1. Introduction

China is one of the largest beer producers and consumers in the world [1]. In 2022, beer production in China was approximately $3.6 \times 10^{10}$ Liters, resulting in 0.27 billion m$^3$ brewery wastewater, which accounts for 1.3% of China’s total wastewater discharge. Brewery wastewater is high-strength organic wastewater, which contains a high concentration of various organic pollutants, such as yeast residues, wheat stillage, protein, sugars, amino acids [2,3]. Although brewery wastewater is non-toxic wastewater, owing to the large annual production and high amounts of organic pollutants, it will lead to serious harmful impacts on the ecological environment if it is not properly treated [4–7]. In China, the construction investment in brewery wastewater treatment facilities is as high as CNY 192.3 billion, and the annual operating cost is CNY 89 million.

Recently, various technologies have been applied for the treatment of brewery wastewater [8,9], including membrane filtration [10], aerobic granular sludge reactor [11,12] and aerobic sequencing batch reactor [13]. The above technologies have the common disadvantage of high energy consumption, which significantly increases the investment and
the operating cost. Additionally, accompanied by greenhouse gases’ emission, the energy resources contained in the wastewater are wasted [8,14]. Therefore, how to recover energy from the wastewater attracts increasing amounts of attention from brewery operators and researchers. Anaerobic digestion is attractive in the brewing industry since it can combine the treatment of wastewater with energy recovery as biogas [15–18]. The anaerobic digestion of wastes to produce methane is also considered an effective way to reduce carbon dioxide levels in the atmosphere and help mitigate climate change.

On the other hand, primary sedimentation and activated sludge processes are the most commonly applied processes in municipal wastewater treatment plants (MWWTPs) worldwide, which generates large amounts of municipal sewage sludge [19]. At present, the treatment technology of municipal sewage sludge in China is mainly based on anaerobic digestion, which decomposes the biodegradable organic matters in municipal sewage sludge to produce methane and carbon dioxide, thus stabilizing the municipal sewage sludge and recovering the biomass energy [20,21]. However, at present, the anaerobic digestion of municipal sewage sludge in China’s WWTPs still faces the bottlenecks of low organic matter conversion efficiency, long residence time, and low engineering operational efficiency [22,23]. Hence, anaerobic co-digestion of municipal sewage sludge with various high organic-containing solid wastes (e.g., municipal solid wastes, food industrial wastes, pig manure, and fruit wastes) has been applied to improve biogas production [24–27]. Considering that the brewery wastewater contains large amounts of biodegradable organic matters, it is expected to integrate the anaerobic digestion of municipal sewage sludge with brewery wastewater treatment to improve biogas production [28]. Mudzanani et al. [29] reported that the anaerobic co-digestion of sewage sludge and brewery spent grain could be considered a cost-effective solution that could contribute to the energy self-efficiency of WWTPs and sustainable waste management for breweries. Rasmeni et al. [30] confirmed that the co-digestion of wastewater sludge with brewery spent yeast could improve biomethane production and breweries’ waste yeast could mitigate some limitations associated with mono-digestion of sewage sludge. It is recommended to scale up the co-digestion of the feed for the plant. However, most studies have been conducted in laboratory-scale biomethane potential tests, and there is little relevant information on full-scale co-digestion of municipal sewage sludge with brewery wastewater. Moreover, an acidification problem may occur in the anaerobic system due to the high COD content and the low pH of brewery wastewater, thus inhibiting the methanogenic process. Additionally, how the operating conditions such as the reaction temperature and the mixing ratio of brewery wastewater affect the anaerobic digestion of municipal sewage sludge needs further investigation.

Based on the above, in this study, biogas production was monitored during the operation of the anaerobic digesters in a plant-scale WWTP for two years, with 2% brewery wastewater mixed in municipal sewage sludge. Additionally, the effects of temperature and the mixing ratio of brewery wastewater on the anaerobic co-digestion performance including biogas production, organic matter degradation, and the variations in the concentrations of ammonia and phosphate were investigated. The microbial mechanism was further explored using a high-throughput sequencing technique to provide a theoretical basis and data support for the practical operation of anaerobic co-digestion of municipal sewage sludge with brewery wastewater.

