Research Article

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Potential of anaerobic co-digestion of acidic fruit processing waste and waste-activated sludge for biogas production

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Abstract: The potential of acidic fruit processing waste (FPW) and waste-activated sludge (WAS) co-digestion for methane production was investigated using batch and continuous experiments. First, batch experiments showed the co-digestion of FPW and WAS solved acid accumulation and increased cumulative biogas production. When the volatile solid (VS) ratio of FPW to WAS was 2:1, the cumulative biogas production was the highest (4,695.47 mL), which increased by 5.2% and 10.5% compared with the VS ratios of 3:1 and 1:1, respectively. Methane production was the limiting step when the FPW percentage was high, and hydrolysis was the rate-limiting step when the WAS percentage was high. Second, the continuous experiments showed fermentation was the most stable when the VS ratio was 2:1, without acid accumulation or excessive total alkalinity consumption. Additionally, the ammonia nitrogen content in the system was above 138.34 mg L\(^{-1}\), which solved the slow WAS hydrolysis rate and low nitrogen supply efficiency. Microbial community structure of the reactor was correlated with substrate composition greatly. On the 180th day, the relative abundance levels of Bacteroides, Paludibacter, Litorilinaea, Levilinea, and Smithella were higher than those on the 120th day and the 240th day. The enrichment of those bacterial groups was beneficial to improve the substrate hydrolysis rate and reduce the influence of organic acids on the anaerobic system.

Keywords: acidic fruit processing waste, waste-activated sludge, anaerobic co-digestion, kinetic parameters, microbial community dynamics

1 Introduction

Apples are in high demand worldwide because of their desirable taste, nutritional value, and unique properties [1]. Globally, almost 54.2 million tons of apples are produced yearly [2], of which more than 50% are produced in China. Most of the apples are eaten as fresh fruits, and 25–30% of apples are converted into processed products, especially concentrated apple juice which is the most important product [3]. There are mainly two kinds of solid wastes generated from apple juice production. One is fruit processing waste (FPW), which is direct solid waste such as rotten apples and apple pomace generated in apple cleaning, screening, crushing, and other steps. The other is waste-activated sludge (WAS), which is indirect solid waste generated during the aerobic treatment of apple juice wastewater [4,5].

Both FPW and WAS have high fermentation characteristics and biodegradability [6,7]. If discarded or landfilled, they can cause environmental problems such as greenhouse gas emissions, landfill leachate emissions, pathogen transport, and groundwater pollution [8]. In recent years, there have been many studies on the recycling of WAS and FPW. WAS is rich in fructose, glucose, protein, and organic acids [9], and is often used for feed [10], pectin recovery [11,12], and enzyme [13] and phenolic [14] extraction. There are also a few studies on the biomethanation of apple waste, but only the fruit seeds and peels screened from wastewater [15] or the residues
from apple juice ultrafiltration are used as raw materials [9]. Anaerobic digestion of rotten apples produced during the washing and sorting stages is rarely studied. FPW is typically treated by incineration, landfills, composting [16,17], and anaerobic digestion [18–20]. In particular, the anaerobic digestion of FPW is well studied and is the best treatment method for sludge reduction. The methane produced therefrom is a clean energy source and can replace some fossil fuels for power generation, boiler combustion, etc.

Biogas preparation by anaerobic digestion of FPW or WAS alone still faces many challenges. When FPW is used as a single fermentation substance, its high carbon-nitrogen ratio may make the digestive system unbalanced, which will decrease microbial activity, resulting in microbial cell dysfunction and process deterioration [21] that are not conducive to the digestion progress. In addition, FPW has a high organic acid content and low pH, but the optimum pH for methanogens is neutral [22]. Too low of a pH can subject the anaerobic digestion reactor to inhibition or failure. Consequently, NaOH or Ca(OH)₂ is often added as a buffer, but this method is only suitable for laboratory research and will raise the cost in large-scale industrial operations, and excessive Na⁺ and Ca²⁺ will inhibit methanogens [23]. When WAS is used as the single fermentation material, first, its low carbon-nitrogen ratio is also unfavorable for the digestion process. Second, hydrolytic acidification becomes the rate-limiting step since sludge contains intractable exopolymer materials and biomass cell envelopes [24]. This unfavorable condition results in low digestion efficiency, with specific methane yields of 150–190 L·g⁻¹ volatile solid (VS). For this reason, thermal pretreatment to improve sludge degradation [25] or alkaline pretreatment to improve sludge solubility and facilitate hydrolytic acidification [26,27] is usually needed. However, this approach increases cost and leads to Maillard reactions that inhibit methanogens [28]. Given the properties of the aforementioned two substrates, co-digestion of FPW and WAS may be able to avoid acidification, and improve the efficiency of WAS hydrolysis and acidification, thereby achieving efficient and stable anaerobic fermentation. Although Fonoll [29] studied the feasibility of co-digestion of sewage sludge and fruit wastes (peach, banana, or apple), there were no particular studies on the optimal condition, fermentation mechanism, and microbial community structure for co-digestion of FPW and WAS.

