Hydrogen and methane production by co-digesting liquid swine manure and brewery wastewater in a two-phase system

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Abstract

Co-digesting liquid swine manure and brewery wastewater for hydrogen and methane production was studied using an integrated, two-phase system with different organic loading rates (OLR) under mesophilic conditions. The highest volumetric hydrogen and methane production rates achieved were 294.06 ± 3.06 mL H2 L−1 d−1 and 497.94 ± 10.01 mL CH4 L−1 d−1, respectively, at the OLR of 8613.6 mg COD L−1 d−1, together with the highest hydrogen-COD and -TVS yields (34.14 ± 0.36 mL g−1 COD and 45.46 ± 0.71 mL g−1 TVS) and the maximum methane-TVS yield (261.42 ± 8.41 mL g−1 TVS). The highest hydrogen and methane concentrations in the biogas (31.86 ± 0.68% and 82.66 ± 0.33%, respectively) were also obtained at this OLR. The maximum methane-COD yield (270.3 ± 3.95 mL g−1 COD), however, was from the lowest OLR (1148.6 mg COD L−1 d−1). The two-phase system removed 75.54 ± 0.19% of COD from the influent.

1. Introduction

Manure from various livestock is being produced in increasing amounts due to the rising human population and parallel increase in quality of life. Manure contains abundant nitrogen, carbon, phosphorus, and ammonia, which can cause harm to various aspects of the environment such as water sources, soil, and air (He et al., 2016). The simplest method to deal with manure is to directly use it as fertilizer. However, the volume of manure produced today is much more than can be utilized as fertilizer. Other methods must be used to accommodate the excess manure. Reactors utilizing anaerobic digestion (AD) can offset manure’s environmental impact by removing much of its harmful substances as well as producing biogas, which can be used as an alternative energy source to traditional, environment-damaging fossil fuels (Battini et al., 2014). Treating manure via AD is a well-established practice, and there are many reports on this technique, mainly on cattle (Li et al., 2018; Neshat et al., 2017; Ozbayram et al., 2017) and poultry (Bayrakdar et al., 2018; Rodriguez-Verde et al., 2018) manure. Since AD produces valuable biogas as a byproduct that has very little impact to the environment, it is the most cost-effective treatment option for wastes such as manure (Kothari et al., 2014).

More recently, researchers have started to perform anaerobic digestion in two phases. A hydrogen-producing dark fermentation phase is followed by a second phase in which the AD process relies on the products of dark fermentation to produce methane. There are several advantages of this approach. First, there are two types of bacteria that are crucial for AD, i.e., acidogenic and methanogenic bacteria. Separating the AD process into two phases allows for the separate cultivation of these two types of bacteria, which thrive at different pH levels and grow at different rates (Cooney et al., 2007). Second, during the first phase, complex substrates can be efficiently broken down by certain bacteria responsible for hydrogen production (Hawkes et al., 2007), so that in the second phase, i.e., the methanogenic phase, there is more matter readily available to be converted to methane (Giordano et al., 2011). Hidalgo et al. (2014) found that a two-phase system yielded more bioenergy than a one-phase system. This process also yields hydrogen gas, which is a cleaner, more efficient energy source than methane, a major benefit of the two-phase process. Also, it is possible to utilize both products such as a combination of methane and hydrogen gas as fuel for natural gas driven vehicles (Bauer and Forest, 2001).

In 2018, there were 73.5 million swine in the USA, generating about 138 billion liters of manure per year (Wang et al., 2019). Swine manure contains a lot of lignocellulose, making it difficult to decompose via anaerobic digestion (Cheng et al., 2014). A study by Sun et al. (2015) suggested that swine manure had a better potential to produce methane during AD than cattle manure.

It is inefficient to digest manure alone via AD. Riaño et al. (2011)
suggested that co-digesting manures with agricultural (or otherwise plant-derived) residues that contained a large amount of carbon, such as certain wastewaters, was an effective method to efficiently digest both substances. Manures have a lot of ammonia as well as nutrients to help methanogenic bacteria flourish. And agricultural residues with a low pH could help balance the manure’s high pH and prevent ammonia inhibition, which is very detrimental to biogas production. Past research showed that the two substances, when digested together, had a much higher buffering capacity and were much more easily digested (Riaño et al., 2011). Also, because the manure is high in nitrogen and the residue is high in carbon, combining the two for co-digestion results in a better carbon-nitrogen ratio (Mao et al., 2017), which, as confirmed by many authors, is an important factor that contributes to the AD process efficiency (Mao et al., 2017; Wu et al., 2010; Zhang et al., 2011).