2. Materials and Methods

2.1. Operation of the Plant-Scale Anaerobic Digesters

In this study, biogas production was monitored for two years (since July 2020 to June 2022) without/with the involvement of brewery wastewater in the anaerobic digestion of municipal sewage sludge in three plant-scale anaerobic digestion tanks of Tuan dao WWTP in Qingdao, Shandong province, China. Each anaerobic digestion tank had a working volume of 7500 m$^3$. Additionally, the average hydraulic residence time was about 25 days and the pH in the anaerobic digesters was controlled at 7.1 ± 0.1. The influent municipal sewage sludge was mainly composed of primary sludge and excess activated sludge, with
an average total quantity of 1035 ± 95 m$^3$/d for the three digesters. Since July 2021, the brewery wastewater was transported from Tsingtao Brewery Company Limited in Qingdao, to Tuandao WWTP using tank trucks. Additionally, the brewery wastewater was evenly added into three sludge anaerobic digesters with an average mixing ratio ($v/v$) of 2%. The real-time mixing ratio fluctuated in the range of 0–10%. The characteristics of the influent primary sludge, excess activated sludge and brewery wastewater are shown in Table 1.

Table 1. The characteristics of primary sludge, excess activated sludge and brewery wastewater as the influent of the plant-scale anaerobic digesters during long-term operation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Primary Sludge</th>
<th>Excess Activated Sludge</th>
<th>Brewery Wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (g/L)</td>
<td>35.5 ± 5.6</td>
<td>38.5 ± 3.8</td>
<td>25.3 ± 5.1</td>
</tr>
<tr>
<td>VS (g/L)</td>
<td>16.7 ± 3.9</td>
<td>19.6 ± 2.2</td>
<td>19.5 ± 4.2</td>
</tr>
<tr>
<td>SCOD (mg/L)</td>
<td>312.2 ± 52.3</td>
<td>126.4 ± 18.9</td>
<td>30,576 ± 6547</td>
</tr>
<tr>
<td>NH$_4^+$-N (mg/L)</td>
<td>93.8 ± 13.5</td>
<td>1.25 ± 0.2</td>
<td>95.6 ± 35.7</td>
</tr>
<tr>
<td>PO$_4^{3-}$-P (mg/L)</td>
<td>37.1 ± 2.4</td>
<td>0.6 ± 0.2</td>
<td>85.6 ± 18.5</td>
</tr>
<tr>
<td>pH</td>
<td>6.4 ± 0.2</td>
<td>6.8 ± 0.2</td>
<td>5.0 ± 0.7</td>
</tr>
</tbody>
</table>

2.2. Batch Tests Operation

The effects of temperature and mixing ratio of brewery wastewater on the anaerobic co-digestion with municipal sewage sludge were further studied in 30-day batch tests.

2.2.1. Substrates and Inoculum

In batch tests, the municipal sewage sludge (volume ratio of primary sludge and excess activated sludge = 7:3) and brewery wastewater were used as substrates. The municipal sewage sludge was collected from Tuandao WWTP, and the brewery wastewater was collected from Tsingtao Brewery Company Limited. Additionally, digested sludge obtained from the anaerobic digester of Tuandao WWTP was used as the inoculum. The characteristics of the substrates and inoculum are shown in Table 2. The substrates and inoculum were stored in a refrigerator at 4 °C before being used.

