Coproduction of hydrogen and methane in a CSTR-IC two-stage anaerobic digestion system from molasses wastewater
Qiaoyan Li and Yongfeng Li

ABSTRACT

A continuous hydrogen and methane production system in a two-stage process has been investigated to increase energy recovery rate from molasses wastewater in this study. This system consisted of a continuous stirred-tank reactor for hydrogen production and an internal circulation (IC) reactor for methane production, and was studied under the influent organic loading rate (OLR) of 18, 24, 30 and 36 kg COD/(m³·d) (COD: chemical oxygen demand). The maximum volumetric hydrogen production rate of 2.41 L/(L·d) was obtained at the OLR of 30 kg COD/(m³·d) with a hydrogen content of 42%, and the maximum volumetric methane production rate of 2.4 L/(L·d) with a methane content of 74.45% was obtained at the OLR of 36 kg COD/(m³·d) using the effluents of hydrogen fermentation as substrate. The maximum of 71.06% of the molasses wastewater energy was converted to biogas (hydrogen and methane) at the OLR of 30 kg COD/(m³·d).

Key words | energy recovery, hydrogen, methane, two-stage anaerobic process

INTRODUCTION

Hydrogen has been recognized as a major world renewable energy source to replace declining fossil fuel resources, and it appears to be an attractive energy source for two main reasons: firstly, the calorific value is 122 kJ/g, which is three times greater than petrol, 3.9 greater times than ethanol and 4.5 times greater than coal (Fayaz et al. 2012); secondly, the combustion products have no secondary pollution, resulting in no net releases of carbon dioxide (CO₂). Hydrogen production methods include water electrolysis, microbial photosynthesis (Holladay et al. 2009), which are energy intensive and unsustainable, and therefore further hydrogen production methods need to be studied.

Anaerobic digestion is one of the environmentally friendly techniques for hydrogen and methane production, where anaerobic bacteria can decompose organic wastes to produce energy (hydrogen and methane) (Tahti et al. 2015). Anaerobic digestion has unique characteristics such as high ecological adaptability, simple reaction condition and low nutrient requirement (Puyol et al. 2017), and microbes utilize the organic matter in wastewater to extract hydrogen under anaerobic environments (Han et al. 2012). And the end products, ethanol and volatile fatty acids (VFAs), are the ideal substrate for methane fermentation (Isa et al. 1986), which makes it possible to produce hydrogen and methane simultaneously (Nualsri et al. 2016). The key point of the coproduction of hydrogen and methane is to separate the fermentation process in two reactors by adjusting process parameters, such as pH and hydraulic retention time (HRT). In the hydrogen production reactor, organic compounds are converted to hydrogen and soluble metabolic products (SMP), and in the methane production, these SMP are converted to methane improving both energy recovery rate and chemical oxygen demand (COD) removal rate (Corona & Razo-Flores 2018).

The anaerobic digestion process is often limited by two major steps depending on the nature of the substrate, i.e. hydrolysis and methanogenesis. The methanogens are characterized as slow growing and vulnerable, which leads to the methanogenesis stage as the key stage of the anaerobic digestion process (Aydin et al. 2015). A number of challenges in operating the methanogenic reactor have been widely documented, foremost of which were instability and lower
methane yields. Accordingly, a number of experiments have studied the methanogenic reactor to enhance stability and methane yields (Khemkhao et al. 2016; Sen et al. 2016). Several studies have associated this poor performance to the difficulty of maintaining a sufficiently high concentration of methanogenic Archaea in the reactor due to their slow growth rate on the one hand and wash-out on the other hand (Schmidt et al. 2014; Khemkhao et al. 2016). Therefore, different reactors have been used as the hydrogen production reactor or methane production reactor in a two-stage system, such as two laboratory continuous tank stirred reactors (CSTRs) (Dareioti & Kornaros 2014), a CSTR and a fixed-bed anaerobic filter (AF) (Lindner et al. 2016), and two up-flow anaerobic sludge blanket reactors (UASBs) (Kongjan et al. 2011). But few researchers used the internal circulation (IC) reactor in a two-stage anaerobic digestion system. The IC reactor is one of the excellent aeration reactors of the third generation: it was developed by the Paques Company in the 1980s (Kassam et al. 2005) and it integrates two UASBs vertically (Luo et al. 2016). Due to the unique structure of the IC system driven by the produced gas, the IC reactor has a unique advantage in treating wastewater and wastes with high organic contents, such as swine waste and wastewater (Deng et al. 2006) and brewery wastewater (Xu et al. 2013). However, few studies have employed IC in a two-stage anaerobic digestion process.

