Effect of organic loading rate on hydrogen (H2) and methane (CH4) production in two-stage fermentation under thermophilic conditions using palm oil mill effluent (POME)

Santhana Krishnan a, Lakhveer Singh a, Mimi Sakinah a, Sveta Thakur a, Zularisam A. Wahida a,⁎, Johan Sohailib

a Faculty of Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300, Gambang, Kuantan, Pahang, Malaysia
b Faculty of Civil Engineering Universiti Teknologi Malaysia Skudai Johor Malaysia

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The present study dealt with hydrogen and methane production in a sequential up-flow anaerobic sludge blanket (UASB) and continuous stirred tank reactor (CSTR) at thermophilic temperature. The POME was used as a suitable substrate of carbon source. The effect of various OLR was investigated from 25 kg-COD/m3·d to 125 kg-COD/m3·d at a constant hydraulic retention time (HRT) of 6 h. The UASB-H2 reactor was operated successfully at the OLR of 75 kg-COD/m3·d when the proportion of H2 in biogas, volumetric production rate of H2, specific hydrogen production rate (SHPR) and H2 yield reached the maximum values of 35%, 2.1 l/d, 175.15 ml H2/g COD applied, and 35% respectively. Further, the effluent from the UASB was directly fed into the CSTR at various OLR ranging from 4 kg-COD/m3·d to 20 kg-COD/m3·d for CH4 production. The maximum CH4 content, volumetric production rate of CH4, specific methane production rate (SMPR) and CH4 yield obtained were 65%, 13 l/d, 325.13 ml CH4/g MLVSS-d, and 155.87 ml CH4/g COD applied respectively. The effluent from CH4 reactor was recycled at feed flow rate recycle ratio of 1:1 to UASB unit and pH was maintained at 5.5, subsequently resulted in COD removal of 85%.

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Introduction

Rapid depletion of fossil fuels, anthropogenic emissions of the greenhouse gases (CO and NOx) and harmful effects of climate change has warned researchers to develop an alternative, renewable and sustainable energy from a wide range of organic wastes (Lee et al., 2010; Nasr et al., 2015). H2 has been widely considered as an ideally clean energy substitute for fossil fuels. It has high energy intensity per unit weight (142 kJ/g) and has high conversion efficiency without the generation of pollutants. It can be used as a pivotal raw material in industrial hydrogenation processes. Among different biological H2 producing methods, dark fermentation is considered to be the most feasible because in dark fermentation the H2 production rate is always greater than photo-fermentation and bio-photolysis (Dareioti and Kornaros, 2014). Especially, the two-stage fermentation including H2 and CH4 production with phase separation has a great potential to enhance the economic benefits of waste treatment. It has several advantages, including high H2 production rate, waste volume reduction and stabilization (Nathoa et al., 2014).

In two-stage fermentation, the unique acid formers and methane formers are developed in two separate bioreactors. In such system, only fast-growing acidogenic and H2 producing microorganisms are spotted in the first stage which involves the production of H2 and volatile fatty acids (VFAs). While in the second stage, slow-growing acetogenic and methanogenic bacteria are found in which VFAs are converted to CH4 and CO2 (Singh and Wahid, 2015a, 2015b). Generally, the first stage (fermentative hydrogenation) does not greatly reduce the organic content of the feed. The remaining un-degraded COD can be reduced in a subsequent second stage (methanogenesis) with the conversion of organic matter into CH4. Cavinato et al. (2012) showed that the chemical oxygen demand (COD) removal was below 36% during the first stage H2 production, corresponding to a yield of 2.5 mol/mol glucose. Moreover, the H2 fermentation stage can also be used as an independent H2 production unit but not as a precursor/pre-treatment for the methanogenic reactor.