Table 2. The characteristics of the substrates and inoculum used in the batch tests.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Municipal Sewage Sludge</th>
<th>Brewery Wastewater</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (g/L)</td>
<td>37.5 ± 3.5</td>
<td>19.2 ± 3.4</td>
<td>42.1 ± 3.2</td>
</tr>
<tr>
<td>VS (g/L)</td>
<td>16.2 ± 1.6</td>
<td>18.1 ± 1.9</td>
<td>27.5 ± 2.4</td>
</tr>
<tr>
<td>SCOD (mg/L)</td>
<td>239 ± 18</td>
<td>28,851 ± 485</td>
<td>423 ± 52</td>
</tr>
<tr>
<td>Carbohydrate (mg/L)</td>
<td>12.8 ± 1.3</td>
<td>23,700 ± 520</td>
<td>11.5 ± 1.8</td>
</tr>
<tr>
<td>NH$_4^+$-N (mg/L)</td>
<td>45.0 ± 3.4</td>
<td>153.8 ± 15.5</td>
<td>440.3 ± 27.2</td>
</tr>
<tr>
<td>PO$_4^{3-}$-P (mg/L)</td>
<td>47.8 ± 3.1</td>
<td>94.3 ± 3.9</td>
<td>24.2 ± 1.6</td>
</tr>
<tr>
<td>Alklinity (mg/L)</td>
<td>1567.2 ± 92.7</td>
<td>999.6 ± 56.4</td>
<td>112.9 ± 1.5</td>
</tr>
<tr>
<td>pH</td>
<td>6.5 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>7.8 ± 0.2</td>
</tr>
</tbody>
</table>

2.2.2. Batch Anaerobic Reactors and Operations

The batch tests were developed based on the method described by Yin et al. [31]. A series of 500 mL glass-made serum bottles were used as anaerobic digestion reactors, with an effective volume of 400 mL. The reactors were sealed with rubber stoppers that were connected to aluminum foil bags using rubber pipe to collect biogas. A total of 100 mL of digested sludge was added to each reactor as the inoculum. Then, brewery wastewater and the municipal sewage sludge were added into the reactors with different mixing ratios of brewery wastewater. The mixing ratio in the batch tests ranged from 0% to 50%, which was selected and determined based on the plant-scale real-time mixing ratio (0–10%) and possible high load of brewery wastewater in the future (20–50%). The brewery wastewater mixing ratio could also result in a change in the SCOD/NH$_4^+$-N ratio in the feed (Table 3). After evenly mixing the substrates in the reactor, aeration using nitrogen gas
was conducted for 3 min, and the reactors were then rapidly sealed with a rubber stopper to maintain the anaerobic environment. Each group of reactors was placed in three different water bath shakers to keep a constant temperature of 34 °C, 37 °C, and 40 °C, respectively, with a rotation rate of 100 r/min. The pH values of the mixture in the reactors were periodically monitored and regulated to 7.0–7.5, which was suitable for the metabolism of methanogenic microorganisms, by adding the solution of HCl or NaOH. All the batch tests were operated in triplicate and the average values were calculated for analyses of the results. The biogas production of reactors was measured daily using injection syringes, and the mixed samples were collected from the reactors to determine the water quality. The intermediate samples were collected via an injector, which was connected to the rubber stopper of the reactors using rubber pipe, thus guaranteeing the anaerobic environment in the reactors. The samples were stored in a refrigerator at 4 °C before the analyses.

### Table 3. The SCOD/NH$_4^+$-N of the feed with different brewery wastewater mixing ratio.

<table>
<thead>
<tr>
<th>Mixing Ratio</th>
<th>0%</th>
<th>2%</th>
<th>5%</th>
<th>10%</th>
<th>20%</th>
<th>35%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCOD/NH$_4^+$-N</td>
<td>5.3 ± 0.2</td>
<td>17.2 ± 0.6</td>
<td>33.1 ± 1.4</td>
<td>55.5 ± 2.8</td>
<td>89.3 ± 3.6</td>
<td>123.4 ± 3.8</td>
<td>146.3 ± 3.4</td>
</tr>
</tbody>
</table>

### 2.3. Analytical Methods

The concentration of total solids (TS), volatile solids (VS), soluble chemical oxygen demand (SCOD), NH$_4^+$-N and PO$_4^{3-}$-P was determined according to standard methods [32]. pH was detected using a portable pH meter (LEICI PHB)-260, INESA Scientific Instrument Co., Ltd, Shanghai, China. The analysis of variance (ANOVA) was conducted using Origin software (version 2021, https://www.originsoftware.co.uk/) to analyze the effects of the process parameters and their possible interactions.