Various raw materials generated from the wastewater of different industries can be used as the main substrates for hydrogen production. Molasses wastewater, a high strength waste, is a liquid by-product generated in large amounts by food industries. Molasses wastewater is a promising feedstock for hydrogen production because it has a high organic strength and relatively high bioavailability.

In this study, the CSTR-IC two-stage fermentation system was installed to study hydrogen and methane production and the total bioenergy recovery rate under various organic loading rates (OLRs) (18, 24, 30, 36 kg COD/(m³·d)) using molasses wastewater as the substrate.

### MATERIALS AND METHODS

**Continuous two-stage CSTR-IC anaerobic hydrogen–methane fermentation system**

A continuous two-stage anaerobic digestion system (Figure 1) consisting of a CSTR for hydrogen production and an IC reactor for methane production was investigated in this study. The CSTR with a working volume of 6.0 L was installed with a magnetic stirrer at the top to control the stirring speed at the rate of 50 rpm and a temperature controller inside to control the inner temperature at 35 °C. The CSTR was started up at the OLR of 18 kg/(m³·d) under a continuous mode (HRT of 6 h), and the OLR was increased in accordance with Table 1 until steady state conditions were achieved, which was based on constant products with a variation of less than 10%. Each fixed OLR should last over 10 days under steady state conditions.

The laboratory-scale IC reactor established for methane production had a working volume of 8.5 L, and used the effluent of the CSTR as the only carbon source for

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Operational conditions of CSTR-IC for evaluation of increasing OLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (d)</td>
<td>Phase 1</td>
</tr>
<tr>
<td>OLR (kg COD/(m³·d))</td>
<td>18</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>4,500</td>
</tr>
</tbody>
</table>

Figure 1 | Diagram of CSTR-IC two-stage anaerobic digestion system. 1 – influent water tanks; 2 – constant-flow pump; 3 – continuous tank stirred reactor (CSTR); 4 – magnetic stirrer; 5 – temperature controller; 6 – water-sealed drainage bottle; 7 – wet gas flow meter; 8 – internal circulation (IC) reactor; 9 – effluent water tanks; 10 – thermocouple; A – water pipe; B – GasHydrogen pipe; C – GasMethane pipe.
methanogen production after adding NaOH (4 M) to maintain the pH at 7. The IC reactor was operated in continuous mode and the HRT was controlled at 12 h and inner temperature was controlled at of 35 °C. The operating temperature and HRT were selected based on the work of Buitron & Carvajal (2010).

### Substrate and seed sludge

The molasses wastewater was collected from the local sugar refinery, and the wastewater composition is shown in Table 2. The fermentation substrate was maintained at COD:N:P ratio of 200–800:5:1 by diluting molasses wastewater and adding NH4Cl and KH2PO4. The feed also contained NaHCO3, MgCl, Na2MoO4, CaCl2, MnCl2 and FeCl2.

The anaerobic seed sludge was collected from the local municipal wastewater treatment plant (Wenchang, Harbin, China). The seed sludge was settled at room temperature and aerated for 20 days to inhibit the methanogen activity and then was added seed sludge was aerated for 6 h and the supernatant was removed and new molasses water was added before aeration. The seed sludge was aerated for 20 days to inhibit the methane-producing bacteria activity and then was added into the CSTR. During the aerobic cultivation process, the COD:N:P was kept at 100:5:1, while adding Fe2+ into the CSTR. During the aerobic cultivation process, the methane-producing bacteria activity and then was added seed sludge was aerated for 20 days to inhibit the methanogen activity and then was added.

The substrate conversion efficiency and bioenergy recovery rate

The substrate conversion efficiency (SCE) in the fermentative hydrogen and methane production process can be calculated from detected VFAs according to Equation (1).

$$SEC = \frac{\sum Q_i \cdot COD_i}{Q_{in} \cdot COD_{in}}$$  

where $Q_i$ is the product content (hydrogen, ethanol, acetic acid, propionic acid and butyric acid), mg/L or L; $COD_i$ is COD equivalent of product, of which hydrogen, ethanol, acetic acid, propionic acid and butyric acid was 0.714, 2.09, 1.07, 1.51 and 1.82 g O2, respectively; $Q_{in}$ is the influent volume (L); $COD_{in}$ is COD content (mg/L).