Fermentative H2 production is highly dependent on the reactor operation conditions such as pH, temperature, OLR, HRT, solid retention time (SRT) and H2 partial pressure, which in turn affects the microbial metabolic balance and subsequently the fermentation end products.
Generally, it is considered that high SRT results in the growth of H2 consumers, including methanogens and non-H2-producing acidogens (Dareioti and Kornaros, 2015). However, less SRT could reduce active biomass retention, substrate uptake, and overall process efficiency (Intanoo et al., 2014). Changing the OLR can either increase or decrease the H2 yield. However, there is dissent in the literature as to whether higher H2 yields are achieved with lower or higher OLRs. In some cases, high OLRs decreased the H2 yield whereas in others higher OLRs increased the H2 yield (Venetsaneeas et al., 2009). The temperature is also another important factor that affects the process performance. The thermophilic temperature at 55 °C gives a greater biogas production rate than a mesophilic temperature at 35–37 °C because under the high temperature the reaction rate is higher as compared to lower temperature (Badiei et al., 2012). Similarly, Mamimin et al. (2005) investigated biohythane production using POME at 60 °C and found that high temperature increased the total energy yield to 15.34 MJ kg COD⁻¹.

In Malaysia, it is estimated that the annual production of POME is about 50 million tons. It is an agro-industrial waste generated from palm oil milling activities which require effective treatment before discharging into water bodies due to its highly polluting properties (Chu et al., 2013). Previously, many researchers have utilized POME as a carbon source to produce either H2 or CH4 separately (Zhao et al., 2012; Xie et al., 2008). Singh and Wahid (2015a, 2015b) investigated continuous H2 production using POME in a single stage and obtained the yield of 0.35 l H2/g COD. Similarly, methane was produced from palm oil waste using a consortium of Methanoculleus and Methanobrevibacter with the yield of 28.0 ± 0.34 ml g⁻¹ VS (Walter et al., 2015). Unfortunately, existing literature pertaining to the production of H2 from CH4 from the two-stage process using POME remains unexplored. Based on this information, a sequential H2 and CH4 production from POME was studied in the present study by using a two-stage UASB–CSTR process at a constant thermophilic temperature of 55 °C at various OLR. The effluent from CH4 reactor was recycled at feed flow rate recycle ratio of 1:1 to UASB unit and pH of H2 reactor was maintained at 5.5.

Materials and methods

Feedstock preparation

The POME was collected from receiving tank of Felda Palm Oil Industry, Lepar Hilir, Gambang, Malaysia and its characteristics are summarized in Table 1. It was then sieved to remove coarse particles and stored at 4 °C before use. Then POME was supplemented with Fe²⁺ iron of 275 mg Fe²⁺ l⁻¹. The COD:N:P ratio was maintained at an average of 250:5:2 with the addition of Na₂HPO₄·2H₂O. The pH was adjusted to 5.5 by adding NaOH prior to transfer to UASB reactor.

Table 1

<table>
<thead>
<tr>
<th>Parameter#</th>
<th>Concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>Biochemical oxygen demand (BOD)</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Chemical oxygen demand (COD)</td>
<td>76.5 ± 0.3</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>16.4 ± 0.2</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>0.81 ± 0.1</td>
</tr>
<tr>
<td>Ammonium-nitrogen</td>
<td>0.04 ± 0.001</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>0.11 ± 0.001</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.022 ± 0.001</td>
</tr>
<tr>
<td>Oil</td>
<td>10.9 ± 0.02</td>
</tr>
<tr>
<td>Total solids (TS)</td>
<td>32.0 ± 0.3</td>
</tr>
<tr>
<td>Volatile solids (VS)</td>
<td>25.0 ± 0.4</td>
</tr>
<tr>
<td>Suspended solids (SS)</td>
<td>8.3 ± 0.2</td>
</tr>
<tr>
<td>Ash</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>Fe</td>
<td>0.002 ± 0.0001</td>
</tr>
</tbody>
</table>

