Maintaining the long term stability of anaerobic digestion of maize straw in a continuous plug flow reactor by verifying the key role of trace elements

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Abstract

A mesophilic anaerobic digestion bioreactor fed on maize straw was investigated in a plug flow reactor for 150 days. The process performance was evaluated through the stable, unstable, and recovery phases. Results showed that the system maintained stable performance within approximately two months but the following trace elements; Fe, Co, and Ni gradually declined and the volatile fatty acids accumulated to 8.07 g/L by the 120th day of operation. Pig manure containing higher trace elements, therefore, was mixed with maize straw at a ratio of 1:4 on a dry matter basis before feeding. The reactor recovery was observed with a significant downtrend of volatile fatty acids and an uptrend in biogas production. Upon recovery of the reactor and stable operation condition, a methane yield of 0.21 L/g-VSadded was obtained. Methane content stabilized at 54%. The quantitative utilization of the three elements on a unit mass of COD degraded was determined. Maintaining long-term stability was still a challenge without determining the minimum additional pig manure required. Conclusively, the co-digestion of trace elements-rich substrate or the addition of the trace elements into the substrate is required for the anaerobic digestion of straw.

1. Introduction

In China, the production of crop straw is estimated at 8.6×10^8 tons in 2020 (China Biogas Society, 2021). Maize straw is the major crop residue. Maize straw is an important biomass source for biological and thermal-chemical processing for producing bioenergy owing to the abundant biodegradable materials (Herrero et al., 2019). Producing biogas by anaerobic digestion is one of the most practical technologies to utilize maize straw, and it provides considerable potential for resource sustainability (Paul et al., 2019). Nevertheless, there are challenges faced when mono-digesting maize straw in an anaerobic system, is experienced during the operation of large-scale plants. Maize straw is mainly composed of cellulose, hemicellulose, and lignin, which are carbohydrate polymers. The complex structure of the carbohydrate polymers results in slow biodegradation and low conversion efficiency. Furthermore, maize straw has an unsuitable value of carbon to nitrogen ratio (C/N), and also lacks essential trace elements, which is a hindrance to anaerobically treating maize straw. Previous studies pointed out that anaerobic digestion of maize silage faces; reactor instability, low biogas production, and accumulation of volatile fatty acids owing to a lack of essential trace elements (Fahlbusch et al., 2018).

The trace elements are essential for the growth of methanogens and fundamentally determine the conversion efficiency of an anaerobic process (Cai et al., 2022). An appropriate amount of trace elements is conducive to the growth of methanogens, otherwise. Comparing animal manure, sewage sludge, and municipal solid waste, maize straw contains minimal quantities of trace elements (Jiang et al., 2022). For this reason, maize straws are normally co-digested with animal manure. However, if the anaerobic digestion can be continuously operated by fed with maize straw was still lack direct results.

Plug flow reactor is known for lower cost, more stable performance, and tolerating environmental stress (Lansing et al., 2010). Plug flow reactor has also been reported to be used for the anaerobic treatment of dry materials (Patinvoh et al., 2020). Previous studies have demonstrated that the COD removal efficiency of animal manure in a plug flow reactor was 44%, which was 16% higher than the conventional completely mixing reactor (Yue et al., 2011). In a plug flow reactor, functional microbes exist along the materials flowing. Hydrolysis, acidogenesis, acetogenesis, and methanogenesis may occur under a more desired environment. Subsequently the biogas production in a plug flow reactor may have advantageous over traditioin reactor. The role of trace elements in a plug flow reactor would be different but still not yet to be known. The begging question is whether the anaerobic digestion of maize straw and long-term operation without co-digestion and additional essential trace elements would be sustainable in a plug flow reactor.
reactor. Besides, whether the reactor can be restored by adding other raw materials when volatile fatty acids accumulate and the impact of trace elements dosing has not been extensively reported. Most previous studies focused on the demand for trace elements fed with food waste (Zhang et al., 2019) manure (Wang et al., 2021), and sewage sludge (Bardi and Aminirad, 2020). However, few studies examined the impact of trace elements on a plug flow reactor. The dosage and demand of trace elements also vary among the different feedstock. If the addition of trace elements is mandatory for maize straw in a plug flow reactor was still uncertain.