In this study, the potential of FPW and WAS co-digestion to produce biogas was evaluated using two fermentation modes, including batch experiments and continuous experiments. The effects of VS ratios on biogas, methane content, pH, NH₄⁻N, and total alkalinity (TA) during the fermentation process were analyzed. Additionally, the experimental data obtained at different VS ratios that yielded better biogas production were kinetically tested using Monod and first-order dynamics models. The changes in microbial community structure during continuous experiments were also analyzed.

## 2 Materials and methods

### 2.1 Feedstock and seeding sludge

FPW (rotten apple and apple pomace) and WAS were collected from Haisheng Fresh Fruit Juice Co. (Shaanxi province, China). FPW was shredded in a mashing device into small particles with a diameter of less than 2 mm. The detailed properties of FPW and WAS are presented in Table 1.

The seeding sludge from a working pilot-scale mesophilic continuous stirred tank reactor (CSTR) for FPW digestion was used as the inoculum. The VS concentration and pH of the inoculum were 30.8 g·L⁻¹ and 7.24, respectively. Before the experiment, starvation treatment was performed for a week, and no biogas production was observed. Hence, the inoculum can be used for anaerobic fermentation.

### Table 1: Physicochemical characterization of FPW and WAS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FPW</th>
<th>WAS</th>
<th>Parameter</th>
<th>FPW</th>
<th>WAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.2 ± 0.2</td>
<td>7.27 ± 0.3</td>
<td>C/N ratio</td>
<td>51.2 ± 0.6</td>
<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>Total solids (g·L⁻¹)</td>
<td>150.1 ± 2.2</td>
<td>83.84 ± 0.9</td>
<td>TP (mg·g⁻¹TS)</td>
<td>12.05 ± 0.3</td>
<td>9.79 ± 0.1</td>
</tr>
<tr>
<td>Volatile solids (g·L⁻¹)</td>
<td>145.3 ± 4.3</td>
<td>51.56 ± 0.5</td>
<td>NH₄⁻N (mg·L⁻¹)</td>
<td>50.1 ± 1.2</td>
<td>229.0 ± 1.8</td>
</tr>
<tr>
<td>SCOD (mg·L⁻¹)</td>
<td>7.7615 ± 39.2</td>
<td>89.2 ± 2.3</td>
<td>NO₃⁻N (mg·g⁻¹TS)</td>
<td>n.d.</td>
<td>12.4 ± 0.1</td>
</tr>
<tr>
<td>COD (mg·g⁻¹TS)</td>
<td>1.249.6 ± 19.4</td>
<td>681.6 ± 11.3</td>
<td>Carbohydrates (% TS)</td>
<td>87.3 ± 1.1</td>
<td>17.7 ± 0.8</td>
</tr>
<tr>
<td>TOC (mg·g⁻¹TS)</td>
<td>462.6 ± 0.8</td>
<td>470.4 ± 1.2</td>
<td>Protein (% TS)</td>
<td>5.3 ± 0.4</td>
<td>21.2 ± 0.6</td>
</tr>
<tr>
<td>TN (mg·g⁻¹TS)</td>
<td>9.0 ± 0.2</td>
<td>51.3 ± 0.5</td>
<td>Lipid (% TS)</td>
<td>4.0 ± 0.2</td>
<td>12.3 ± 0.5</td>
</tr>
</tbody>
</table>
2.2 Reactor and operation