# All in g/l except pH

Inoculum

A granulated sludge sample was taken from the full-scale UASB unit treating POME and used as inoculum for starting up of UASB reactor. The sludge was heat-treated at 90 °C for 60 min in order to enrich indigenous H2 producing bacteria and inhibit the methanogens. It was then used as inoculum for the H2 production. The acclimated inoculum has 4.6 g-SS/l mixed liquor suspended solids (MLSS) and 5.6 g-VSS/l of mixed liquor volatile suspended solids (MLVSS). The heat-treated sludge was gradually acclimatized with POME in order to develop a stable microbial consortium. The acclimatized sludge was operated by discharging 50% cultured broth and the addition of 50% fresh POME every 24 h. Anaerobic sludge without heat treatment was used as inoculum for CH4 production. It was maintained in an incubator for 5 d until no biogas production was seen in order to minimize the organics’ degradation present in the inoculum. The inoculum was contained of 15.4 g-l⁻¹ of total solids (TS), 12.1 g-l⁻¹ of volatile solids (VS) and 10.4 g-l⁻¹ of volatile suspended solids (VSS). Both the reactors were inoculated with 10 g-VSS l⁻¹ of 400 ml thermally treated sludge and 600 ml non-treated sludge for H2 and CH4 production, respectively.

Reactor operation and monitoring

Fig. 1 illustrates the schematic diagram of the two-stage reactor system. A borosilicate glass made reactor consisted of sequential UASB–CSTR with an effective working volume of 4 l and 8 l for H2 and CH4 production, respectively. The temperature inside both reactors was controlled uniformly at 55 °C using temperature controller. The POME was continuously fed into the bottom of UASB reactor in an upward flow direction using a peristaltic pump to achieve various OLR such as 25, 50, 75, 100, 125 kg-COD/m³·d (based on the feed organic loading). The temperature of VFAs and the ethanol. Helium was used as another carrier gas with linear flow rate of 40 ml min⁻¹. The heat-treated sludge was mixed thoroughly using the vortex [G-560 Scienti Industries, USA] prior COD analysis. The temperature of the column was 100 °C, the injection and detection temperatures were 120 °C. The concentrations of H2, CH4, CO2 and N2 were determined using a 3-point calibration with standard gas (GL Sciences). A gas chromatograph (GC 16, Shimadzu, Kyoto, Japan) connected to flame ionization detector (FID) Stabilwax-DA capillary column (60 m x 0.53 mm ID, Resteck, USA) was used to measure the contents of VFAs and the ethanol. Helium was used as another carrier gas with the flow rate of 40 ml min⁻¹. The heat-treated sludge was mixed thoroughly using the vortex [G-560 Scientific Industries, USA] prior COD test, carbohydrate test and protein assay. The COD in the POME, effluent samples from UASB and CSTR were measured using dichromate method.

Analysis

The gas mixtures of H2, CH4, CO2, and N2 were quantified using a Shimadzu GC-8A gas chromatograph attached to a thermal conductivity detector (TCD) and a stainless-steel column (2 m x 0.3 mm) packed with molecular sieve (80/100 mesh, Shimadzu GLC Ltd., Japan). The argon was used as carrier gas and the flow rate and pressure were maintained at 50 ml min⁻¹ and 6 kg/cm² respectively. The temperature of the column was 100 °C, the injection and detection temperatures were 120 °C. The concentrations of H2, CH4, CO2 and N2 were determined using a 3-point calibration with standard gas (GL Sciences). A gas chromatograph (GC 16, Shimadzu, Kyoto, Japan) connected to flame ionization detector (FID) Stabilwax-DA capillary column (60 m x 0.53 mm ID, Resteck, USA) was used to measure the contents of VFAs and the ethanol. Helium was used as another carrier gas with the flow rate of 40 ml min⁻¹. The heat-treated sludge was mixed thoroughly using the vortex [G-560 Scientific Industries, USA] prior COD test, carbohydrate test and protein assay. The COD in the POME, effluent samples from UASB and CSTR were measured using dichromate method.
according to Zhu et al. (2008). The carbohydrate concentration was estimated by the phenol-sulphuric acid method, using glucose as a standard (Dubois et al., 1956). The protein content was analysed using the Lowry protein assay rapid kit, (Sigma, USA). The NH$_4^+$–N and total nitrogen (TN) were determined by an auto-analyser TN 3000 using a colorimeter to detect colour changes in the analytes (Thermo Electron Corp, USA). The sludge samples were centrifuged at 12,000 rpm for 15 min, then it was filtered through a 0.45 μm membrane preparatory to analyse the VFAs, ethanol, and NH$_4^+$–N concentrations. The oil, ash, Fe, BOD, TS, MLVSS, VSS and alkalinity concentrations were estimated using procedures outlined in APHA standard methods (APHA, 2005). The total phosphorous contents in the feed and effluent samples were determined by the molybdovanadate method with acid persulfate digestion.

