Control of C/N ratio for butyric acid production from textile wastewater sludge by anaerobic digestion

Bo Fu, Jingjing Zhang, Jinfeng Fan, Jin Wang and He Liu

ABSTRACT

Increasing textile wastewaters and their biotreatment byproduct–waste activated sludge are serious pollution problems. Butyric acid production from textile wastewater sludge by anaerobic digestion at different C/N ratios was investigated. Adding starch to textile wastewater sludge with a C/N ratio of 30 increased the butyric acid concentration and percentage accounting for total volatile fatty acids (TVFAs) to 21.42 g/L and 81.5%, respectively, as compared with 21.42 g/L and 10.6% of textile wastewater sludge alone. The maximum butyric acid yield (0.45 g/g VS), conversion rate (0.74 g/g VSdigest) and production rate (2.25 g/L d) was achieved at a C/N ratio of 30. The biological toxicity of textile wastewater sludge also significantly decreased after the anaerobic digestion. The study indicated that the anaerobic co-digestion of textile wastewater sludge and carbohydrate-rich waste with appropriate C/N ratio is possible for butyric acid production.

Key words | anaerobic digestion, butyric acid production, C/N ratio, textile wastewater sludge

INTRODUCTION

When treating the textile wastewaters, the disposal of textile sludge is a problem of growing importance. This is not only because of the colour, but also because many dyes from wastewater or sludge and their breakdown products are toxic and/or mutagenic to life (Dos Santos et al. 2007). Anaerobic digestion plays an important role in sludge disposal for their abilities to further transform organic matter, and also reduces the amount of final sludge solids for disposal. It is thought that the anaerobic digestion process consists of hydrolysis, acidogenesis, acetogenesis and methanogenesis (Appels et al. 2008). Butyric acid is one of the key intermediates in the course of the anaerobic digestion, and also a high-value product that can be used as a raw material for food additives, plastic material and pharmaceutical industries (Zigová & Šturdík 2000). Therefore, a practical method for the selective production of butyric acid from textile wastewater sludge controlling the operating parameters is strongly desired.

It was reported that the accumulation of acidogenic bacteria in the anaerobic system was influenced by many operating parameters, especially pH, resulting in different types of anaerobic digestion (Horiuchi et al. 2002; Yang et al. 2005). Our previous study indicated that the initial C/N ratio had significant effects on the distribution pattern of VFAs produced from sewage sludge and a high C/N ratio lead to the enhancement of butyric acid yield (Liu et al. 2008a). Given that the anaerobic co-digestion of textile wastewater sludge and carbohydrate-rich wastes for butyric acid production may simultaneously provide economic and environmental benefits, the anaerobic digestion of textile sludge and starch at different C/N ratios was investigated. The biological toxicity after anaerobic digestion was also studied.

METHODS

Seed sludge

Seed sludge was collected from an anaerobic digester of Lucun municipal sewage treatment plant (Wuxi, China). The characteristics of seed sludge were described elsewhere (Liu et al. 2008a). In order to kill the non-spore-forming methanogens, the sludge was concentrated by setting for about 24 h at room temperature, and then boiled for 2 h at 100 °C (Oh et al. 2005; Park et al. 2005).
Substrate sludge and pretreatment

Substrate sludge was collected from the textile wastewater treatment plant of Wuxi Pacific Group Co., LTD. To increase the accumulation of VFAs at the subsequent acidogenic stage, the sludge was pretreated by thermo-alkaline methods according to Liu et al. (2008b). The characteristics of the pretreated sludge are shown in Table 1.

Anaerobic digestion

One litre conical flasks with working volume of 500 mL were used for sludge anaerobic digestion. The pretreated sludge was inoculated with seed sludge of 10 g VS/L. The soluble starch was added to the pretreated sludge, resulting in six different C/N ratios of 3.2 (without starch), 20, 30, 40, 50 and 60. According to the reported results (Liu et al. 2004), the initial pH of all samples was adjusted to 10.0. Oxygen in the flasks was removed from the headspace by nitrogen gas sparging for 1 min. The flasks were immediately capped with rubber stoppers and placed in an air-tight shaker (120 rpm) at 35 ± 1 °C for 25 days of incubation. All experiments were done in triplicate.