The bioenergy recovery rate of the two-stage fermentation system can be calculated from the hydrogen/methane production according to Equation (2).

$$\text{Bioenergy recovery rate} = \frac{\sum Q_i \cdot COD_i}{Q_{in} \cdot COD_{in}}$$  

where $Q_i$ is the bioenergy volume of hydrogen and methane, L/d; $COD_i$ is COD equivalent, of which hydrogen and methane was 0.714 and 2.86 g O2/L, respectively; $Q_{in}$ is the influent volume (L); $COD_{in}$ is COD content (mg/L).

### Operation index analysis

Biogas produced in the CSTR and IC reactor was collected and measured daily at a room temperature using a wet gas meter (Model LML-1, Changchun Filter, Changchun, China). The biogas was analyzed by a gas chromatograph (SC-7, Shandong Lunan Instrument Factory). The gas chromatograph was equipped with a thermal conductivity detector and a stainless steel column (2 m × 5 mm) filled with Porapak Q (50–80 mesh). Nitrogen was used as the carrier gas at a flow rate of 40 mL/min. VFAs (acetic acid, volumetric hydrogen production rate (VHPR), butyric acid) and ethanol in the fermentation solution were analyzed by a gas chromatograph (GC 112, Shanghai Analytical Instrument Co., Ltd) with a flame ionization detector. A 2 m stainless steel column was packed with the support GDX-103 (60–80 mesh). The temperatures of the injection port, oven, and detector were 220, 190, and 220 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 30 mL/min.

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### Table 2 | The composition of molasses wastewater

<table>
<thead>
<tr>
<th>Composition</th>
<th>Percentage (%)</th>
<th>Composition</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>75–85</td>
<td>MgO</td>
<td>0.01–0.1</td>
</tr>
<tr>
<td>Total sugar</td>
<td>48–58</td>
<td>K2O</td>
<td>2.2–4.5</td>
</tr>
<tr>
<td>Total organic carbon</td>
<td>28–34</td>
<td>SiO2</td>
<td>0.1–0.5</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen</td>
<td>0.2–2.8</td>
<td>Al2O3</td>
<td>0.05–0.06</td>
</tr>
<tr>
<td>P2O5</td>
<td>0.02–0.07</td>
<td>Fe2O3</td>
<td>0.001–0.02</td>
</tr>
<tr>
<td>CaO</td>
<td>0.15–0.8</td>
<td>Ash</td>
<td>4–8</td>
</tr>
</tbody>
</table>
The COD, pH, oxidation reduction potential (ORP), SS, VSS and biogas production volume were monitored and measured daily according to standard methods (Gilcreas 1966). All the samples obtained from this study were analyzed in triplicate.

RESULTS AND DISCUSSION

Coproduction of hydrogen and methane from molasses in CSTR-IC system

Hydrogen production in the CSTR

Coproduction of hydrogen and methane of the CSTR-IC system was started up at the OLR of 18 kg COD/(m³·d) using molasses wastewater as the substrate. The time courses of OLR, hydrogen production and hydrogen content are shown in Figure 2. In the CSTR, a maximum VHPR of 2.41 L/(L·d) was obtained at the OLR of 30 kg COD/(m³·d), and as the OLR continued rising to 36 kg COD/(m³·d), VHPR decreased to 1.38 L/(L·d). This can be explained by the relatively higher OLR of 36 kg COD/(m³·d) causing insufficient time for hydrolysis in the CSTR (Wang et al. 2013). Moreover, the accumulation of propionic acid under high OLR typically resulted in the failure of hydrogen production of the CSTR. Thus the OLR of 30 kg COD/(m³·d) can be determined to be the optimum condition for hydrogen production in the first phase of the two-stage anaerobic process. This result was consistent with Han et al. (2012), who obtained the maximum VHPR at the OLR of 32 kg COD/(m³·d) using molasses as substrate in a single CSTR for hydrogen production. Through the sludge acclimation and HRT control, the CSTR was stabilized as the first-stage of the two-stage anaerobic process, and no methane was detected throughout the experiment, mainly because the pH and HRT were both controlled at extremely low values not suitable for methanogens to grow (Antonopoulou et al. 2008).