Beer is one of the most popular alcoholic beverages in the world. In 2014, the world’s top ten beer-producing countries produced 119 billion liters of beer (Arantes et al., 2017). Considering that each liter of beer produced required 4.5–10 L of water, a massive amount of wastewater resulted. This wastewater is generally high in chemical oxygen demand (COD), suspended solids, and carbon (Arantes et al., 2017), making it a good candidate for co-digestion with manure.

The purpose of this study was to investigate the efficiency of the two-phase system for producing hydrogen and methane via anaerobic co-digestion of swine manure and brewery wastewater. To our knowledge, there are presently no other studies that use these two substrates for co-digestion in a two-phase system. The experiment tested the effect of different organic loading rates (OLRs) to determine the most effective rate for co-digestion.

2. Materials and methods

2.1. Construction and configuration of the reactors

For the experiment, the anaerobic sequencing batch reactor (ASBR) and upflow anaerobic sludge blanket (UASB) reactor were chosen for hydrogen and methane production, respectively. Both reactors were assembled by hand. The ASBR reactor (Fig. 1a) was made by assembling a cylinder made of PVC, one PVC plate, two PVC flanges with eight bolts, and a gasket. The PVC plate and one flange were glued to the bottom and top of the cylinder using PVC glue. Finally, the two flanges were bolted together against a gasket. The ASBR had an inner diameter of 20.32 cm and a total height of 43.18 cm, with a total volume of 14 L (working volume: 7 L). During the experiment, the ASBR’s internal temperature was maintained at 37 ± 2 °C. This was achieved by keeping a water bath in a five-gallon bucket with a heater immersed in the water and using pumps to circulate the heated water through plastic tubes wrapped around the reactor, which formed the water jacket, and back to the water bath. A temperature controller modulated the heating and pumping of the water to maintain the temperature. To mix the liquid inside the ASBR, a centrifugal water pump was used. A pH controller with two low-flow chemical metering pumps (McMaster-Carr, 4049KR8, USA), one for pumping 0.5 M NaOH and another for 0.2 M HCl, maintained a pH level of 5 inside the ASBR.

The UASB (Fig. 1b) was made with the same materials as the ASBR, except the bottom of the UASB was composed of a piece of acrylonitrile butadiene styrene (ABS) shaped like a cylinder that tapered into a cone at the bottom. The cone had a height of 7.62 cm and a diameter of 15.24 cm, the same diameter as the ASBR. The UASB had a total height of 1.40 m and a total volume of 25 L. Approximately 88% of the UASB’s volume was the digestion section located in the bottom part of the reactor, and the remainder of the volume at the top of the reactor was the solid-liquid-gas separation section, where the three-phase separator was placed. The height of the three-phase separator was 15.0 cm. The length of the defectors was 4.4 cm, and they formed a 45° angle with each side of the reactor such that each deflector’s end was 2.2 cm away from the inner wall of the reactor. A funnel was used at the top of the UASB to help the gas exit the reactor. The funnel was inverted so that the neck was facing upward. The bottom of the inverted funnel was 1 cm away from the inner wall of the reactor. The height of the inverted funnel from its bottom to the top of the reactor was 11.6 cm; 2.5 cm of the neck was inside the reactor, and the remainder of the neck came out of the reactor, leading the gas into a tube for collection. Temperature regulation was done in the same way as the ASBR, using the same method and the same temperature of 37 ± 2 °C.

2.2. Preparation of substrates

The swine manure (SM) used as substrate was obtained from the University of Arkansas Swine Farm. The swine manure was mixed for 10 min, then pumped from the pit and immediately transported to the lab and stored in the freezer to be thawed right before use. The swine manure had an average residence time in the pit of approximately 5 days. The brewery wastewater (BW) was collected from the sparging stage of beer production at the Core Brewery & Distilling Company, Springdale, Arkansas. Characteristics of both the original swine manure and the prepared co-fermentation substrate, including total solids (TS), total volatile solids (TVS), total suspended solids (TSS), reactive phosphorus (RP), total phosphorus (TP), total ammonia nitrogen (TAN), total nitrogen (TN), and sugars, were presented in Table 1.

2.3. Inoculum, reactor integration, and startup

The seed sludge for both reactors was collected from a bench-scale UASB reactor at Little Rock Wastewater Utility, AR, USA. This reactor was operated for methane production from municipal wastewater. The TS and TVS of the sludge were 54.44 and 32.99 g L⁻¹, respectively.