2.2.1 Batch experiments

Batch experiments were carried out in 500 mL reactors (effective volume of 400 mL), each covered with a rubber septum. Triplicate bottles were used in all batch experiments, and all data were expressed as the mean of triplicate ± standard deviation. Among the various samples, FW1 and FW7 were 100% FPW and 100% WAS as controls. Then, the effects of the VS ratios of FPW and WAS on biogas production were investigated. Typically, 250 mL of inoculated sludge and feedstock under different feeding ratios of FPW and WAS at the same organic load was added to each group of reactors, which were then added with water to a volume of 400 mL. After stirring evenly, it was purged with 99.99% pure nitrogen for 5 min so that the oxidation-reduction potential reached to −300 mV to ensure an anaerobic environment. The fermentation proceeded at 35 ± 0.5°C for a period of 40 day under 20 min of stirring at 100 rpm every 12 h. Biogas and methane production was detected online using a fully automated methanogenesis potential testing system (AMPTS II, Bioprocess Control). The feed-to-microbe (F/M) ratio was calculated based on the initial VS of the substrate and inoculum. Detailed experimental conditions and mixing ratios are summarized in Table 2. The F/M ratio was calculated based on the initial VS of the substrate and inoculum in Eq. 1:

\[
F/M = \frac{\text{substrate added (g VS)}}{\text{Inoculum added (g VS)}}
\]

2.2.2 Continuous experiments

A lab-scale one-phase anaerobic digester (Bioprocess® CSTR-5S, Sweden) with a 4 L working volume was used for the co-digestion of FPW and WAS (Figure 1). A water bath was used to maintain the digester in a mesophilic environment (35 ± 0.5°C). The reactor was mixed using dedicated heaters (3619 Aquarium Heater 300 W, Eheim Jager, Germany) until a homogeneous state was reached. Then, 4 L of the inoculum was added initially and flushed with N₂, ensuring that the oxidation-reduction potential reached to −300 mV to establish anaerobic conditions. FPW was diluted with water to a VS concentration of 8% before feeding from Day 1 to Day 230.

Five operation stages were applied (Table 3). The feedstock was added once a day, and the digestate was

Table 2: Experimental design for batch experiment

<table>
<thead>
<tr>
<th>Samples</th>
<th>FW1 FPW 100%</th>
<th>FW2</th>
<th>FW3</th>
<th>FW4</th>
<th>FW5</th>
<th>FW6</th>
<th>FW7 WAS 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS ratio (FPW:WAS)</td>
<td>/</td>
<td>3:1</td>
<td>2:1</td>
<td>1:1</td>
<td>1:2</td>
<td>1:3</td>
<td>/</td>
</tr>
<tr>
<td>Total VS (g)</td>
<td>7.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F/M ratio</td>
<td>1:1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substrate loading (gVS L⁻¹)</td>
<td>19.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental duration</td>
<td>40 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>35 ± 0.5°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Figure 1: Schematic and material object diagram of the lab-scale FPW and WAS co-digestion processes.
discharged every three days. The effluent pH, ammonium nitrogen, volatile fatty acids (VFAs), and alkalinity were analyzed every three days. Biogas and methane were detected online.

2.3 Physicochemical analysis

As described in Standard Methods (APHA/AWA/WEF 2005), TS, VS, chemical oxygen demand (COD), pH, and ammonium nitrogen (N–NH₄⁺) were measured. VFAs and bicarbonate alkalinity (BA) were quantified by titration with 0.1 N hydrochloric acid (HCl) and 0.1 N sodium hydroxide (NaOH) [30]. Biogas and methane were measured using µFlow Meters (Bioprocess Control, Sweden) and recorded online by AMPTS equipment (Bioprocess Control, Sweden), expressed at standard state. Carbohydrates were analyzed using the Dubois method with glucose as a standard [31]. Total protein content was estimated by multiplying organic nitrogen (TKN–N–NH₄⁺) by 6.25. Fat was measured by the Soxhlet extraction method. Organic elemental analysis (C, H, and N) was carried out on a Flash EA 1112 Elemental Analyzer (Thermo Finnigan, Italy). The metal elements of RA including K, Ca, and Fe were analyzed prior to anaerobic digestion by AAS (atomic absorption spectrophotometer, VarianSpectrAA55-B, Palo Alto, USA).