The sludge samples from the anaerobic reactors operated at 37 °C with mixing ratios of 0%, 2%, 20%, 35% and 50% were collected for microbial community analyses. The supernatant of the samples was removed using a high-speed centrifuge at 13,000 rpm for 30 min. DNA extraction was conducted using Power Soil® DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) within 24 h of sampling. Additionally, the quality of isolated DNA was evaluated using 1% agarose gel electrophoresis. The first PCR amplification of the bacterial or archaeal 16S rRNA included 3 min of initial template denaturation and polymerase activation at 95 °C, 25 cycles processed via denaturation at 95 °C for 30′′, primers annealing at 60 °C for 30′′ and primers’ elongation at 72 °C for 30′′, followed by a final elongation step at 72 °C for 10 min. At the end of the reaction, 2.0% agarose gel was used to detect PCR products. Then, the Illumina bridge PCR-compatible primers were introduced in the second round of amplification (95 °C for 3 min, followed by 8 cycles at 95 °C for 30′, 55 °C for 30′, and 72 °C for 30 s, and a final extension at 72 °C for 5 min). The mixture of the amplicons extracted from 2% agarose gels was purified using the AxyPrep DNA gel extraction kit (Axymgen Biosciences, Union City, CA, USA) according to the manufacturer’s instructions, and quantified using QuantiFluorTM-ST (Promega, Madison, WI, USA). The purified amplicons were pooled in equimolar amounts, and then they were paired-end sequenced (2 × 300 bp) on the Illumina MiSeq platform according to standard protocols. The obtained paired-end reads were spliced according to the overlap relationship. After performing quality control and filtering, the sequences were clustered into operational taxonomic units (OTUs) with 97% similarity using the MOTHUR program (version 1.43.0) with an RDP 16S database. Based on the results of OTUs’ clustering analysis and taxonomic information, a statistical analysis of community structure was performed at each taxonomic level.

### 3. Results and Discussion

#### 3.1. Plant-Scale Anaerobic Digestion Performance of Municipal Sewage Sludge without/with the Involvement of Brewery Wastewater

As shown in Figure 1A, the VS concentrations in the influent municipal sewage sludge fluctuated around 16 g/L. The VS reduction efficiencies were below 10% before September.
2020, and gradually increased and fluctuated around 30% in November 2020–June 2021. When brewery wastewater was introduced from July 2021, the VS reduction decreased to around 20% in the first 3 months, and it then recovered to about 40%. In addition, the VS reduction efficiencies were more stable than those without the feeding of brewery wastewater.

Figure 1. (A) The VS reduction, (B) the biogas production before the brewery wastewater was introduced (in black) and after the brewery wastewater was introduced (in red) and (C) the biogas composition in the plant-scale anaerobic digesters without/with the involvement of brewery wastewater in the influent municipal sewage sludge.
Accompanied by VS reduction, biogas was produced during anaerobic digestion. As shown in Figure 1B, with only municipal sewage sludge as an influent in July 2020–June 2021, the average monthly biogas production was about $14.5 \times 10^4 \text{m}^3$. After adding brewery wastewater into the influent municipal sewage sludge with a mixing ratio of 2% since July 2021, the biogas production of anaerobic digestion decreased significantly in the first 3 months, which was in accordance with the performance of VS reduction (Figure 1A). It was also accompanied by the decreasing pH in the anaerobic digesters, indicating the occurrence of acidification. However, in this study, along with the recovery of VS reduction, the monthly biogas production gradually recovered and increased to a higher level (about $18 \times 10^4 \text{m}^3$) after 5 months of operation, which was 24% higher than that when brewery wastewater was not involved. Brewery wastewater contained a high concentration of organic pollutants, which was 80–85 times higher than that in the municipal sewage sludge. Even though the average mixing ratio of brewery wastewater was low (2%), the involvement of brewery wastewater had significantly contributed to biogas production. Rapoll et al. [33] proposed Sherry wine distillery wastewater as a co-substrate of sewage sludge and also verified the use of co-digestion technology as a breakthrough solution to increase biogas production.