Methane production in the IC reactor

The acidification and hydrolysis of organic matter mainly occurred in the CSTR, and ethanol-type fermentation (Ren et al. 1997) provided ideal substrates (ethanol, acetate, butyrate and propionate) for utilization of methanogens. After the hydrolysis phase in the CSTR, the IC reactor had a lower OLR; the average influent OLRs in each stage were 6.093, 7.86, 9.496 and 11.97 kg COD/(m³·d), respectively. The volumetric methane production rates (VMPRs) of each stage were linear with influent OLRs of the IC reactor, which were 1.32, 1.74, 2.17 and 2.4 L/(L·d), respectively (Figure 2). Through all the fermentation progress, the methane content was between 57.32% and 74.51%, and carbon dioxide and sulfur dioxide could also be detected in the fermentation gas.

Comparison with other two-stage anaerobic digestion processes

There are various reactor combinations in two-stage anaerobic digestion processes (Table 3), in which the VHPR was between 0.46 L/(L·d) and 2.51 L/(L·d) and the VMPR was between 0.33 L/(L·d) and 5.04 L/(L·d). That difference was mainly owing to the different substrates, operating OLRs, HRTs control and reactor combinations. To the best of our knowledge, few studies have used the IC reactor in a two-stage anaerobic digestion process. In this experiment, a two-stage CSTR-IC system for the coproduction of hydrogen and methane was successfully established, and moreover, this system was stable and efficient in

Figure 2 | OLR-dependent profile of the CSTR-IC two-stage system. (a) Hydrogen and methane production rate. (b) Hydrogen and methane content.
fermentating the cost-effective molasses wastewater in contrast with other reactor combination.

The configuration of CSTR-IC in this study had little difference with the configuration of CSTR-UASB (Wang et al. 2015), the main reason is because the CSTR had higher OLR than the methane reactor owing to a relatively shorter HRT and higher influent COD; however, CSTR had a relatively lower OLR capability than the UASB and IC reactors. Han et al. (2009) obtained the maximum OLR of 36 kg COD/(m³·d) using molasses as substrate in a single CSTR for hydrogen production. Shen et al. (2013) stated that their CSTR was overloaded with respect to glucose utilization at an OLR of 30 g COD/(L·d). In this study, the IC reactor used the effluent of the CSTR as the only carbon source for methanogen production after adding NaOH (4 M) to maintain the pH of 7; the concentration of the CSTR effluent decided the methane performance of IC reactor. Methane performance could improve only when the hydrogen reactor had good performance to supply optimal fermentation substrate.

CSTR-IC system performance

The CSTR-IC system was operated at 35 °C, and the pH was not artificially controlled in the CSTR, and the influent pH of the IC reactor was controlled at 7 by adding NaOH 4 M. The overall performance of the CSTR-IC system at steady OLRs is summarized in Table 4.

The COD removal efficiency of the CSTR was between 32% and 36%, because the organic substances produced (VFAs and ethanol) from the acidification and hydrolysis of organics remained in the effluents and methane production had not occurred (Ueno et al. 2007). The COD removal mainly occurred in the methane production process, and it could be greater than 68% during the two-stage operation. In this experiment the total COD removal efficiency of the CSTR-IC system was over 78%, and improve a maximum COD removal efficiency of 87.51% was obtained at the OLR of 36 kg COD/(m³·d).

According to the hydrogen production theory, fermentation type in an acidification reactor is very sensitive to the pH changes in anaerobic system; the butyrate-type, propionate-type and ethanol-type are favored by the pH of 4.8–5.2 or 5.8–6.5, 5.5 and 4.0–5.0, respectively (Zheng et al. 2015). In this study, the pH remained approximately stable in both the CSTR and IC reactor; the pH of the CSTR was stable between 4.31 and 4.62, which was a desirable pH range for both ethanol-type fermentation and hydrogen production (Ren et al. 2011). In this study, the effluent of the CSTR was firstly supplemented with NaOH to

Table 3 | Comparison of experimental data of VHPR and VMPR in two-stage anaerobic process