Control experiment design

In order to investigate whether the butyric acid was converted only from starch or not, a control experiment was carried out. Experiment 1 used the pretreated sludge as the substrate with C/N ratio of 3.2; experiment 2 used the pretreated sludge and starch solution as the mixed substrate with C/N ratio of 30; experiment 3 used the starch solution as the only substrate. The TN amount of experiment 1 was the same as that of experiment 2, and the TOC amount of experiments 2 and 3 were same. The operation conditions of the control experiment were similar with the aforementioned anaerobic digestion experiment. All experiments were set up in triplicate.

Biological toxicity test

Euglena gracilis purchased from Research Center for Analysis & Measurement of Fudan University (Shanghai, China) was used for biological toxicity test. The cells were harvested by centrifugation, and inoculated into the diluted supernate obtained from sludge sterilization at 121 °C. Distilled water was used as the control. The inoculation was performed at 25 °C for 72–96 h with light/dark ratio of 12:12. The cell death rate was determined with a phase-contrast microscope. The chlorophyll content was measured by the method of Vernon (1960).

Analytical methods

Total solids (TS) and volatile solids (VS) were determined according to APHA (1998). The concentrations of VFAs were measured as in Liu et al. (2008a). The total VFA (TVFA) concentration was calculated by summing up each individual VFA. VS0 refers to the VS before the fermentation, while VS1 indicated the VS after the fermentation. The yield and conversion rate of butyric acid and TVFAs were calculated as follows:

\[
\text{TVFA conversion rate} = \frac{\text{TVFA}}{\text{VS}_0 - \text{VS}_1} \quad (\text{g} / \text{g VS}_{\text{digest}})
\]

\[
\text{Butyric acid conversion rate} = \frac{\text{IBu}}{\text{VS}_0 - \text{VS}_1} \quad (\text{g} / \text{g VS}_{\text{digest}})
\]

\[
\text{TVFA yield} = \frac{\text{TVFA}}{\text{VS}_0} \quad (\text{g} / \text{g VS})
\]

\[
\text{Butyric acid yield} = \frac{\text{IBu}}{\text{VS}_0} \quad (\text{g} / \text{g VS})
\]

Total carbon values were determined with a solids module of a liquid TOC analyzer (Elementar Analysensysteme GmbH, Germany).

Table 1 | Characteristics of the textile wastewater sludge

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Substrate sludge</th>
<th>Pretreated sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (TS, wt%)</td>
<td>5.32</td>
<td>3.25</td>
</tr>
<tr>
<td>Volatile solids (VS, wt%)</td>
<td>5.01</td>
<td>2.01</td>
</tr>
<tr>
<td>Total proteins (g/g VS%)</td>
<td>39.31</td>
<td>32.60</td>
</tr>
<tr>
<td>Total carbohydrates (g/g VS%)</td>
<td>2.20</td>
<td>1.34</td>
</tr>
<tr>
<td>Total lipids (g/g VS%)</td>
<td>2.89</td>
<td>2.41</td>
</tr>
<tr>
<td>NH₄⁺–N (mg/L)</td>
<td>37.68</td>
<td>NT</td>
</tr>
<tr>
<td>Total nitrogen (TN, mg/L)</td>
<td>552.60</td>
<td>561.28</td>
</tr>
<tr>
<td>Total phosphorus (TP, mg/L)</td>
<td>52.20</td>
<td>NT</td>
</tr>
<tr>
<td>SCOD (mg/L)</td>
<td>1,447.00</td>
<td>17,790.40</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>1,412.10</td>
<td>1,525.64</td>
</tr>
<tr>
<td>pH</td>
<td>7.42</td>
<td>12.00</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>3.10</td>
<td>3.20</td>
</tr>
</tbody>
</table>