Therefore, the main objective of this study is to investigate the performances of the anaerobic mono-digestion of maize straw at a mesophilic condition in a continuous plug flow reactor for a lab-scale long-term operation, and study the changes of Fe, Co and Ni during long-term continuous operation to provide more accurate technical data for guiding industrial application.

2. Materials And Methods

2.1 Feedstock and inoculum

The maize straw used in this study was collected from Shang Zhuang Experimental Station of China Agricultural University, Beijing, China. After taking back, the maize straw was ground and sieved using 18\(^{th}\) mesh sieve (1.0 mm size). The maize straw was mixed with a liquid fraction of centrifuged biogas plant effluent treating cattle manure to ensure that the TS of the mixture was 10% and soaked at 37\(^{\circ}\)C for one day to prepare the substrate. The biogas slurry used for soaking the maize straw was taken from a full-scale mesophilic biogas plant treating cattle manure. The liquid fraction of the slurry was obtained after centrifuging at 4000 rpm for 20 minutes. The inoculum was obtained from a pilot scale anaerobic digestion reactor that treats waste activated sludge at mesophilic temperature. The pig manure added was taken from a pig farm in Beijing, China. The characteristics of feedstock and inoculum are summarized in Table 1.

2.2 Calculation of trace element requirements

The quantitative requirement of trace elements was calculated in Eq. (1).

\[
\frac{X}{COD} = \frac{[S(X)_{in} - S(X)_{eff}]}{[COD_{in} - COD_{eff}]} \tag{a}
\]

Where X is the trace elements concentration, in milligram per liter; S(X)\(_{in}\) is the concentration of Fe, Co, and Ni in the influent, respectively, in milligram per kilogram; S(X)\(_{eff}\) is the concentration of iron, cobalt and nickel in the effluent, respectively, in milligram per liter; COD\(_{in}\) is the concentration of COD in the influent, in kilogram per liter; and COD\(_{eff}\) is the concentration of COD in the effluent, in kilogram per liter.

2.3 Experimental set-up

Figure 1 shows the plug flow reactor which had a capacity of 30 L with a working volume of 21 L. Intermittent stirring using a motor (Oriental Motor, Japan) was realized by a timer, working for five minutes per half an hour at 60 rpm. The reactor was operated at a hydraulic retention time of 42 days, and draw and fill were operated once per day. The reactor consisted of a water bath interlayer with a thickness of three centimeters to maintain the temperature at 37\(^{\circ}\)C by circulating hot water through a water pump (Sensen HQB-2200, China). It was divided into four chambers owning the same volume. Each chamber had an effluent port at the bottom and a gas pipe connected with a gas bag. The
reactor started with an organic loading rate (OLR) of 1.4 g-TS/(L·d) and increased to 2.4 g-TS/(L·d) for long term operation.

2.4 Analytical methods

Total solid (TS), volatile solid (VS), ammonium nitrogen, total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), and other biochemical parameters were determined according to the American Public Health Association (APHA) methods. pH was measured by using the pH meter (Mettler Toledo, Switzerland), volatile fatty acids (VFAs) and the components of CH\textsubscript{4} and CO\textsubscript{2} in the biogas were tested by the gas chromatography (Shimadzu GC-2010 plus, Japan), and the gas chromatography (Shimadzu GC-8A, Japan), respectively. The trace elements were analyzed by the atomic absorption spectrometer (AAS, PerkinElmer 900T, USA).

3. Results And Discussion

3.1 Biogas production performance

Figure 2 shows the biogas production at different stages. The biogas production increased gradually during the start-up stage and then level to a maximum of 0.52 L/(g-VS). The average biogas and methane yield reached 0.39 and 0.23 L/ g-VS with a methane content of 60%, which was similar to a previous study treating maize straw in a plug flow reactor at 38°C (Dong et al., 2020). Similar results in other types of reactors using maize straw as raw material are listed in Table 3. After 99 days of operation, the biogas production rate decreased to some degree compared with the relatively stable stage, the accumulation of volatile fatty acids and biogas production declined continuously which was observed when the reactor operated till the 120th day. By stopping feeding from 120th days for 12 days, the concentration of VFAs declined to approximately 340 mg/L. Then 2 liters of pig manure were added to replace the reactor content. Subsequently feeding of the reactor was done by a mixture of straw and pig manure substrate (TS ratio of straw: pig manure=4:1) from 134-150 days. The biogas production and methane yield resumed to 0.32 and 0.21 L/(g-VS gradually till 150th days. The observed performance was comparable to a previous study for mono-digestion of maize straw at 37±1°C (Aimee et al., 2018).