The biogas composition was also influenced by the involvement of brewery wastewater in the anaerobic digestion of municipal sewage sludge. As shown in Figure 1C, when municipal sewage sludge was used as feed without brewery wastewater, the CH$_4$ proportion was 59.5% on average. Additionally, the co-digestion of municipal sewage sludge with 2% brewery wastewater increased the percentage of CH$_4$ to 61.6%. The quality of biogas was promoted, with the CO$_2$/CH$_4$ ratio increasing from 0.52 to 0.57. The 2.1% increase seemed small, though it could result in $3.8 \times 10^3 \text{m}^3$ of CH$_4$ production per month, indicating the huge significance of sewage sludge of anaerobic co-digestion with brewery wastewater.

Furthermore, the plant-scale operational results demonstrated that the VS reduction and the biogas production were fluctuant during anaerobic co-digestion, which could be attributed to the dynamic changes in the characteristics and the mixing ratio of brewery wastewater, and seasonally affected the operational temperature (Figure 1B). It was suggested that those operating parameters should be carefully controlled in the full-scale plant. Therefore, the effects of the brewery wastewater mixing ratio and temperature on the performance of anaerobic co-digestion were further investigated, as shown in Section 3.2.

3.2. Effects of Brewery Wastewater Mixing Ratio and Temperature on Anaerobic Co-Digestion of Municipal Sewage Sludge

3.2.1. Biogas Production Process

The variation in the cumulative biogas production with different brewery wastewater mixing ratios and different reaction temperatures is shown in Figure 2. As shown in the figure, the cumulative biogas production was significantly influenced by the brewery wastewater mixing ratio and temperature. At a temperature of 34 °C, the biogas production could be effectively improved by appropriately increasing the mixing ratio of brewery wastewater in the correct range (<20%) (Figure 2A). The highest biogas production could be achieved when the mixing ratio was 20%, which was 44.6% higher than that without brewery wastewater. Rapoll et al. [33] also reported that the biodegradability and biomethane potential of the mixture of sewage sludge and Sherry wine distillery wastewater were determined by the optimal co-substrate ratio (50%). However, in this study, when the mixing ratio was further increased to a higher level (≥35%), biogas production was inhibited, although more organics in brewery wastewater were introduced into the reactors, mainly due to the acidification phenomenon that occurred in the reactor, which led to a significant decrease in pH in the first two days (Figure 3A), thus forming an unfavorable environment to anaerobic methane production. Additionally, the higher the mixing ratio of brewery wastewater was, the faster the pH dropped. It should also be noted that the biogas production plateau would last for about 10–15 days even though the pH values
had been regulated to 7.0, demonstrating a continuous inhibition of severe acidification on methanogenic microorganisms. Therefore, an appropriate mixing ratio of brewery wastewater is a crucial factor regarding the performance of anaerobic digestion. When pH was adjusted to the range of suitable anaerobic digestion (7.0–7.5), the biogas production could slightly recover but still failed to reach the highest value, which was consistent with the plant-scale operation.

Figure 2. Variation in cumulative biogas production of anaerobic co-digestion of brewery wastewater and municipal sewage sludge with different mixing ratios under different temperatures.
Figure 3. Variation in pH in the reactors during anaerobic co-digestion of brewery wastewater and municipal sewage sludge.