Before being applied as inoculum, the sludge to be used in the ASBR was first sieved with a 1.4 mm pore size sieve (No. 14) to eliminate big chunks of debris. To enhance its active bacterial population, 3.5 L of the prepared co-fermentation substrate, including total solids (TS), total volatile solids (TVS), total suspended solids (TSS), reactive phosphorus (RP), total phosphorus (TP), total ammonia nitrogen (TAN), total nitrogen (TN), and sugars, were presented in Table 1.

The swine manure was sieved through the same sieve used for the ASBR sludge, then 6.25 L of Nutrient Solution B was used as inoculum, 3.5 L of another nutrient solution (called Nutrient Solution B in this paper) was pumped into the reactor to boost the growth of the sludge and make the acclimatization step more efficient. Each liter of Nutrient Solution B contained 10 g glucose, 1.5 g KH₂PO₄, 3.2 g Na₂HPO₄·7H₂O, 0.5 g NH₄Cl, 0.22 g MgSO₄·7H₂O, 0.5 g CaCl₂, 1.5 g yeast extract, and 1.5 g peptone. The sludge was then left at room temperature for 24 h. Afterwards, the pH of the sludge was adjusted to 5.0 with 1 mol L⁻¹ HCl, and the sludge was heated at 100 °C for 30 min to select for acidogenic bacterial species. Then, excess liquid was drained until the sludge mixture was reduced to 3.5 L before being placed in the ASBR.

After the ASBR’s inoculation, 3.5 L of another nutrient solution (called Nutrient Solution B in this paper) was pumped into the reactor to boost the growth of the sludge and make the acclimatization step more efficient. Each liter of Nutrient Solution B contained 1 g glucose, 0.064 g NH₄Cl, 0.015 g KH₂PO₄, 0.0306 g MgSO₄·7H₂O, 0.025 g CaCl₂, 0.0064 g MnSO₄·H₂O, 0.0025 g CuSO₄·5H₂O, 0.32 g ZnSO₄·7H₂O, and 0.0025 g COCl₂·6H₂O. After three days, 3.5 L of liquid was pumped out of the ASBR, and it was ready for the experiment.

The sludge to be used in the UASB was sieved through the same sieve used for the ASBR sludge, then 6.25 L of Nutrient Solution B was mixed with the same amount of sludge inside the UASB for 3 days at 37 ± 2 °C. After that, the reservoir holding the effluent from the ASBR was connected to the UASB via a peristaltic pump when the experiment started.

The substrate was stored in an influent tank and mixed with a magnetic mixer, and a pump was used to recirculate the liquid. The substrate (influent) into the ASBR and the effluent from the ASBR were moved by peristaltic pumps (Core-Parmer, 7551-20, USA) and modulated in order to control the hydraulic retention time (HRT). The same type of microtube pump that controlled the ASBR’s pH also maintained the pH of the ASBR effluent (i.e., the UASB’s influent) at 7.5. The feeding, decanting, and settling of the ASBR were automatically controlled by a digital time controller (CR1000, Campbell Scientific, USA).
Fig. 1. Diagram of (a) ASBR’s and (b) UASB’s components.

Table 1
Composition of experimental substrates.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>TS (g L⁻¹)</th>
<th>TVS (g L⁻¹)</th>
<th>TSS (g L⁻¹)</th>
<th>COD (mg L⁻¹)</th>
<th>RP (mg L⁻¹)</th>
<th>TP (mg L⁻¹)</th>
<th>TAN (mg L⁻¹)</th>
<th>TN (mg L⁻¹)</th>
<th>Sugars (g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM</td>
<td>7.6</td>
<td>13.2</td>
<td>8.4</td>
<td>8.6</td>
<td>15433.0</td>
<td>514.3</td>
<td>667.5</td>
<td>1320.8</td>
<td>1839.8</td>
<td>0.0</td>
</tr>
<tr>
<td>BW</td>
<td>5.7</td>
<td>38.9</td>
<td>38.1</td>
<td>5.7</td>
<td>49525.0</td>
<td>41.3</td>
<td>61.6</td>
<td>85.1</td>
<td>166.0</td>
<td>34.5</td>
</tr>
</tbody>
</table>
The control module, LoggerNet 4.4 (Campbell Scientific, USA), of the computer system was programmed to control the time of the sequence of events in the following way: 4 min for influent feeding, 200 min for reaction, 32 min for settlement, and 4 min for effluent decanting.