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Acidification reactor (HRT)</th>
<th>Methanization reactor (HRT)</th>
<th>Maximum VHPR (L/(L·d))</th>
<th>Maximum VMPR (L/(L·d))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese whey</td>
<td>CSTR (24 h)</td>
<td>PBR (4.4 d)</td>
<td>2.51</td>
<td>5.04</td>
<td>Antonopoulou et al. (2008)</td>
</tr>
<tr>
<td>Olive pulp</td>
<td>CSTR (7.5 h)</td>
<td>CSTR (10 d)</td>
<td>0.46</td>
<td>1.13</td>
<td>Koutrouli et al. (2009)</td>
</tr>
<tr>
<td>Municipality biowaste</td>
<td>CSTR (5 h)</td>
<td>CSTR (13 d)</td>
<td>0.85</td>
<td>1.75</td>
<td>Cavinato et al. (2011)</td>
</tr>
<tr>
<td>Mixed wastewater</td>
<td>CSTR (0.75 d)</td>
<td>CSTR (25 d)</td>
<td>1.72</td>
<td>0.33</td>
<td>Dareioti &amp; Kornaros (2014)</td>
</tr>
<tr>
<td>Molasses (OLR = 28.8 kg COD/(m³·d))</td>
<td>CSTR (5 h)</td>
<td>UASB (15 d)</td>
<td>3.06</td>
<td>2.01</td>
<td>Wang et al. (2013)</td>
</tr>
<tr>
<td>Molasses (COD = 28 g/L)</td>
<td>PBR (6 h)</td>
<td>PBR (6 d)</td>
<td>2.8</td>
<td>1.94</td>
<td>Park et al. (2010)</td>
</tr>
<tr>
<td>Molasses (OLR = 30 kg COD/(m³·d))</td>
<td>CSTR (6 h)</td>
<td>IC (12 h)</td>
<td>2.41</td>
<td>2.17</td>
<td>This study</td>
</tr>
</tbody>
</table>

PBR: packed-bed reactor.

Table 4 | Process performances of the CSTR-IC two-stage system

<table>
<thead>
<tr>
<th>OLR (kg COD/(m³·d))</th>
<th>CODin (mg/L)</th>
<th>COD removal efficiency (%)</th>
<th>pH</th>
<th>ORP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSTR</td>
<td>IC</td>
<td>CSTR</td>
<td>IC</td>
</tr>
<tr>
<td>18</td>
<td>4,500</td>
<td>3,020</td>
<td>32.31</td>
<td>68.26</td>
</tr>
<tr>
<td>24</td>
<td>6,000</td>
<td>3,930</td>
<td>34.53</td>
<td>73.44</td>
</tr>
<tr>
<td>30</td>
<td>7,500</td>
<td>4,740</td>
<td>36.72</td>
<td>78.37</td>
</tr>
<tr>
<td>36</td>
<td>9,000</td>
<td>5,985</td>
<td>33.59</td>
<td>80.21</td>
</tr>
</tbody>
</table>
neutralize the pH to 7 and then fed into the IC reactor, and the inner pH of the IC reactor was between 6.94 and 7.38, which was a desirable pH for the methanogens. The ORPs in the CSTR and the IC reactor were approximately stable at -430 and -645 mV, respectively.

Substrate conversion efficiency

During the progress of fermentation molasses, various kinds of SMP are generated along with the hydrogen production, and the main SMP were found to be ethanol, acetate, butyrate and propionate (Puyol et al. 2011). Syntrophic propionate-oxidizing bacteria and syntrophic butyrate-oxidizing bacteria in a methane production reactor oxidized propionate acid and butyric acid into acetate, and synthesized ATP through substrate level phosphorylation (Kato & Watanabe 2013), and methanogens use acetate to produce methane. The SCE of short-chain fatty acids in a methane production reactor determines the total bioenergy recovery rate.

The CSTR in this experiment was controlled at the HRT of 6 h and the fermentation type was kept stable at ethanol-type fermentation (Ren et al. 2013) under various OLRs, and the main end products were ethanol, acetate, butyrate, and propionate. The concentration of SMP and the SCE of the CSTR-IC system at each steady OLR are summarized in Table 5. As can be seen, the variation of OLRs had little effect on the SCE, and the average SCE was ethanol (100%) > acetate (92.37%) > butyrate (85.87%) > propionate (62.04%). These end products of ethanol-type fermentation can be used as appropriate substrates for methane production, and according to the equilibrium of the NADH/NAD⁺ ratio inside the fermentation microorganism (Han et al. 2015), the ethanol-type pathway was a more energy-efficient metabolic pathway than the butyrate-type or propionate-type pathway (Zhang et al. 2017).

Bioenergy recovery

Hydrogen and methane were simultaneously produced from the CSTR-IC two-stage system under various OLRs (18, 24, 30, 36 kg COD/(m³·d)) in this study. The bioenergy recovery rate was estimated in terms of the hydrogen COD equivalent (0.71 g O₂/L) and methane COD equivalents (2.86 g O₂/L). The OLR-dependent bioenergy recovery from the CSTR-IC system at each steady state is summarized in Table 6. The bioenergy recovery rate of the CSTR-IC system was between 59.98% and 71.06%, and the maximum bioenergy recovery of 71.06% was obtained at the OLR of 30 kg COD/(m³·d).