Table 4 lists the performances of the reactor at different stages. Volatile fatty acids were detected at a low concentration of around 300 mg/L during days 1~26. Biogas production and methane production stabilized at an average of 0.33, 0.20 L/(L·d) with a methane content of 61%. The VFAs concentration increased to 0.48 g/L with a pH value of 7.22 during days 27-42. From day 43-99, biogas production and methane production decreased to 0.80, 0.47 L/(L·d) with a methane content of 60%. The VFAs concentration in the reactor gradually increased from 0.21 to 1.90 g/L on the 99th day. As a result, the instability of the system was probably due to the inhibition of methanogenesis. VFAs concentration continued to increase and accumulate to a new average level of almost 8.07 g/L on the 120th day, the reactor was stopped for 12 days. During the recovery period, pig manure was added to the feedstock as an additional essential trace element source. VFAs were decomposed and declined to around 0.60 g/L. In this study, ammonia nitrogen concentration was almost constant, at an average value of 1500 mg/L over the rest of the time, and didn't pose inhibition for the microbes.

3.2 Operational performance of the four chambers

Biogas production of the four chambers is illustrated in Figure 3. It was observed that most of the gas production in the reactor was mainly in the 3rd and the 4th chambers. The biogas production increased to the maximum of 1.1 L/(L·d) gradually and then decreased, which was determined at an average value of 0.80 L/(L·d) during 43-90 days.
Subsequently, biogas production continued to drop to the lowest value. The trend changed and the gas production rate increased to 0.71 L/(L·d) after re-feeding. The methane production corresponded to the biogas production changing trend.

The biogas composition of the four chambers is shown in Figure 4. It can be observed that the fluctuation magnitude in the 1st chamber was larger than in other chambers. It may be attributed to the closeness of the 1st chamber to the feeding port, resulting in the impact of the feeding on the microorganisms. During the early 99 days of operation, the methane concentration in the four chambers varied within the range of 50%~70%, the CH$_4$/CO$_2$ in the four chambers first rising, then decreasing gradually and stabilized at an average ratio of 1.66 rapidly from day 43 to day 99. After 99 days, the methane concentration decreased rapidly to 34%, the proportion of CO$_2$ in biogas components gradually increased and the average CH$_4$/CO$_2$ in the four chambers decreased to the minimum of 0.93 on the 120th day. On the contrary, upon the addition of pig manure after re-feeding, the methane concentration began to show an upward trend and resumed to 54% once again with an average value CH$_4$/CO$_2$ ratio of 3.39.

Based on the results above, there were no significant differences in biogas production and methane concentration among the four chambers of the plug flow reactor. It is speculated that the hydrolysis and methanogenesis in the reactor were not well separated. The plug flow reactor was designed to distinguish hydrolysis limiting the anaerobic digestion rate of straw theoretically and methanogenesis. In theory, hydrolysis acidification occurred in the front section of the plug flow reactor, and methane production occurred in the rear section, which resulted in basically no gas production and only acid production in the front section. However, it has been reported that hydrolysis seems not the rate-limiting step of anaerobic digestion of straw, but the methanogenesis stage (Mahmoud et al., 2021). Therefore, the hydrolysis and methanogenesis didn’t take place in one part of the reactor, but in the entire reactor. This may have resulted in low separation efficiency between hydrolysis and methanogenesis.