2.4 Definition of conversion efficiency

The conversion efficiency of hydrolysis, acidogenesis, acetogenesis, and methanogenesis were calculated based on the COD in Eqs. 2–5, respectively [32]:

\[
\text{Hydrolysis} (\%) = \frac{\text{SCOD}_{\text{out}} - \text{SCOD}_{\text{in}} + \text{COD}_{\text{CH}_4}}{\text{TCOD}_{\text{in}} - \text{SCOD}_{\text{in}}} \quad (2)
\]

\[
\text{Acidogenesis} (\%) = \frac{\text{COD}_{\text{VFA-out}} - \text{COD}_{\text{VFA-in}} + \text{COD}_{\text{CH}_4}}{\text{TCOD}_{\text{in}} - \text{COD}_{\text{VFA-in}}} \quad (3)
\]

\[
\text{Acetogenesis} (\%) = \frac{\text{COD}_{\text{Acetate-out}} - \text{COD}_{\text{Acetate-in}} + \text{COD}_{\text{CH}_4}}{\text{TCOD}_{\text{in}} - \text{COD}_{\text{Acetate-in}}} \quad (4)
\]

\[
\text{Methanogenesis} (\%) = \frac{\text{COD}_{\text{CH}_4}}{\text{TCOD}_{\text{in}}} \quad (5)
\]

where TCOD and SCOD are the total COD and soluble COD, respectively. COD_{VFA} is the total VFA concentration calculated by the COD of individual VFAs. COD_{Acetate} is the concentration of acetic acid represented by COD. COD_{CH4} is calculated based on the principle of 0.35 LCH₄·kg⁻¹·g⁻¹ COD. The footnotes “in” and “out” indicate the COD of the influent and effluent, respectively.

2.5 Kinetic modeling

The kinetics of anaerobic co-digestion of FPW and WAS were investigated using two models. Cumulative biogas production during batch anaerobic co-digestion was estimated using the model in Table 4. Curve fitting and calculation of kinetic parameters were performed in Origin.

2.6 Microbial analysis

Fermentation samples were collected from CSTR digester on Days 120, 180, and 240, respectively, and processed for microbial DNA extraction with the Fast DNA SPIN Kit for Soil (MP Biomedicals, Illkirch, France) in accordance with the manufacturer’s instructions. Then, the universal primer 341 F (5’ Adaptor–CTACGGGNGGCWGCAG–3’) and reverse primer 805 R (5’ Adaptor–GACTACHVGGGTATCTAATCC–3’) were used for the polymerase chain reaction (PCR) amplification of V3–V4 hypervariable regions in 16 S rRNA. After purification and quantification, the amplicons were operated on a MiSeq sequencing platform (Illumina Inc, California, USA) by a company of microbial analysis (Majorbio Bio-Pharm Technology Co. Ltd. Shanghai, China). The raw data were merged, quality

### Table 3: Influent characteristic and operational parameters for FPW and WAS co-digestion

<table>
<thead>
<tr>
<th>Stage</th>
<th>Day</th>
<th>FPW VS (%)</th>
<th>WAS VS (%)</th>
<th>Feed volume (mL)</th>
<th>VS ratio (FPW:WAS)</th>
<th>C/N ratio</th>
<th>OLR (kg VS·m⁻³·day⁻¹)</th>
<th>pH</th>
<th>Retention time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1–60</td>
<td>8</td>
<td>5.16</td>
<td>100</td>
<td>4:1</td>
<td>22.2</td>
<td>1.80</td>
<td>4.21</td>
<td>40</td>
</tr>
<tr>
<td>II</td>
<td>61–120</td>
<td>8</td>
<td>5.16</td>
<td>109</td>
<td>3:1</td>
<td>19.8</td>
<td>1.92</td>
<td>4.51</td>
<td>37</td>
</tr>
<tr>
<td>III</td>
<td>121–180</td>
<td>8</td>
<td>5.16</td>
<td>128</td>
<td>2:1</td>
<td>16.8</td>
<td>2.16</td>
<td>4.76</td>
<td>31</td>
</tr>
<tr>
<td>IV</td>
<td>181–240</td>
<td>8</td>
<td>5.16</td>
<td>165</td>
<td>1.2:1</td>
<td>14.2</td>
<td>2.64</td>
<td>5.14</td>
<td>24</td>
</tr>
<tr>
<td>V</td>
<td>241–300</td>
<td>8</td>
<td>5.16</td>
<td>184</td>
<td>1:1</td>
<td>13.4</td>
<td>2.88</td>
<td>5.32</td>
<td>22</td>
</tr>
</tbody>
</table>
filtered by FLASH software and Mothur platform, and the taxonomic level was aligned using PyNAST and Greengenes [35].