The behavior of cumulative biogas production at the higher temperature of 37 °C and 40 °C was different from that at 34 °C. The biogas production was hardly improved with a mixing ratio of brewery wastewater below 20%, as shown in Figure 2B,C. Additionally, only with the mixing ratio higher than 20% could the final biogas production be promoted.
It should be also noted that when the mixing ratio was higher than 20%, the plateau of biogas production started to be observed from the third day at a temperature of 37 °C and 40 °C, which was in agreement with the occurrence of acidification at the beginning of anaerobic digestion (Figure 3B,C). In addition, even though the pH was manually adjusted, the duration of the plateau would last 7–15 days; this length was positively associated with the mixing ratio of brewery wastewater. Additionally, the biogas was recovered to be produced after this lag phase, indicating that the methanogenic microorganisms gradually adapted to the feed and the environment.

The increase in operating temperature would generally promote the biogas production, probably owing to the fact that a higher temperature could promote the hydrolytic acidification of insoluble organic matters in the municipal sewage sludge, thus producing more substrates for biogas production [34]. However, the relationship between biogas production and the mixing ratio of brewery wastewater showed a different pattern at different temperatures. As shown in Figure 4, with low brewery wastewater mixing ratios (<10%), the specific biogas production at 37 °C was higher than that at 34 °C, while a temperature of 40 °C would reduce it. This further demonstrated that the higher temperature was a double-edged sword, which would accelerate acidogenesis to provide a substrate for biogas production, while severe acidification would also inhibit methanogenesis. Additionally, when the mixing ratio was 10%, the biogas production decreased with increasing temperature due to acidification. Although a high mixing ratio (>35%) introduced high concentrations of organic matter, the enhancement in biogas production could only be achieved at temperatures of 37 °C and 40 °C. On the contrary, the kinetic study conducted by Rapoll et al. [35] showed that mesophilic anaerobic co-digestion of sewage sludge and wine distillery wastewater could increase the methane production rate and enhance the acetate consumption step because of the higher amounts of biodegradable compounds. Additionally, another study demonstrated that under thermophilic conditions, anaerobic co-digestion of sewage sludge and Sherry wine distillery wastewater almost doubled the methane yield in comparison to sewage sludge [36]. The inconsistency in the findings might be due to different soluble biodegradability fractions in the co-substances and operating temperatures [29].

![Figure 4. Specific biogas production during anaerobic digestion of municipal sewage sludge with different mixing ratios of brewery wastewater at different temperatures.](image-url)
much more significantly. The results also showed that the interaction between the temperature and the mixing ratio was also significant in this study. Additionally, considering that the batch scale reactors were smaller than suggested in the literature for biomethane potential tests, the laboratory study still faced potential limitations for its scale up. The full-scale results might not be in complete conformity with the laboratory results, which requires further investigation.

Table 4. The overall results of two-way ANOVA concerning specific biogas production.

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>2</td>
<td>184.94148</td>
<td>92.47074</td>
<td>0.09721</td>
<td>0.90807</td>
</tr>
<tr>
<td>Mixing ratio</td>
<td>6</td>
<td>13,564.24872</td>
<td>2260.70812</td>
<td>2.37656</td>
<td>0.09517</td>
</tr>
<tr>
<td>Interaction</td>
<td>12</td>
<td>11,415.01938</td>
<td>951.25161</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>20</td>
<td>25,164.20957</td>
<td>1258.21048</td>
<td>1.80672</td>
<td>0.17148</td>
</tr>
<tr>
<td>Error</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>20</td>
<td>25,164.20957</td>
<td>--</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2.2. The Variation in Organic Matter, Ammonia and Phosphate Concentrations in the Reactors