2.4. Experimental setup

Table 2 showed the composition of the influent, the HRT schedule, and the resulting OLR used throughout each stage in the experiment. The influent started out as a modified Nutrient Solution B that contained three times the concentration of all its ingredients per liter as the original, denoted NS-B3. Then, NS-B3 was gradually replaced with a swine manure and brewery wastewater mixture diluted with tap water. The ratio of SM to BW was always 2:1. The experimental design kept the OLR relatively low during the start-up stage to allow the biological system to acclimate.

The start-up stage consisted of the first three stages (S-I, S-II, S-III). Each change to the influent after the start-up stage (experiment stage, Table 2) was done to observe the system's response to different conditions.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Time (d)</th>
<th>NS-B3 (%)</th>
<th>SM/BW (%)</th>
<th>Tap water (%)</th>
<th>Total COD (mg L⁻¹)</th>
<th>HRT (h)</th>
<th>OLR (mg COD L⁻¹ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-I</td>
<td>1–27</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>3000</td>
<td>24</td>
<td>1285.7</td>
</tr>
<tr>
<td>S-II</td>
<td>28–54</td>
<td>50</td>
<td>5</td>
<td>45</td>
<td>2840</td>
<td>24</td>
<td>1217.1</td>
</tr>
<tr>
<td>S-III</td>
<td>55–79</td>
<td>0</td>
<td>10</td>
<td>90</td>
<td>2680</td>
<td>24</td>
<td>1148.6</td>
</tr>
<tr>
<td>E-I</td>
<td>80–99</td>
<td>0</td>
<td>20</td>
<td>80</td>
<td>5359</td>
<td>16</td>
<td>3445.1</td>
</tr>
<tr>
<td>E-II</td>
<td>100–111</td>
<td>0</td>
<td>30</td>
<td>70</td>
<td>8039</td>
<td>16</td>
<td>5167.9</td>
</tr>
<tr>
<td>E-III</td>
<td>112–128</td>
<td>0</td>
<td>40</td>
<td>60</td>
<td>10,719</td>
<td>16</td>
<td>6890.8</td>
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<tr>
<td>E-IV</td>
<td>129–167</td>
<td>0</td>
<td>50</td>
<td>50</td>
<td>13,399</td>
<td>16</td>
<td>8613.6</td>
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<tr>
<td>E-V</td>
<td>168–199</td>
<td>0</td>
<td>60</td>
<td>40</td>
<td>16,078</td>
<td>16</td>
<td>10335.9</td>
</tr>
<tr>
<td>E-VI</td>
<td>200–208</td>
<td>0</td>
<td>70</td>
<td>30</td>
<td>18,758</td>
<td>16</td>
<td>12058.7</td>
</tr>
</tbody>
</table>

Fig. 2. Experimental data from the ASBR throughout the experiment, i.e., (a) total volume of biogas produced daily and hydrogen concentration in the biogas, (b) daily hydrogen volumetric production rate, (c) hydrogen yield per gram COD and TVS, and (d) all of the measured VFAs' concentrations throughout the experiment. The separating lines in each chart denote each individual stage in the experiment.
E-I, E-II, E-III, E-IV, E-V, E-VI) was made only when the steady state was reached. This served to ensure the stability of the system and enhance the reliability of the experimental data. The steady state was defined as in a seven-day period in which the maximum value of daily hydrogen gas production was no more than 5% higher than the minimum value within that period. The influent composition was modified to increase the OLR after each steady state. Daily hydrogen and methane volumes were measured to analyze the evolution of biogas production in the experiment, and the daily biogas volumes that occurred during a steady state were averaged for each steady state, producing a metric of hydrogen and methane production potential for each OLR in the respective stage when the steady state occurred.

2.5. Analytical techniques

The biogas production from the ASBR and UASB reactors was measured every day using a gas meter (Changchun Automobile Filter Co. Ltd, China), and collected in gas bags (Cel Scientific Corp, USA). Gas composition (CH₄, H₂, N₂, and CO₂) was analyzed with a Shimazu 2014 gas chromatograph equipped with a thermal conductivity detector (TCD). Helium (40 mL min⁻¹) was used as the carrier gas and the temperatures of the column, detector, and injector were 40, 100, and 100 °C, respectively. VFAs were analyzed using the same gas chromatograph equipped with a flame-ionization detector (FID) and a stabilwax-DA column (30 m × 0.53 mm × 0.25 μm). Nitrogen was used as a carrier gas and the temperatures of the column, detector, and injector were 50, 250, and 250 °C, respectively. TS, TVS, and TSS for all liquid samples were determined following standard methods (Wu et al., 2013). The pH was measured with a Fisher Scientific Accumet XL600 pH meter. COD, TP, TP, TAN, and TN were analyzed using analysis kits from the Hach Company, Inc. (Colorado, USA) using a HACH DR 3900 Spectrometer.