Cheng et al. (2012) obtained the total energy recovery of 67.1% by coproduction of hydrogen and methane with a CSTR-UASB two-stage anaerobic digestion of cornstalk. Corona & Razo-Flores (2018) obtained the total energy recovery of 56% by coproduction of hydrogen and methane with a CSTR-UASB two-stage anaerobic digestion of agave bagasse, compared to one-stage hydrogen production of 8.2%. In the CSTR-IC two-stage system, more than 80% of the total energy came from methane, and hydrogen had a much lower energy recovery rate than methane; this can be owing to the lower heating value per volume (Tahti et al. 2016) and the relative lower production rate.

CONCLUSION

Hydrogen and methane were simultaneously produced from molasses wastewater by the two-stage system composed of a CSTR and an IC reactor in this study. The HRT of 6 h for hydrogen and HRT of 12 h for methane production with

Table 5  Concentration of VFAs and ethanol and SCE of the CSTR-IC two-stage system

<table>
<thead>
<tr>
<th>OLR (kg COD/(m³·d))</th>
<th>TVFA (mg/L)</th>
<th>Ethanol (mg/L)</th>
<th>Acetate (mg/L)</th>
<th>Propionate (mg/L)</th>
<th>Butyrate (mg/L)</th>
<th>CSTR SCE (%)</th>
<th>IC SCE (%)</th>
<th>CSTR SCE (%)</th>
<th>IC SCE (%)</th>
<th>CSTR SCE (%)</th>
<th>IC SCE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>1,360.74</td>
<td>130.15</td>
<td>552.05</td>
<td>0</td>
<td>100</td>
<td>441.02</td>
<td>38.59</td>
<td>90.31</td>
<td>152.41</td>
<td>63.47</td>
<td>215.17</td>
</tr>
<tr>
<td>24</td>
<td>1,487.72</td>
<td>140.51</td>
<td>602.27</td>
<td>0</td>
<td>100</td>
<td>484.61</td>
<td>42.40</td>
<td>91.25</td>
<td>159.12</td>
<td>59.38</td>
<td>241.15</td>
</tr>
<tr>
<td>30</td>
<td>1,586.12</td>
<td>152.08</td>
<td>635.14</td>
<td>0</td>
<td>100</td>
<td>520.17</td>
<td>45.51</td>
<td>93.95</td>
<td>176.85</td>
<td>62.74</td>
<td>254.42</td>
</tr>
<tr>
<td>36</td>
<td>1,302.45</td>
<td>97.49</td>
<td>419.45</td>
<td>0</td>
<td>100</td>
<td>341.18</td>
<td>19.85</td>
<td>93.73</td>
<td>112.37</td>
<td>62.17</td>
<td>159.45</td>
</tr>
</tbody>
</table>

Table 6  Bioenergy recovery from the CSTR-IC two-stage system

<table>
<thead>
<tr>
<th>OLR (kg COD/(m³·d))</th>
<th>COD (g/L)</th>
<th>Hydrogen (L/d)</th>
<th>Methane (L/d)</th>
<th>Bioenergy recovery rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSTR</td>
<td>IC</td>
<td>Hydrogen (L/d)</td>
<td>Methane (L/d)</td>
<td>Bioenergy recovery rate</td>
</tr>
<tr>
<td>18</td>
<td>4.50</td>
<td>3.02</td>
<td>7.21</td>
<td>11.23</td>
</tr>
<tr>
<td>24</td>
<td>6.00</td>
<td>3.93</td>
<td>9.92</td>
<td>14.85</td>
</tr>
<tr>
<td>30</td>
<td>7.50</td>
<td>4.74</td>
<td>14.45</td>
<td>18.47</td>
</tr>
<tr>
<td>36</td>
<td>9.00</td>
<td>5.98</td>
<td>8.34</td>
<td>20.41</td>
</tr>
</tbody>
</table>
the OLR of 30 kg COD/(m³·d) can be employed as the optimum condition in the two-stage system. Through the continuous two-stage process for hydrogen and methane production, a maximum of 71.06% of the energy of the molasses wastewater was converted to the biogas. This study provides suitable operational conditions for industrial application of the continuous two-stage sequential process to maximize energy recovery from molasses wastewater.

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