The variation in the SCOD concentration within each batch reactor is shown in Figure 5. It can be seen that a higher dosing ratio of brewery wastewater introduced more SCOD (mainly carbohydrates) into the reactors. Additionally, it was observed that there was a significant decrease in the SCOD concentration in each reactor, demonstrating that the efficient removal of organics could be achieved via the anaerobic co-digestion of municipal sewage sludge with brewery wastewater. It was particularly noteworthy that when the mixing ratio was at a high level (35% and 50%), the SCOD concentrations slightly increased in the early phase of the tests, further indicating that the production of soluble organics through acidogenesis was faster than the consumption via methanogenesis, which coincided with little cumulative biogas production during the plateau (Figure 2). Additionally, the SCOD concentration began to decrease when the pH was adjusted to neutral, thus recovering biogas production. Additionally, generally, the higher the mixing ratio, the more residual soluble organic matter there was left in the reactor. However, the theoretical COD reduction via biogas production was much lower than the decrease in SCOD concentration. It was speculated that large amounts of organics were adsorbed in municipal sewage sludge, which might be released again for biogas production when the hydraulic retention time was longer.

As shown in Figure 6A, the release of ammonia occurred with the hydrolysis of large amounts of nitrogen-containing organic matters (such as protein) during anaerobic digestion in all tests. It could be observed that at a temperature of 34 °C, the ammonia release level of municipal sewage sludge was nearly 200%, and it increased slightly with a low mixing ratio (<5%) of brewery wastewater into municipal sewage sludge, while less ammonia was released when more brewery wastewater was involved in the anaerobic digestion, which was also consistent with the trend of biogas production. In particular, compared with municipal sewage sludge digestion (mixing ratio = 0%), no more than half of the ammonia release level was obtained, which was also found in the tests at 37 °C and 40 °C. This should be attributed to the fact that the majority of the released ammonia was consumed for a faster growth of microorganisms with more biodegradable substrates in the reactor, even though more nitrogen would be introduced with brewery wastewater. Furthermore, the ammonia release level at 37 °C slightly increased in the reactors with the mixing ratio of 0–2%, indicating that anaerobic hydrolysis could be enhanced under appropriate temperature, which resulted in more ammonia being released.
Figure 5. Variation in the SCOD concentrations in the reactors during the co-digestion of municipal sewage sludge with brewery wastewater.
Surprisingly, in this study, phosphate could be removed during anaerobic digestion of municipal sewage sludge, which was more remarkable at a higher temperature (Figure 6B). Additionally, the phosphate removal efficiencies with a low mixing ratio (<10%) of brewery wastewater were similar. However, the effects of an increased amount of brewery wastewater performed differently at different temperatures. When the anaerobic reactors were operated at 34 °C, the phosphate removal could be promoted significantly with an increasing mixing ratio of brewery wastewater. However, at a temperature of 37 °C and 40 °C, larger quantities of brewery wastewater would further reduce the phosphate removal efficiencies. Meanwhile, phosphate might also react chemically with some metal ions such as calcium and magnesium in the anaerobic system to form precipitation. Additionally, the acidification could provide acidity to the reactor so that the precipitation would be redissolved with a high mixing ratio of brewery wastewater.

3.3. Microbial Community

The mixing ratio of brewery wastewater was influential to the distribution of the microbial community in the anaerobic digesters. As shown in Figure 7, when municipal sewage sludge was fed for anaerobic digestion without brewery wastewater, Chloroflexi, Proteobacteria and Bacteroidetes were the dominant bacteria, accounting for more than 60% of the total bacterial population. With the increasing mixing ratio of brewery wastewater, the Firmicutes gradually became dominant, while the proportions of Chloroflexi and Bacteroidetes were reduced. When the mixing ratio of brewery wastewater was 50%, Chloroflexi was completely eliminated, indicating that Chloroflexi was significantly inhibited by a large quantity of influent brewery wastewater. However, Proteobacteria appeared to be less affected by the co-digestion with brewery wastewater, whose relative abundance was kept around 20% in each test.
As shown in Figure 7B, the acidogenic bacteria accounted for 8.5% of the total bacterial population in the anaerobic digester using municipal sewage sludge as the influent. By increasing the mixing ratio of brewery wastewater, the relative abundance of the acidogenic bacteria increased significantly. When the mixing ratio was 50% for co-digestion, the percentage of acidogenic bacteria in the total population was 21.3%, which was 2.5 times that in municipal sewage sludge digester. This provided further evidence that the involvement of brewery wastewater would promote the acidification process, which was in agreement with the results discussed in Section 3.2.1. In addition, the composition of the bacterial communities changed with the introduction of brewery wastewater. It was obvious that the proportions of Aminicenantes_genera_incertae_sedis decreased with increasing the mixing ratio of brewery wastewater, while the relative abundances of Candidatus_Cloacamonas, Smithella and Syntrophomonas increased. The genera of Candidatus_Cloacamonas and Smithella could facilitate the conversion of propionic acid into acetic acid [37]. Additionally, Syntrophomonas-related species were identified to be linked to degradation as the degraders of long-chain fatty acids such as oleate [38]. It should be noted that some specific genera were present in large quantities only with a high mixing ratio of 35% or 50%, such as Clostridium_sensu_stricto, Longilinea, Clostridium_III, Romboutsia, Acetobacter, and Gracilibacter, which could hydrolyze insoluble organics to provide more substrates (e.g., volatile fatty acids (VFA)) for the metabolism of methanogens [39–44].