Results and discussion

H$_2$ production in UASB unit

H$_2$ production efficiency

The biogas composition, H$_2$ production rate and SHPR inside UASB unit are shown in Fig. 2a and b. The biogas produced was mainly composed of H$_2$ and CO$_2$ with a small amount of CH$_4$. The CH$_4$ content steadily decreased with increase in OLR, corresponding to the HRT reduction from 12 h at OLR of 25 kg/m$^3$.d to 3 h at OLR of 125 kg/m$^3$.d. The increase in the OLR from 25 to 75 kg/m$^3$.d positively increased H$_2$ content, H$_2$ production rate and SHPR. This is due to the fact that increasing COD loading rate provides more carbon source for the microorganisms that might have enhanced the microbial activity to produce more H$_2$. Further increasing OLR from 75 to 125 kg/m$^3$.d positively increased the H$_2$ content, H$_2$ production rate and SHPR. This is due to the fact that increasing COD loading rate provides more carbon source for the microorganisms that might have enhanced the microbial activity to produce more H$_2$. Further increasing OLR from 75 to 125 kg/m$^3$.d, the system alkalinity slightly decreased. The decrease in alkalinity has resulted due to an increase of VFA in the system. It should be indicated that the alkalinity range in the H$_2$ UASB reactor was considerably less because of the low pH 5.5 in the system.

VFAs composition

The total VFA concentration against OLR in H$_2$-UASB unit is shown in Fig. 2e. The total VFA significantly increased with the increase in OLR from 25 to 100 kg-COD/m$^3$.d and moderately increased with further increasing OLR beyond 100 kg-COD/m$^3$.d. The maximum total VFA concentration of 8800 mg/l as acetic acid was at the highest OLR of 125 kg-COD/m$^3$.d. From Fig. 2e, it can be understood that the toxicity level of VFA to H$_2$-producers, was around 9000 mg/l as acetic acid and is in agreement with results reported in previous studies by Walter et al. (2015) and Chong et al. (2009). Also, the produced VFA contained butyric acid (H$_{Bu}$), valeric acid (H$_{Va}$), acetic acid (H$_{Ac}$) and propionic acid (H$_{Pr}$). All individually produced VFAs were reported to have the similar pattern like total VFA concentration, except the H$_{Pr}$ concentration slightly increased in the highest OLR. When H$_{Bu}$ concentration was at its peak, the H$_{Pr}$ concentration was the lowest, which contributed the system to achieve high H$_2$ production performance (Cavinato et al., 2011). According to the microbial metabolic pathway, all VFAs are produced along with the H$_2$ production, while the consumption of H$_2$ results in the formation of H$_{Pr}$ (Intanoo et al., 2012) as shown in the following equation

\[
\text{CH}_3\text{COOH} + \text{H}_2 \rightarrow \text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O}
\]

Also, ethanol production shows an inhibitory effect during H$_2$ production. Under anaerobic condition, production of 1 mol ethanol consumes
In this study, only a small amount of ethanol was produced and its concentration remained constant throughout the COD loading rate range of 25–125 kg/m$^3$·d. Hence, the effect of ethanol production may be negligible.