3 Results and discussion

3.1 Batch experiments

3.1.1 Performance of biogas production and methane content in batch experiments

To study the methanogenesis capacity of substrates with different VS ratios, the cumulative biogas production obtained from the reactors under 7 feeding ratios of FPW to WAS is illustrated in Figure 2. Under the same total feeding VS, the biogas production first increased and then decreased following a gradual increase in the proportion of WAS. Because of its low pH and easy degradation, FPW fermented alone can be rapidly converted into small molecular acids at the early stage, resulting in abundant acid accumulation that inhibits the activity of methanogens and leads to fermentation failure (Figure 2a). With the addition of WAS, the acidification during fermentation was significantly improved. When the VS ratio was 2:1, the amount of cumulative biogas produced in the reactor was maximized to 4695.47 mL (Figure 2c), which increased by 5.2% and 10.5% compared with the VS ratios of 3:1 and 1:1, respectively. Then, with a further increase in the WAS proportion, the biogas production gradually decreased, because the hydrolysis stage is the rate-limiting step in WAS methane production. When WAS was fermented alone (Figure 2g), the cumulative biogas production was only 2,234.4 mL, which is consistent with some previous studies [15].

With an increase in the proportion of WAS, the overall methane content gradually rose (Figure 2h). Raising the proportion of WAS improved the lipid and protein contents of the feed, including lipid (1,014 mL CH₄ g VS⁻¹), carbohydrate (415 mL CH₄ g VS⁻¹), and protein (496 mL CH₄ g VS⁻¹) contents, according to theoretical methane production (TMP) [36]. Hence, changes in the components of the feed will affect TMP and thus the overall methane content (Table 5).

### Table 4: Equation of the kinetic model used for data analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Mathematical definition</th>
<th>Abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monod [33]</td>
<td>( B(t) = B_{\text{max}} \left( \frac{kt}{1+kt} \right) )</td>
<td>( B(t) ): cumulative biogas production (mL)</td>
</tr>
<tr>
<td>First-order dynamics [34]</td>
<td>( B(t) = B_{\text{max}}(1 - \exp(-kt)) )</td>
<td>( B_{\text{max}} ): ultimate biogas production potential (mL)</td>
</tr>
<tr>
<td>( k ): rate constant (day⁻¹)</td>
<td></td>
<td>( t ): co-digestion day (day)</td>
</tr>
</tbody>
</table>

3.1.2 Conversion efficiency of hydrolysis, acidogenesis, acetogenesis, and methanogenesis in anaerobic co-digestion

To analyze the rate-limiting step in the anaerobic co-digestion process, we calculated the conversion ratio of the four stages of hydrolysis, acidogenesis (VFA), acetogenesis (acetic acid, CO₂, and H₂), and methanogenesis [37]. The conversions were calculated from the initial and final COD values of anaerobic co-digestion at different VS ratios. As shown in Figure 3, as the proportion of sludge in the substrate increased, and the methane conversion ratio showed a trend of first increasing and then decreasing. For FW1, FW2, and FW3, apple content was higher, and apple is a substrate that is highly anaerobically digestible. Apples are rich in a variety of soluble sugars (fructose, sucrose, glucose, and sorbitol), which can be converted to pyruvate through the Embden–Meyerhof–Parnas pathway [38]. It is rich in a variety of organic acids, such as malic acid content of 29.4–32.2 mg·g⁻¹ TS and citric acid content of 4.44–5.19 mg·g⁻¹ TS, in addition to lactic acid, succinic acid, fumaric acid, oxalic acid, and other small-molecule organic acids, so that the initial concentration of organic acids in apple raw materials is relatively high. In addition, the macromolecular carbohydrates (pectin, cellulose, and hemicellulose), proteins, and fats contained in apples are also easily converted into small molecules such as monosaccharides, amino acids, and fatty acids, and finally into acetic acid, CO₂, and H₂, which can be used by methanogens. FW1 and FW2 rapidly accumulated a large amount of VFA during the early stage of fermentation, which inhibited the conversion of acetic acid and the production of methane so that VFA could not be converted into methane smoothly. The conversion rate of the four stages is hydrolysis > acidogenesis > acetogenesis > methanogenesis. The methane conversion ratio
was the highest in FW3, and there was no inhibition of the methane conversion process.