From the perspective of the archaeal community, as shown in Figure 8, Methanotrix and Methanolinia were the dominant methanogens for the anaerobic digestion of municipal sewage sludge when no brewery wastewater was introduced, accounting for 90% of the archaeal community. Additionally, the population of Methanotrix was much larger than that of Methanolinia. The anaerobic co-digestion of municipal sewage sludge with brewery wastewater gradually changed the distribution of the archaeal community. With a mixing ratio of brewery wastewater ranging from 0% to 35%, the proportion of Methanotrix was significantly reduced, and Methanolinia gradually dominated. However, by adding much more...
brewery wastewater into municipal sewage sludge (mixing ratio = 50%), *Methanosarcina* became the dominant archaeal group instead of *Methanothrix* and *Methanolinea*. Mathai et al. [45] also observed that during the startup phase characterized by high VFA concentrations and low pH values, *Methanosarcina* replaced *Methanothrix* as the dominant acetoclastic methanogen. It was also reported that the several physiological features of *Methanosarcina* that were not found in *Methanothrix* offered adaptability to high-VFA-concentration stressed conditions [46]. With a greater abundance of *Methanosarcina*, the anaerobic system with the highest brewery wastewater mixing ratio of 50% was better able to tolerate the elevated acetate concentrations and low pH values that resulted from the overload.

Figure 8. The composition of archaeal community at the genus level.

4. Conclusions

This study investigated the potential of the anaerobic co-digestion of municipal sewage sludge and brewery wastewater. The effects of anaerobic digestion under different mixing ratio and temperature conditions were explored, providing a theoretical basis for the integrated treatment of brewery wastewater and improvement of the biogas production efficiency of municipal sewage sludge. Moreover, the microbiological mechanism was further analyzed. The main conclusions are as follows:

1. Within a certain range (0–20%), an increased ratio of brewery wastewater is beneficial to increase biogas production. The highest biogas production was achieved when the mixing ratio was 20%, which was 44.6% higher than that without brewery wastewater. However, a further increase in the mixing ratio could lead to the acidification of the anaerobic system, thus inhibiting biogas production.

2. With increasing temperature, the biogas production effect of the reactor with a high mixing ratio of brewery wastewater was significantly improved, indicating that high temperature is beneficial for improving the synergistic anaerobic digestion effect between brewery wastewater and municipal sewage sludge.
(3) The anaerobic co-digestion of municipal sewage sludge with brewery wastewater was accompanied by the release of ammonia nitrogen and the removal of phosphate, which was also influenced by the mixing ratio and the temperature.

(4) The microbial community structure was significantly changed with the introduction of brewery wastewater. With the increasing mixing ratio of brewery wastewater, Firmicutes gradually become dominant instead of Chloroflexi. Meanwhile, the proportion of *Methanothrix* was significantly reduced, and *Methanolinea* and *Methanosarcina* gradually dominated.

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