### 3.3 Volatile fatty acids accumulation and system stability

The VFA concentration is shown in figure 5. VFAs maintained a low level and floated up and down around 0.5 g/L which did not change significantly, it indicated that the system had sufficient buffer capacity until 99 days. The pH in the digester gradually fell from 7.70 to 7.21 at this stage. After that, the VFAs concentration continued to increase about 10 times higher than the stable stage and reached the maximum value of 8.07 g/L with acetic acid dominating at a concentration of 5.95 g/L, while the concentration of propionic acid was 0.63 g/L till 120 days. Generally, in an anaerobic digestion system, acid inhibition will occur when the total VFA concentration reaches 8 g/L or the acetic acid concentration is above 2 g/L (Karthikeyan and Visvanathan, 2013). Hence, it was preliminarily considered that VFA accumulation occurred in the reactor. The absence of sufficient trace elements was among the major reason to check if the anaerobic system will poorly perform and the main cause of adverse reactor performance (Björn, 1985). Previous studies have demonstrated that acidification occurred and the reactor could not operate normally at 2.5 g-VS/(L·d) owing to the lack of trace elements during anaerobic mono-digestion of maize straw after 70 days (Aimee et al., 2018). Similar results were also obtained in the anaerobic system treating wheat silage (Schmidt et al., 2014). Therefore, it is reasonable to conclude that a lack of trace elements occurred in the reactor, resulting in VFA accumulation. The analysis of VFAs in this study indicated that acids were accumulated at different levels, the results were different from previous studies while treating wheat stillage under mesophilic reported that propionic acid accumulated to concentrations exceeding 7000 mg/L in less than 40 days of operation, while acetic acid concentration started to increase to more than 2000 mg/L adversely impacting and causing the collapse of reactor operation on 60th day. However, upon depleted of the Fe in the reactor, both the propionic and acetic acids increased...
in concentration to 1500 mg/L. In another reactor, depleted of Ni (No concrete value was given) the same performance was observed (Schmidt et al., 2014). After stopping to feed the reactor for 12 days, VFA concentrations declined to the initial level and remained below 0.5 g/L till the end of the experiment. It may be construed that exogenous trace elements in pig manure played an important role. Previous studies have reported that adding trace elements could solve VFAs accumulation when food waste is used as substrate and decreases VFAs concentration from around 6000 mg/L to 300 mg/L under an organic loading rate (OLR) of 10.0 kg-COD/(m$^3$·d) (Jiang et al., 2022). Trace elements play an important role in the degradation effect of VFAs by methanogens (Karlsson et al., 2012). When sufficient trace elements exist, the conversion efficiency of VFAs is stable and suitable (Moestedt et al., 2016), which corresponded to the changing trend of volatile fatty acids above.

### 3.4 Trace elements deficiency

Previous studies report the following trace elements; Fe, Co, and Ni being capable of promoting the biosynthesis and methanogenesis of microbial chambers (Baek et al., 2014; So-Jeong et al., 2014). Trace elements are closely related to the activities of microorganisms and are one of the key factors to ensure the stable operation of the reaction. Scherer et al determined the elemental composition of 10 methanogenic species and found that the trace elements content of Fe, Co, and Ni in the chambers of methanogenic were 0.07-0.28%, 10-120 ppm, 65-180 ppm (Scherer et al., 1983), which are vital to methanogens. It has been reported that Fe, Co, and Ni were the main required trace elements to stimulate the activity of methanogens in the process of anaerobic digestion (Paula et al., 2004). The lack of Fe, Co, and Ni had observable effects on anaerobic digestion (Fermoso et al., 2008).

Figure 6 shows the trace elements at different stages in the reactor. In conjunction with the progress of the reaction, Fe, Co, and Ni showed a gradual downward trend and reached the lowest value during 100-120 days. After operation for 120 days, the concentrations of Fe, Co, and Ni dropped to 0.5, 0.002, and 0.036 mg/L, respectively. VFAs concentration rose to 8.07 g/L, the reactor was acidified at the same time. Some studies have pointed out that low trace element contents cause instabilities, and low biogas production rates and pose the inhibition due to acid accumulation during anaerobic digestion with maize silage as feedstock (Fahlbusch et al., 2018). Stop feeding the reactor and operation for 12 days and then add pig manure to the feedstock. In Table 1, the contents of Fe, Co, and Ni in maize straw was 407. 0.5 and 3.0 mg/kg-TS, significantly lower than those in pig manure i.e. 4012, 3.0, and 10.0 mg/kg-TS. It was found that these three elements were restored to the initial value of the experiment. However, with continuous feeding and discharging, microbial metabolism and utilization, result in the decrease of trace elements ceaselessly in the reactor.

After resuming feeding, the trace elements concentration continued declining, which was in agreement with a previous study pointing to the impact of trace elements in the co-digestion of straw and manure keep a continually decreasing trend for the entire digestion time (Muhayodin et al., 2021). Consequently, whether it can be maintained or not requires a long-term operation to prove.