On the other hand, in the fermentation process of FW3, FW4, FW5, FW6, and FW7, the organic matter produced by hydrolysis can quickly enter the stages of acid production and acetic acid production, and finally be converted into biogas. The conversion rates of the four stages are basically equal. In Figure 3, the hydrolysis conversion ratios in the system decreased with the decrease in apple feed components, and the influence of the hydrolysis
process as the rate-limiting step became increasingly obvious. This is because WAS is a microbial matrix (floc) composed of microorganisms and exopolymers. These microbial-originated extracellular polymeric substances (EPS) are a complex mixture of biopolymers comprising polysaccharides, proteins, nucleic acids, uronic acids, humic substances, and lipids, amongst others [28]. It is well known that certain compounds in EPS are resistant to anaerobic digestion, resulting in hydrolysis as the rate-limiting step. Overall, when the VS ratio is 2:1, the problems of VFA accumulation and WAS hydrolysis rate-limiting can be alleviated at the same time.

### Table 5: Theoretical methane production with different VS ratios

<table>
<thead>
<tr>
<th>Formula</th>
<th>FW1</th>
<th>FW2</th>
<th>FW3</th>
<th>FW4</th>
<th>FW5</th>
<th>FW6</th>
<th>FW7</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMP mL CH₄ VS</td>
<td>3,406</td>
<td>3,505</td>
<td>3,542</td>
<td>3,606</td>
<td>3,671</td>
<td>3,707</td>
<td>3,806</td>
</tr>
</tbody>
</table>

### Table 6: Kinetic parameters for anaerobic co-digestion of selected mixtures

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>FW2</th>
<th>FW3</th>
<th>FW4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monod</td>
<td>B(t)-experimental (mL)</td>
<td>4,339.13</td>
<td>4,578.89</td>
<td>4,015.12</td>
</tr>
<tr>
<td></td>
<td>B(t)-predicted (mL)</td>
<td>4,478.73</td>
<td>4,660.35</td>
<td>4,013.63</td>
</tr>
<tr>
<td></td>
<td>Bₘₐₓ (mL)</td>
<td>7,987.57</td>
<td>7,036.63</td>
<td>6,096.57</td>
</tr>
<tr>
<td></td>
<td>k (day⁻¹)</td>
<td>0.043</td>
<td>0.065</td>
<td>0.060</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.9916</td>
<td>0.9992</td>
<td>0.9998</td>
</tr>
<tr>
<td>First-order dynamics</td>
<td>B(t)-experimental (mL)</td>
<td>4,339.13</td>
<td>4,578.52</td>
<td>4,015.89</td>
</tr>
<tr>
<td></td>
<td>B(t)-predicted (mL)</td>
<td>4,432.94</td>
<td>4,601.44</td>
<td>3,968.68</td>
</tr>
<tr>
<td></td>
<td>Bₘₐₓ (mL)</td>
<td>5,352.51</td>
<td>5,010.5</td>
<td>4,337.75</td>
</tr>
<tr>
<td></td>
<td>k (day⁻¹)</td>
<td>0.059</td>
<td>0.076</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.9901</td>
<td>0.9991</td>
<td>0.9989</td>
</tr>
</tbody>
</table>

### 3.1.3 Kinetics analysis

To further study the fermentation efficiency of different VS ratios, we chose FW2, FW3, and FW4 with higher cumulative biogas yields for kinetic simulation analysis. The results of the applied kinetic models are shown in Figure A1 (in the Appendix), while the calculated kinetic parameters are listed in Table 6. For FW2, FW3, and FW4, the correlation coefficients obtained by the Monod model are above 0.9914, 0.9992, and 0.9998, respectively. The correlation coefficients obtained by the first-order kinetic model were higher than 0.9901, 0.9991, and 0.9989, respectively. This indicates that the Monod model is best suited for FPW and WAS co-digestion of cumulative biogas production trends. The correlation coefficients of the two models of FW2 were lower, which was due to the slight fluctuation of the cumulative biogas curve due to acid suppression in the early stage of fermentation.