**Substrate degradation and gas production rate**

Fig. 2c shows gas production rate and COD removal percentage of the H$_2$-UASB unit. The COD removal increased with the increase in OLR and achieved a maximum value of 40% at an OLR of 75 kg-COD/m$^3$·d. Beyond the OLR of 75 kg-COD/m$^3$·d, the COD removal decreased with further increase in the OLR. The rate of gas production also showed a similar direction of the COD removal. The maximum gas production rate of 4.5 l/d was found at the same OLR of 75 kg-COD/m$^3$·d. It is known that POME is enriched with high organic matter in terms of a COD value around 85,500 mg/l. Hence, a higher OLR could have provided a greater organic load for microbial growth and its activities, which resulted in the increased COD removal and hydrogen production rate (Ma et al., 2015). However, with further increase in the OLR to 100 kg-COD/m$^3$·d, both COD removal and gas production rate were decreased. This was resulted due to the increase in toxicity from VFA accumulation (feedback inhibition) in the system (Chuang et al., 2011).

**CH$_4$ production in CSTR unit**

**CH$_4$ production efficiency**

The CSTR was operated successfully throughout the study and the best results were obtained when the OLR in a hydrogenic reactor was 75 kg-COD/m$^3$·d or 12 kg-COD/m$^3$·d based on the feed organic load.
and CH4-CSTR unit working volume. Fig. 3a shows produced gas from the CH4-CSTR unit mainly contained CH4 and CO2 with a very small amount of H2 (<0.5%). Both CH4 content and CH4 production rate increased with increasing in OLR from 4 to 12 kg-COD/m3·d. However, it started decreasing with any further increasing OLR from 12 to 20 kg-COD/m3·d (Fig. 4b). In contrast, the CO2 content in the produced gas showed an opposite pattern. The maximum CH4 content and CH4 production rate of 65% and 13 l/d, respectively, were found at an OLR of 12 kg-COD/m3·d, corresponding to the unchanged optimum OLR of 75 kg/m3·d in UASB unit. Fig. 3b shows the specific CH4 production rates (SMPR) and CH4 yields. The maximum values of SMPRs (600 ml CH4/l d or 325.13 ml CH4/g MLVSS d) and CH4 yields (155.87 ml CH4/g CODremoved or 90.25 ml CH4/g CODapplied) were at an OLR of 12 kg-COD/m3·d. Hence, the OLR of 12 kg/m3·d was considered to be an optimum for both productions of H2 and CH4 by the two-stage thermophilic UASB–CSTR system. In comparison with other researchers, the maximum CH4 yield found in this study was about 20% higher than that from municipal solid waste using the two-stage process at 37 °C (266 ml/g VS) by Giuliano et al. (2014). The pH and alkalinity in CSTR unit increased with increasing OLR and reached a maximum level at an OLR of 12 kg-COD/m3·d (Fig. 3d). With further increasing OLR from 12 to 20 kg/m3·d, the system pH from 7.2 to 6.1 and alkalinity sharply declined. This was resulted due to the VFA increase in the system and affected the activity of methanogenic bacteria, which in turn decreased the CH4 production rate.

VFAs composition
The total VFA composition versus OLR effect inside CSTR unit is shown in Fig. 3e. The maximal total VFA of 540 mg/l as acetic acid was observed at the highest OLR of 20 kg/m3·d. Based on the results of gas production rate, total VFA and COD removal the toxic level of VFA to the methanogenic bacteria was found to be around 350 mg/l which is similar to the work of Mamimin et al. (2005). The VFAs produced in the CSTR unit contained HAc, HPr, Hbu and Hva, and all produced VFAs also increased gradually when the OLR was increased with respect to the order of HAc > HPr > Hbu > Hva. The maximum HAc concentration has resulted due to further breakdown of both HPr and Hbu. Basically, the production of CH4 mainly results from the two basic bioconversion reactions of hydrogenotrophic and acetotrophic pathways, as shown in the following equations:

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} & \rightarrow 2\text{CH}_3\text{COOH} + 2\text{H}_2 + 2\text{H}_2 \\
\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} & \rightarrow \text{CH}_3\text{COOH} + \text{CO}_2 + 3\text{H}_2 \\
\text{CO}_2 + 4\text{H}_2 & \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \\
\text{CH}_3\text{COOH} & \rightarrow \text{CH}_4 + \text{CO}_2
\end{align*}
\]

