas methane was increased to 35 kPa.

**Effect of pH**

Figure 3 illustrates the effect of pH on: (a) VSS reduction efficiency; (b) net TOC concentration; and (c) net VFA concentration, whereas Figure 4 illustrates: (a) degradations of carbohydrate, protein and lipid; (b) concentration of acetate, propionate, butyrate and i-butyrate in the effluent; and (c) partial pressures of \( \text{H}_2 \), \( \text{CH}_4 \) and \( \text{CO}_2 \) in the biogas at various pH values.

Figure 3a illustrates that VSS was reduced with an efficiency exceeding 60% at pH 5.0, or higher; after pH was lowered to 4.5, the VSS reduction decreased to 48%; a further decrease of pH to 4.0 resulted in a significant decrease of VSS reduction to 34%. This indicates that the VSS reduction was sensitive to pH at low levels. The responses of net TOC and VFA productions to changes of pH are similar to that obtained for the VSS reduction, as shown in Figures 3b and 3c.

As illustrated in Figure 4a, the degradations of carbohydrate, protein and lipid were also
significantly influenced by variation of pH. At pH 4.0, 31%, 20% and 13% of carbohydrate, protein and lipid were degraded. Degradation of carbohydrate increased to 50% at pH 5.5, reaching 65% at pH 6.5. Degradation efficiency of carbohydrate was pH sensitive at pH less than 5.5; this is consistent with a previous finding that fermentation of lactose was mainly regulated by pH, and was independent of HRT and lactose concentration (Fang and Yu, 2001). 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 lower degrees of degradation at any given pH, but the variation with pH had a trend similar to those of carbohydrate and protein below pH 6.0. However, the lipid degradation at pH 6.5 was 22%, slightly lower than 24% at pH 6.0.

The relative amount of individual products was strongly dependent on pH. At pH 4.0, the effluent products were composed of 23% acetate, 42% propionate, 10% butyrate, and 7% i-butyrate. These four products represented 88% of the total VFA at pH 5.0, and 90% at pH 6.5. Other VFA included formate, valerate, i-valerate, and caproate. 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 (McInerney, 1988); acidogenesis of non-proteinaceous substrates produced little of these three VFA (Zoetmeyer et al., 1982). Acetate, butyrate, and i-butyrate all increased with pH, whereas production of propionate was depressed by low pH levels, also propionate production decreased with the increase of pH, as reported by many others, from 42% at pH 4.0 to 18% at pH 6.5. In contrast, the fractions of acetate and butyrate in effluent products both decreased with pH, from 37% and 19% at pH 6.5, to 23% and 10%, respectively, at pH 4.0. These results clearly show that 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.

Figure 4c shows that the variation of pH also had a substantial effect on the production of biogas. At pH 4.0 and 4.5, the hydrogen partial pressures were 43 and 29 kPa, respectively, and there was no detectable methane in the biogas. Figure 4c also illustrates that the hydrogen partial pressure decreased, along with the increase of methane, as pH increased. At pH 6.5, the methane partial pressure was 49 kPa, and the biogas became free of hydrogen. In the acidogenesis of sewage sludge, hydrogen production was favoured in lower pH, whereas methane production was encouraged at higher pH.

**Evaluation of the modified upflow reactor**

The above experimental results demonstrated that the modified upflow reactor used in this study was of high-efficiency for the hydrolysis and acidogenesis of sewage sludge. Table 1 lists the specific COD solubilization rate and specific VFA production rate of this study with those found in the literature. In the other studies, CSTRs were employed hydrolysis and acidogenesis of sewage sludge. The maximum specific COD solubilization rate of 126 mg-COD/g-VSS·d obtained in this study was greater than those reported. In addition, the maximum specific VFA production rate of 102 mg-VFA/g-VSS·d observed in this study, was also considerably higher than those of other studies. Because of its intrinsic structure, especially the installation of a gas–solids–liquid separator, this upflow reactor retained a high level of biomass. Hence, because of its higher concentration of biomass, the upflow reactor possessed much higher capacity for hydrolysis and acidogenesis than the CSTRs. Considering the above results and its stable operation for at least 49 weeks, it might be concluded that this upflow reactor is a more promising biosystem for the hydrolysis and acidogenesis of sewage sludge than CSTR.

**Conclusions**

Results of this study showed that 34–78% of VSS in sewage sludge was hydrolyzed at pH ranging 4.0–6.5, 35°C and 4–24 hours of hydraulic retention. About 31–65% of carbohydrate in sewage sludge, 20–45% of protein and 14–24% of lipid were acidified in this reactor. Hydrogen production was favoured in lower pH and HRT, whereas methane production was encouraged at higher pH and HRT. Acetate, propionate, butyrate, and i-butyrate were the main aqueous acidogenic products. The distribution of these compounds in the effluent was more sensitive to pH, but was less sensitive to HRT. The maximum specific COD solubilization rate and specific VFA production rate were 126 mg-COD/g-VSS·d and 102 mg-VFA/g-VSS·d, respectively. Compared with a CSTR, this modified upflow reactor was shown to be a more promising biosystem for the hydrolysis and acidogenesis of sewage sludge.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Maximum COD solubilization rates (mg-COD/g-VSS·d)</th>
<th>Maximum specific VFA production rate (mg-VFA/g-VSS·d)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upflow reactor</td>
<td>126</td>
<td>102</td>
<td>This study</td>
</tr>
<tr>
<td>CSTR</td>
<td>43</td>
<td>36</td>
<td>Eastman and Ferguson, 1981</td>
</tr>
<tr>
<td>CSTR</td>
<td>53</td>
<td>40</td>
<td>Henry et al., 1987</td>
</tr>
<tr>
<td>CSTR</td>
<td>/</td>
<td>45</td>
<td>Ghosh et al., 1995</td>
</tr>
<tr>
<td>CSTR</td>
<td>70</td>
<td>62</td>
<td>Banister and Pretorius, 1998</td>
</tr>
<tr>
<td>CSTR</td>
<td>73</td>
<td>57</td>
<td>Banerjee et al., 1999</td>
</tr>
</tbody>
</table>
Acknowledgements
The authors wish to thank the Natural Science Foundation of China (Grant No. NSFC-GM 20122203) and the Ministry of Science and Technology, China (Grant No. 2001AA515050) for the financial support of this study.

References