The decreasing trend of three trace elements in the first 99 days of operation is shown in Figure 7. These three kinds of trace elements gradually decreased and decreased from 13.2, 0.032, and 0.284 mg/L to 7.4, 0.01, and 0.063 mg/L on the 99th day, respectively. The reduction of trace elements concentration was accompanied by a high rate of consumption of the essential trace elements, resulting in insufficient trace elements in the reactor and accumulation of volatile fatty acids.
3.5 Determination of the quantitative requirements of trace elements

In this study, the trace elements required per COD removed were calculated. Table 5 lists a comparison of the requirements for Fe, Co, and Ni between earlier studies and this study. The required concentration of trace elements to remove per kilogram of COD differs significantly, which depends on the substrate, digestion temperature, and so on. In this study, the requirements of Fe, Co, and Ni to remove per kilogram COD were 30.26, 0.11, and 1.74 milligrams under mesophilic. Even at the same range of temperature, different digestion materials such as maize straw (this study), glucose (Masanobu et al., 2011), and acetate (Takashima and Speece, 1989), have different requirements for trace elements. The Fe, Co, and Ni requirements per kilogram COD removed in this study were within the range of other studies. After operation for 120 days, pig manure was mixed as trace elements with maize straw to add to the reactor. Subsequently, biogas production gradually recovered, volatile fatty acids decreased significantly, and the system gradually tended to stabilize.

4. Conclusions

The mesophilic anaerobic mono-digestion of maize straw was operated for 150 days through start-up, intermediate stable, unstable, and recovery stages. A well-balanced metabolism between acidogenesis and methanogenesis was observed but was not sustainable due to the lack of trace elements. By mixing pig manure (trace elements rich substrate) with straw, biogas production can be recovered. The quantitative requirement of Fe, Co, and Ni for the anaerobic treatment of maize straw in a plug flow reactor was finally determined. The findings verified the medatory requirement of trace elements for the anaerobic digestion of straw through long-term operation. Further optimizing the dosage of trace elements is still worthy of investigation.

Declarations

Acknowledgment

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Conflict of interest

The authors declare that they have no known conflicting interests or personal relationships that could have appeared to influence the work reported in this paper.

References


Tables

Table 1

Characteristics of feedstock and inoculum
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Maize straw</th>
<th>Biogas slurry</th>
<th>Inoculum</th>
<th>Pig manure</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>%</td>
<td>94.3±0.2</td>
<td>1.1±0.1</td>
<td>4.5±0.3</td>
<td>27.1±5.2</td>
<td>3</td>
</tr>
<tr>
<td>VS</td>
<td>%</td>
<td>89.5±0.3</td>
<td>0.1±0.1</td>
<td>3.0±0.3</td>
<td>21.1±4.8</td>
<td>3</td>
</tr>
<tr>
<td>TCOD</td>
<td>g/L</td>
<td>/</td>
<td>4.5±0.2</td>
<td>48.5±1.2</td>
<td>/</td>
<td>3</td>
</tr>
<tr>
<td>NH$_4^+$-N</td>
<td>g/L</td>
<td>/</td>
<td>1.5±0.1</td>
<td>1.4±0.1</td>
<td>/</td>
<td>3</td>
</tr>
<tr>
<td>VFAs</td>
<td>g/L</td>
<td>/</td>
<td>0.13±0.01</td>
<td>1.20±0.10</td>
<td>/</td>
<td>3</td>
</tr>
<tr>
<td>pH</td>
<td>/</td>
<td>/</td>
<td>8.25±0.05</td>
<td>8.34±0.05</td>
<td>/</td>
<td>3</td>
</tr>
<tr>
<td>Cellulose</td>
<td>%</td>
<td>31.6±1.9</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>3</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>%</td>
<td>33.7±2.1</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>3</td>
</tr>
<tr>
<td>Lignin</td>
<td>%</td>
<td>4.7±0.5</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>3</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>mg/(kg·DM)</td>
<td>407±12</td>
<td>/</td>
<td>/</td>
<td>4012±528</td>
<td>3</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>mg/(kg·DM)</td>
<td>0.5±0.1</td>
<td>/</td>
<td>/</td>
<td>3.0±0.1</td>
<td>3</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>mg/(kg·DM)</td>
<td>3.0±0.5</td>
<td>/</td>
<td>/</td>
<td>10±4.5</td>
<td>3</td>
</tr>
</tbody>
</table>

/: Not detected. DM means dry matter.