The kinetic constant K is an important index to determine the operating efficiency of the anaerobic reactor, which reflects the speed of the co-digestion system. In both the Monod model and the first-order model, the K (0.065 and 0.076 day⁻¹) of FW3 is the largest, indicating that the cumulative biogas production rate of FW3 is the fastest, which is consistent with the analysis in 3.1.2. The K (0.043 and 0.059 day⁻¹) of FW2 was the smallest because the generated excess VFA inhibited the subsequent acetogenic and methanogenic stages and reduced the overall velocity of the reaction system. Similarly, the
K (0.060 and 0.074 day\(^{-1}\)) of FW4 is smaller than that of FW3, which is mainly due to the flocculation of sludge and the difficulty of cell wall degradation, which reduces the overall progress of the reaction system.

### 3.2 Continuous experiments

#### 3.2.1 Continuous experiments performance

In order to further study the performance of continuous co-digestion for biogas production, the co-digestion of FPW and WAS was operated for 300 days over the five stages, and the biogas and methane production under different VS ratios (FPW:WAS) is illustrated in Figure 4. During the whole co-digestion process, as the OLR increased from 1.80 to 2.88 kg VS m\(^{-3}\) day\(^{-1}\), the MPR rose from 0.6 to 0.89 L L\(^{-1}\) day\(^{-1}\), and biogas production increased from 1.16 to 1.48 L L\(^{-1}\) day\(^{-1}\). The methane yield is always above 300 mL g\(^{-1}\), indicating that the continuous anaerobic fermentation process can achieve high-rate co-digestion with FPW and WAS. The pH was maintained between 6.76 and 7.29, and VFA/TA was below 0.16, indicating the reactor was always in a steady state.

In Run 1, the average methane yield was 354.51 mL g\(^{-1}\), but the pH and TA continued to decrease. This is because FPW is easily hydrolyzed organic matter, and the rate-limiting step of its anaerobic fermentation is methanation, so acid accumulation will occur and the TA consumption in the

![Figure 4: Changes in the pH, TA, VFA, NH\(_4\)-N, methane yield, MPR, biogas production, and CH\(_4\) content during acidic FPW and WAS co-digestion.](image-url)
buffer system will be increased. This result is similar to the batch experiment. In Run 2, the average methane yield was 351.87 mL·g⁻¹, and the downtrend of pH in the first 20 days was alleviated, but the downtrend of pH and TA in the reactor remained unchanged with the accumulation of VFA. Similarly, Fonoll [29] found the co-digestion experiment with apple waste and sewage sludge at a mass ratio of 30:70 (ww/ww, VS ratio of 3:1) was also showed the increase of VFA, a decrease of TA and methane production within the first 20 days, and the average methane yield was 260 mL·g⁻¹. In particular, on the 60th and 120th days, the ammonia nitrogen concentration in the reaction system decreased from the initial 227.54–138.11 and 123.67 mg·L⁻¹, respectively. The C/N ratio in the feed was 22.2 and 19.8, respectively, which met the C/N requirements in the general anaerobic fermentation process [39]. However, because WAS is not easily degraded, the consumption of ammonia nitrogen is less than the production, resulting in insufficient nitrogen source supplementation during the reaction.

In Run 3, the VFA was significantly reduced from 533.45 to 438.12 mg·L⁻¹, the TA content increased from 3,755.67 to 3,958.32 mg·L⁻¹, and no acid accumulation occurred. At this time, the average methane yield was 346.69 mL·g⁻¹ and did not change significantly from the previous two stages, indicating that FPW and WAS maintained high hydrolysis efficiency. The ammonia nitrogen content increased from 123.67 to 138.34 mg·L⁻¹, so the problem of nitrogen source consumption was solved. In Runs 4 and 5, as the addition of WAS further increased, the pH in the system rose to 7.37, and the buffering capacity was significantly improved, but the average methane yield decreased significantly to 310.21 and 299.07 mg·L⁻¹ respectively. This is because although co-digestion of FPW and WAS can improve the hydrolysis efficiency of WAS, excessive WAS can reduce the hydrolysis efficiency.

During the whole fermentation process, the methane content increased slightly with a rise in the WAS proportion. This is because WAS is rich in proteins and fats, and the biogas produced by its decomposition contains a higher percentage of methane [39]. This result is the same as that of the batch experiment.