NT: Not tested.
RESULTS AND DISCUSSION

VS degradation

Figure 1 shows the VS degradation rates of sludge anaerobic digestion with different C/N ratios. The VS degradation rates were 49.9, 61.1 and 61.7% for the C/N ratios of 3.2, 20 and 30, respectively, and they decreased from 50.2 through 47.5 to 38.6% as C/N ratios increased from 40 through 50 to 60. The results showed an optimum C/N ratio of 20 or 30 for maximum VS degradation in the sludge anaerobic digestion. Yen & Brune (2007) reported an optimum C/N ratio for co-digestion of algal sludge and waste paper was in the range of 20–25. In this study, the gradual decrease in VS degradation with increasing C/N ratios may be due to the inhibition caused by a secondary metabolite produced in quantities that could be toxic or inhibitory at a certain concentration.

VFA production and composition

The detectable individual VFAs included acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid and valeric acid. The distribution patterns of VFAs at different C/N ratios are illustrated in Figure 2. As shown in Figure 2(a), the TVFA concentrations increased from 4.69 through 17.73, 26.23, 31.96, 37.06 to 38.04 g/L as C/N ratios increased from 3.2 through 20, 30, 40, and 50 to 60. Yen & Brune (2007) also found that anaerobic co-digestion of algal sludge and waste paper with higher C/N ratios exhibited higher TVFA production.

The acetic acid concentrations in the fermentation products with C/N ratios of 3.2, 20, 30 and 40 accelerated initially and then decreased to a relatively steady level of 2.03, 6.16, 2.64 and 3.56 g/L, respectively (Figure 2(b)). The acetic acid of C/N = 50 and 60, which had been increasing significantly within the initial 3 days, also decreased in the following days, but gradually increased to about 8 g/L (Figure 2(b)). Due to the hydrolysis, acidogenesis and acetogenesis, the acetic acid accumulated earlier than the other VFAs, but decreased afterwards as a result of the consumption by methanogens. For the C/N ratio of 50 and 60, the acetic acid amount increased during the final period. The reason might be attributed to the formation of acetic acid from other organic acids and alcohols by acetogens or from CO₂ and H₂ by homoacetogenic bacteria (Batstone et al. 2003; Ni et al. 2011).

The propionic acid concentrations were relatively stable and low throughout the study period, with average values of 0.49, 1.43 and 1.99 g/L for C/N ratios of 3.2, 30 and 40, respectively (Figure 2(c)). However, for C/N ratios of 20, 50 and 60, the low propionic acid yield was significantly enhanced to 4.42, 9.27 and 9.75 g/L at the end. Variations of the butyric acid were similar for the C/N ratios ranged from 20 to 60, which kept low initially, but gradually increased after 8–16 days of fermentation, with the average concentrations reached of 21.42, 25.57, 19.40 and 18.50 g/L at C/N ratios of 30, 40, 50 and 60, respectively (Figure 2(d)). The above results show that propionic acid and butyric acid yields for C/N ratio ≥20 gradually increased after 8–16 days of fermentation, and then remained relatively stable. This phenomenon was related to the slower hydrolysis resulting from an increase in C/N ratio caused by the increase in the starch additions. The hydrolysis is considered as rate limiting, and therefore controls the acidogenesis step forming VFA (e.g. propionic acid and butyric acid) as products (Appels et al. 2008).

The percentages of individual VFA fractions at different C/N ratios are shown in Figure 3. The butyric acid fraction increased from 10.6 through 18.5 to 81.5% with C/N ratio increasing from 3.2 through 20 to 30, but decreased to 77.1, 51.9 and 48.3% for C/N ratios of 40, 50 and 60, respectively. The variation trend of the acetic acid fraction was contrary to that of butyric acid. Compared with acetic acid and butyric acid, the percentage of valeric acid was relatively small. Butyric acid dominated in the fermentation products with C/N ratio ≥30 (Figure 3). Elefniotis et al. (2005) also indicated that the increase of butyric acid production was mainly attributed to the increase in the amount of carbohydrate (starch) during the co-digestion of starch-rich industrial wastewater and municipal wastewater. According
to the study of Liu et al. (2008a), a high C/N ratio ranging from 5 to 30 enhanced butyric acid production from soybean protein isolate and maize starch. However, the distribution patterns of VFAs produced from textile sludge and starch were different from those of the aforementioned substrate despite the similar C/N ratios. This might be because of the difference in organic composition between the two kinds of substrates. The organic matter in textile sludge was mainly in the form of proteins from lysed cells (Table 1).