Table 3
Summary of anaerobic digestion fed with corn maize

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>Fed mode</th>
<th>Running period (d)</th>
<th>Temperature (℃)</th>
<th>OLR kg-TS/(m$^3$·d)</th>
<th>HRT (d)</th>
<th>Biogas production (m$^3$/kg-VS$_{added}$)</th>
<th>Methane yield (m$^3$/kg-VS$_{added}$)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFR</td>
<td>Periodically fed</td>
<td>120</td>
<td>38±1</td>
<td>2.1</td>
<td>15</td>
<td>0.39</td>
<td>0.22</td>
<td>Dong et al., 2020</td>
</tr>
<tr>
<td>CSTR</td>
<td>Periodically fed</td>
<td>90</td>
<td>35±1</td>
<td>1.8</td>
<td>50</td>
<td>/</td>
<td>0.20</td>
<td>Liu et al., 2019</td>
</tr>
<tr>
<td>CSTR</td>
<td>Daily fed</td>
<td>210</td>
<td>35±1</td>
<td>2.3</td>
<td>40</td>
<td>/</td>
<td>0.19</td>
<td>Li et al., 2017</td>
</tr>
<tr>
<td>PFR</td>
<td>Daily fed</td>
<td>150</td>
<td>37±1</td>
<td>2.4</td>
<td>42</td>
<td>0.39</td>
<td>0.23</td>
<td>This study</td>
</tr>
</tbody>
</table>

“/”: Not mentioned, the data from this study adopts the average value from day 43 to day 90.

Table 4
Performances of the reactor at different stages
### Parameters

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>Feeding maize straw</th>
<th>Feeding straw and manure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feeding Materials</strong></td>
<td>/</td>
<td>Start-up</td>
<td>Before acidification</td>
</tr>
<tr>
<td>Durations</td>
<td>d</td>
<td>1~26</td>
<td>27~42</td>
</tr>
<tr>
<td>TS&lt;sub&gt;in&lt;/sub&gt;</td>
<td>g/L</td>
<td>5.8±0.1</td>
<td>10.2±1.4</td>
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<tr>
<td>VS&lt;sub&gt;in&lt;/sub&gt;</td>
<td>g/L</td>
<td>4.6±0.3</td>
<td>8.9±0.8</td>
</tr>
<tr>
<td>OLR</td>
<td>g-&lt;br&gt;TS/(L·d)</td>
<td>1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>CH&lt;sub&gt;4&lt;/sub&gt;</td>
<td>%</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>%</td>
<td>23</td>
<td>35</td>
</tr>
<tr>
<td>Biogas production</td>
<td>L/(L·d)</td>
<td>0.33±0.14</td>
<td>0.84±0.24</td>
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<tr>
<td>Methane production</td>
<td>L/(L·d)</td>
<td>0.20±0.09</td>
<td>0.51±0.14</td>
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<tr>
<td>pH</td>
<td>/</td>
<td>7.56±0.20</td>
<td>7.22±0.09</td>
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<tr>
<td>Acetic acid</td>
<td>g/L</td>
<td>0.17±0.12</td>
<td>0.30±0.15</td>
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<tr>
<td>Propionic acid</td>
<td>g/L</td>
<td>0.02±0.03</td>
<td>0.16±0.04</td>
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<tr>
<td>Butyric acid</td>
<td>g/L</td>
<td>0.01±0.01</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>g/L</td>
<td>0.03±0.07</td>
<td>0±0</td>
</tr>
<tr>
<td>Total VFA</td>
<td>g/L</td>
<td>0.30±0.14</td>
<td>0.48±0.15</td>
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</table>

/: Not detected.

### Table 5

Trace elements requirement of per COD removed (mg/kg-COD removed)

<table>
<thead>
<tr>
<th>Trace elements requirement</th>
<th>Fe</th>
<th>Co</th>
<th>Ni</th>
<th>Temperature</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>17</td>
<td>6.3</td>
<td>35~37</td>
<td>Masanobu et al., 2011</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>54</td>
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**Figures**
Figure 1

Plug flow reactor system
Figure 2

Biogas production at different stages
Figure 3

Volumetric biogas production of four chambers
Figure 4

Biogas components during the whole operation
Figure 5

VFAs at different stages
**Figure 6**

The trace elements at different stages
Figure 7

Decreasing trend of trace elements during different stages

Supplementary Files

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