### 3.2.2 Microbial community dynamics

The species and abundance of bacteria have a close relationship with the hydrolytic and acidogenic capacity to degrade complex organic matter during CSTR digestion. Relative abundance (RA) and distribution of bacterial communities at 120, 180, and 240 days during continuous fermentation were analyzed at the phylum and genus levels. Four main phyla were selected (Figure 5a), including **Firmicutes**, **Bacteroidetes**, **Proteobacteria**, and **Chloroflexi**.

![Figure 5: The RA of bacteria communities at (a) the phylum and (b) genus levels.](image_url)
The RA of _Firmicutes_ increased in the whole digestion, which was 10.2%, 24.3%, and 31.9% at 120, 180, and 240 days, respectively. This phylum is one of the major hydrolytic bacteria that can decompose substrates into SCOD and especially degrade proteins and polysaccharides into soluble organic matter [40]. It helps to promote the hydrolysis of incremental sludge in the feed. _Bacteroidetes_ can degrade complex compounds such as celluloses and hemicelluloses and convert them into glucose and organic acids and further to VFAs [41]. On the 180th day, the RA of _Bacteroidetes_ increased to 21.3%, which helped to improve the hydrolysis efficiency of long-chain compounds. _Proteobacteria_ and _Chloroflexi_ are commonly found in the decomposition reaction of monosaccharides, polysaccharides, and small-molecular compounds to form acetic acids [42]. With the increase in WAS content, the proportion of carbohydrates in the substrate, and the RA of _Proteobacteria_ and _Chloroflexi_ all decreased, which are also consistent with the gradual decrease in VFAs.

The reasons for the changes in hydrolysis efficiency under different VS ratio can also be explained by analyzing the changes in microbial community structure at the genus level. On the 180th day, the RAs of _Bacteroides_, _Paludibacter_, _Litorilinea_, _Levilinea_, and _Smithella_ reached 5.4%, 5.4%, 5.0%, 7.9%, and 3.8%, respectively (Figure 5b), which were higher than those on Day 120 and Day 240. Among them, _Bacteroides_ and _Paludibacter_ are carbohydrate-fermenting bacteria that promote the utilization of carbon sources such as cellulose and hemicellulose during hydrolysis and acidification [43]. _Litorilinea_ can utilize proteins to facilitate WAS hydrolysis. The monosaccharides from the hydrolysis of carbohydrates, peptides, and amino acids from the decomposition of protein can be decomposed into volatile organic acids by _Levilinea_ [44]. At last, _Smithella_ can degrade butyrate and propionate and maintain the stability of the acetogenic methanogenesis reaction [45]. The enrichment of the above-mentioned bacterial groups is conducive to the simultaneous hydrolysis and acidification of carbohydrates, proteins, and other substrates, which can accelerate the feed hydrolysis rate and reduce the impact of organic acids in an anaerobic system.

4 Conclusions

This study focused on the biogas production potential of acidic FPW and WAS co-digestion. FPW was rich in various organic acids and had high acidity. The pH of a single fermentation reached 5.16, which greatly inhibited the activity of methanogens, and the cumulative biogas production was only 1,602.41 mL. The acidification of FPW digestion could be solved by co-digestion with WAS. When the VS ratio of FPW and WAS was 2:1, the buffer capacity of the batch fermentation system was improved, and the highest cumulative biogas yield was 4,695.47 mL. Similarly, the continuous co-digestion process is the most stable when the VS ratio was 2:1, without VFA accumulation as in Run 1 and Run 2, and ammonia nitrogen concentration decreased in the system. The average methane yield reached 346.69 mL·g⁻¹. Compared with the batch fermentation system, continuous fermentation system handle more organic load per day, and it has better adaptability and stability to organic load changes. The enrichment of _Bacteroides_, _Paludibacter_, _Litorilinea_, _Levilinea_, and _Smithella_ was beneficial to improve the hydrolysis rate of the substrate and reduced the impact of organic acids in the anaerobic system.

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Conflict of interest: Author Lulu Zhang is employed by Shaanxi Provincial Land Engineering Construction Group Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References


Appendix

Figure A1: Anaerobic co-digestion kinetics analysis for (a) Monod and (b) first-order dynamics models.