![Figure 2](image_url)  
**Figure 2** | VFAs yield profiles at different C/N ratios, (a) TVFAs; (b) acetic acid; (c) propionic acid; (d) butyric acid, □ C/N = 3.2; ● C/N = 20; Δ C/N = 30; ◆ C/N = 40; ○ C/N = 50; ▼ C/N = 60.

![Figure 3](image_url)  
**Figure 3** | The percentages of individual VFAs accounting for TVFAs at different C/N ratios. HAc: acetic acid; HPr: propionic acid; Bu: iso-butyric acid; HBu: butyric acid; Iva: iso-valeric acid; HVa: valeric acid.

<table>
<thead>
<tr>
<th>C/N</th>
<th>TVFA yield (g/g VS)</th>
<th>TVFA conversion rate (g/g VS&lt;sub&gt;digest&lt;/sub&gt;)</th>
<th>TVFA production rate (g/L d)</th>
<th>Butyric acid yield (g/g VS)</th>
<th>Butyric acid conversion rate (g/g VS&lt;sub&gt;digest&lt;/sub&gt;)</th>
<th>Butyric acid production rate (g/L d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>0.45</td>
<td>0.58</td>
<td>0.34</td>
<td>0.11</td>
<td>0.14</td>
<td>1.57</td>
</tr>
<tr>
<td>20</td>
<td>0.46</td>
<td>0.75</td>
<td>0.50</td>
<td>0.12</td>
<td>0.20</td>
<td>2.00</td>
</tr>
<tr>
<td>30</td>
<td>0.49</td>
<td>0.78</td>
<td>1.95</td>
<td>0.45</td>
<td>0.74</td>
<td>2.25</td>
</tr>
<tr>
<td>40</td>
<td>0.40</td>
<td>0.81</td>
<td>1.66</td>
<td>0.36</td>
<td>0.74</td>
<td>2.02</td>
</tr>
<tr>
<td>50</td>
<td>0.31</td>
<td>0.64</td>
<td>0.85</td>
<td>0.18</td>
<td>0.37</td>
<td>1.95</td>
</tr>
<tr>
<td>60</td>
<td>0.22</td>
<td>0.58</td>
<td>0.91</td>
<td>0.12</td>
<td>0.31</td>
<td>1.82</td>
</tr>
</tbody>
</table>
The explanations for the VFA distribution varying with C/N ratio were given from two aspects. One possible explanation is the presence and structure of different acid-producing microbial communities influenced by C/N ratio. In the present study, a high C/N ratio means carbohydrate-rich substrate, which benefits the butyric-producing bacteria and resulted in their dominance in the microbial population (Bouallagui et al. 2004). The other explanation may be a shift in the metabolic pathway of the acid-producing microorganism (Yu & Fang 2001).

The yield, conversion rate and production rate of butyric acid and TVFAs at C/N ratio of 30 and 40 was higher than those of the other C/N ratios, and the maximum butyric acid yield, conversion rate and production rate was achieved at C/N ratio of 30 (Table 2). The butyric acid yield and conversion rate of C/N = 30 reached 0.45 g/g VS and 0.74 g/g VS_{digest}, respectively, while those of C/N = 3.2 were 0.11 g/g VS and 0.14 g/g VS_{digest}, respectively. This result reveals that a C/N ratio of 30 was optimum for butyric acid production from textile wastewater sludge by anaerobic digestion.