Substrate degradation and gas production rate
As mentioned above, the effluent from the H2-UASB unit was directly transferred to the CH4-CSTR unit for further digestion to produce CH4. Fig. 3c shows the COD removal at different OLR (based on the feed COD load and CH4-CSTR volume). The COD removal increased with increase in OLR and reached a maximum value of 85% at an OLR range of 12 kg-COD/m3·d. Further increase in OLR beyond 12 kg-COD/m3·d decreased the COD removal percentage. The gas production rate was similar to the COD removal percentage. Interestingly, the gas production rate of the CSTR unit was about 3 times higher than that of UASB unit, due to the size ratio of both reactors.
MLVSS and effluent VSS concentration

As shown in Fig. 4 and b, the MLVSS decreased with increase in OLR and the effluent VSS has shown an opposite pattern. Increasing OLR from 25 to 75 kg-COD/m³·d increases the organic load available for microbial activities to produce more gases. Also, VSS concentration in the effluent increased due to microbial washout and this indicated clearly the washout of inactive microbes. With further increase in OLR from 75 kg-COD/m³·d to 125 kg-COD/m³·d decreased the production efficiency with the accumulation of high organic acid concentration of 9000 and 350 mg/l in UASB and CSTR reactors respectively. Generally, the microbial activity depends on the pH concentration. The low level of sodium (210–330 mg/l) stimulates the activity of methanogens whereas high level greater than 15,000 mg/l, exhibits toxicity (Schievano et al., 2012). In this study, the sodium level in both UASB and CSTR units were maintained by effluent recycle in the range of 130–210 mg/l. The difference in sodium level between feed (118 mg/l) and the final effluent (130–210 mg/l) was not significantly varied, clearly showing that effluent recycling strategy could reduce the buffer usage for pH adjustment in the H2-UASB unit.

Nitrogen, phosphorous and iron uptakes and transformation

Fig. 5 shows the nitrogen (N), phosphorous (P) and iron (FeSO₄) uptakes in both steps of acidogenesis and methanogenesis. The uptake of nitrogen and phosphorous and iron concentrations in the two-stage fermentation was increased with increasing COD loading rate. With higher COD loading rate, the nutrient uptakes were also increased. Generally, N, P and FeSO₄ enhance hydrogen production and yield. It is considered that N and P are required for the growth of the microorganisms and the biological hydrogen production depends on the activity of hydrogenase (iron-containing enzyme) (Cheng and Liu, 2012). At an optimal COD loading rate, both N and P uptakes in the H₂-UASB unit were slightly higher than those in the CH₄-UASB unit. Sompong et al.

![Graph a)

![Graph b)

![Graph c)
(2007) reported that bio-stimulants such as nitrogen and phosphorus are required for the growth of bacteria, especially the dominant *Thermoanaerobacterium* species in H$_2$-UASB reactor under thermophilic conditions. Such variations in the N and P uptakes between the two reactors were due to the difference in microbial metabolism, reactor configuration and operation conditions.

Overall performance

Comparison of process performance with some previous studies is shown in Table 2. The best COD removal of 85% was observed in this study. In comparison with the previous reports (Suksong et al., 2015; Mohammadi et al., 2012), the present study indicates that the
maximum H₂ and CH₄ yield obtained was 49.22 ml H₂/g COD and 155.87 ml CH₄/g COD at the optimum OLR of 12 kg-COD/m³·d. The mixed H₂/CH₄ biogas ratio of 1:6.5 was produced. The mixed gas produced known as hythane, contained 7.11% H₂, 54.13% CH₄ and 38.75% CO₂ with the total energy yield of 19.25 MJ/m³. Such good results in SHPR and SMPR were probably due to temperature conditions (55 °C). Similarly, Mamimin et al. (2005) achieved mixed H₂ and CH₄ yield of 4.4 l biogas l⁻¹ d⁻¹ with biogas composting of 51% CH₄, 14% H₂ and 35% CO₂ by the two-stage process. The difference in the production yield and hythane ratio is due to the difference in the reactor design, temperature and feeding ratio.