**Biogas composition**

The biogas production increased with the fermentation time, and stayed relatively stable during the final period. Biogas yield after 25 days of fermentation reached 1,689 ± 114.55 mL at a C/N ratio of 40, and 1,413 ± 91.22 mL at a C/N ratio of 30 (Figure 4(a)). The percentage of H_{2} was much higher than that of CH_{4} (Figure 4(b)), indicating the heating method could not inhibit the methane production completely but was reduced very effectively. H_{2} accounted for 68.5 and 59.3% of the biogas at C/N ratios of 30 and 40, respectively. It was reported that carbohydrate (glucose) fermentation was accompanied by high hydrogen yield (Liu & Fang 2002). The biogas yield and the percentage of H_{2} were positively correlated with the butyric acid yield in this study. It is indicated that C/N ratio could induce a metabolic flow to the pyruvic acid pathway of glycolysis, which was also found in hydrogen and butyric acid production from food waste (Han & Shin 2004).

**The results of the control experiment**

As shown in Table 3, co-digestion of textile sludge and starch resulting in C/N ratio of 30 led to more significant increases in the butyric acid yield, conversion rate and production rate than were observed in starch digestion alone. The reason was that the textile sludge supplied a good source of organic nitrogen and other nutrients such as iron for cell growth (Yen & Brune 2007; Romano & Zhang 2008). The control experiment confirmed that it is available to control C/N.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>TVFA yield (g/g VS)</th>
<th>TVFA conversion rate (g/g VS_{digest})</th>
<th>TVFA production rate (g/L d)</th>
<th>Butyric acid yield (g/g VS)</th>
<th>Butyric acid conversion rate (g/g VS_{digest})</th>
<th>Butyric acid production rate (g/L d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.45</td>
<td>0.58</td>
<td>1.58</td>
<td>0.11</td>
<td>0.14</td>
<td>0.34</td>
</tr>
<tr>
<td>2</td>
<td>0.49</td>
<td>0.78</td>
<td>2.25</td>
<td>0.45</td>
<td>0.74</td>
<td>1.95</td>
</tr>
<tr>
<td>3</td>
<td>0.45</td>
<td>0.63</td>
<td>1.65</td>
<td>0.35</td>
<td>0.50</td>
<td>1.07</td>
</tr>
</tbody>
</table>

**Figure 4** | Biogas yield (a) and percentage of H_{2} and CH_{4} (b) at different C/N ratios.
ratio by adding starch for butyric acid production from anaerobic digestion of textile wastewater sludge. Therefore, the anaerobic co-digestion of textile wastewater sludge and carbohydrate-rich wastes (food wastes, kitchen wastes, etc.) with appropriate C/N ratio is possible for butyric acid production, which might be very attractive for future applications.

Biological toxicity

The biological toxic effect of textile sludge on algae growth is shown in Figure 5. The chlorophyll content of the control was 52.1 mg/L. The sludge supernate with volume concentration more than 11.1% (v/v) resulted in the nearly complete inhibition of the algal growth (Figure 5(a)). When the sludge concentration was lower than 1.5% (v/v), almost all the algal cells survived (Figure 5(a)). The higher chlorophyll content of sludge supernate than the control might be attributed to the organic substrate from sludge supernate for cell growing. Therefore, 3 and 6% (v/v) were accepted as appropriate concentrations for the algae growth inhibition test.

For untreated sludge, the cell death rate after anaerobic digestion changed from 25% to 0% and from 95% to 50% for 3 and 6% (v/v), respectively (Figure 5(b)). Meanwhile, for pre-treated sludge, the values changed from 15% to 0% and from 90% to 70% (Figure 5(b)). The results indicated that the biological toxicity of textile wastewater sludge significantly decreased after the anaerobic digestion. It was suggested that the toxic compounds were degraded or adsorbed by the microorganisms or their extracellular products during the anaerobic process.

CONCLUSIONS

Controlling C/N ratio to 30 by adding starch was optimum for butyric acid production from textile wastewater sludge by anaerobic digestion. The maximum butyric acid yield (0.43 g/g VS), conversion rate (0.74 g/g VS_{digested}) and production rate (2.25 g/L d) were achieved at a C/N ratio of 30. The butyric acid concentration and percentage accounting for TVFAs with C/N of 30 reached 21.42 g/L and 81.5%, respectively. The biological toxicity of textile wastewater sludge also significantly decreased after the anaerobic digestion.

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