**Conclusions**

In the experimental optimisation of the hydrogen production, the process was stable up to 50 kg-COD/m³·d but the best results were achieved when the OLR was in the range of 75 kg-COD/m³·d. The maximum H₂ production rate was 175.15 ml H₂/g MLVSS d, while the highest H₂ content and yield were 35% and 49.22 ml H₂/g COD applied respectively. Methane production using hydrogen effluent as a substrate occurred with the satisfactory yield. In the methanogenic stage, the methane yield was stable when the OLR in the hydrogen UASB reactor was 75 kg-COD/m³·d and 12 kg-COD/m³·d based on the feed COD load and

**Table 2**

Comparison of previous studies on hydrogen and methane production from biomass using mixed culture.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Two stage process</th>
<th>Single stage process</th>
<th>Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of waste water</td>
<td>POME</td>
<td>POME</td>
<td>POME</td>
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<tr>
<td>Inoculum</td>
<td>Decanter cake</td>
<td>Anaerobic seed sludge</td>
<td>Granulated sludge</td>
</tr>
<tr>
<td>Reactor type</td>
<td>500 ml serum bottles</td>
<td>UASB-CSTR</td>
<td>UASB-CSTR</td>
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<tr>
<td>pH</td>
<td>5.5/7</td>
<td>5.5/7.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Temperature</td>
<td>60 °C/60 °C</td>
<td>55 °C/37 °C</td>
<td>55 °C</td>
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<tr>
<td>Optimal OLR</td>
<td>60 g VS/l·d</td>
<td>6.0 g COD/l·d</td>
<td>75 g COD/l·d</td>
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<tr>
<td>HRT</td>
<td>24 h</td>
<td>48 h/15 d</td>
<td>9 h/12 d</td>
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<tr>
<td>COD removal efficiency</td>
<td>62%</td>
<td>84%</td>
<td>40%</td>
</tr>
<tr>
<td>H₂ yield</td>
<td>16.26 ml/g VS</td>
<td>21 ml H₂/g COD⁻¹</td>
<td>24.5 mmol H₂/g COD_removed</td>
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<tr>
<td>H₂ production rate</td>
<td>1.48 L H₂ l⁻¹ d⁻¹</td>
<td>1.84 L H₂ l⁻¹ d⁻¹</td>
<td>1.24 L H₂ L⁻¹ d⁻¹</td>
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<tr>
<td>CH₄ yield</td>
<td>240.65 ml/g VS</td>
<td>315 ml CH₄/g COD⁻¹</td>
<td>0.124 L H₂ L⁻¹ d⁻¹</td>
</tr>
<tr>
<td>CH₄ production rate</td>
<td>51.39 m³/ton waste</td>
<td>2.61 CH₄ l⁻¹ d⁻¹</td>
<td>0.124 L H₂ L⁻¹ d⁻¹</td>
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<tr>
<td>Total energy content</td>
<td>13.9 Mj kg VS</td>
<td>15.34 Mj kg COD⁻¹</td>
<td>4.83 MJ kg COD⁻¹</td>
</tr>
</tbody>
</table>

**Fig. 5.** Effects of OLR on (5a) nitrogen, phosphorous and iron uptakes of the H₂-UASB unit and (5b) nitrogen, phosphorous and iron uptakes of the CH₄-CSTR unit.
CH₄ UASB volume. The maximum CH₄ content, CH₄ yield, and SMPR were 68%, 155.87 ml CH₄/g COD applied and 325.13 ml CH₄/g MLVSS d, respectively. The overall COD removal efficiency of the two-stage process was found to be 85%. These results indicate that the POME could be efficiently used for H₂ and CH₄ production and the two-stage process can operate successfully under these experimental conditions.

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