Sugars were determined by a high-performance liquid chromatograph (HPLC, Waters 2695 Separations Module, Milford, MA) equipped with a Waters 2414 Refractive Index Detector (Milford, MA), Shodex precolumn (SP-G, 8 μm, 6 × 50 mm), and Shodex column (SP0810, 8 μm × 300 mm). Millipore filtered water (0.2 mL min⁻¹) was the mobile phase and the column was heated to 85 °C with an external heater. Data were processed with the Empower 3 chromatography data software.

All samples, including liquid and gas samples, were analyzed in triplicate.

3. Results and discussion

3.1. Hydrogen production

From Day 1 to Day 54, hydrogen production was very low (Fig. 2a, b, and c). In a study by Chang and Lin (2004) studying H₂ production from succrose fermentation using a UASB reactor inoculated with sewage sludge pretreated with heat shock, hydrogen production was almost 0 L·L⁻¹·d⁻¹ for the first approximately 40 days before rising to significant levels. Also, Thanwised et al. (2012) reported hydrogen volumetric production rates seasing from close to 0 to around 200 mL·L⁻¹·d⁻¹ for about 60 days directly after their experiment began, then reaching higher and more consistent levels. Their study used an anaerobic baffled reactor (ABR) to digest tapioca wastewater. VFA concentrations in the ASBR up to Day 54 were also relatively low (Fig. 2d). However, butyric acid, which was the dominant VFA product in this time, was produced by a metabolic pathway that also produces hydrogen (Zhou et al., 2018). Because almost no hydrogen was detected in this time, it must have been consumed by another process. Homocacetogens, which were a type of bacteria that consumed hydrogen to create acetic acid, were resilient, resistant to pretreatment, and common in anaerobic digesters (Saady and Noori, 2013). Since acetic acid levels in this time were almost as high as butyric acid on average, it was likely that the cause of the very low detected hydrogen relative to the VFA concentrations in the ASBR was homoacetogenic activity.

From Day 55 to 128, hydrogen production steadily rose. The most likely reason for this is that the ASBR became a more efficient hydrogen-producing system with time. Suddenly producing much more H₂ starting around Day 55 is in alignment with the two studies mentioned previously, in which H₂ production experienced a sudden boost around Day 40 (Chang and Lin, 2004) and 60 (Thanwised et al., 2012). While the addition of the new substrates into the ASBR starting from Day 28 and doubling in concentration in the influent on Day 55 occurred simultaneously with the hydrogen production increase, the occurrences seemed to be a coincidence because there was no evidence found in literature that the rise in SM/BW concentration would cause an increase in hydrogen production, especially since this influent change was associated with an overall COD decrease. The H₂ concentration in the biogas (H₂-%) rose from 3.36% on Day 55 to 27.65% on Day 128 (Fig. 2a).

The hydrogen volumetric production rate (H₂-VPR) leaped from 3.95 to 149.28 mL·L⁻¹·d⁻¹ from Day 55 to 128 (Fig. 2b). The hydrogen yield per gram of COD and TVS added (H₂-yield₉₀₌ and H₂-yield₉₅₄₈, respectively) both increased during the same time interval, but showed a pattern of decreasing every time the COD and TVS were increased. Then they usually arrived at higher values before the COD and TVS increased again (Fig. 2c). This was due to the adaptation of the biological system to the increase in organics in the reactor. The bacterial population gradually accommodated the addition of increased organics to their environment, which enabled them to utilize more organics and produce more biogas over time (Saner et al., 2016).

From Day 129 to 167, hydrogen production showed a lot of fluctuations, and reached its overall maximum in the experiment. Xing and Zhao (2009) also reported significant fluctuations associated with OLR increases in their study on two-stage food waste fermentation. The fluctuations in biogas production can thus be attributed to the experimental OLR increase, which caused instability in the biological system until it acclimated. H₂-% and H₂-VPR peaked at 39.90% on Day 136 and 405.81 mL·L⁻¹·d⁻¹ on Day 141, respectively. Giordano et al. (2011) reported hydrogen gas concentrations between 45% and 72% (during separate) fermentation of glucose, common wheat, durum wheat, mashed potatoes, and steam-peeled potato wastes, while Fan et al. (2006), using brewery wastewater, reported concentrations between 14% and 47%. Therefore, the results obtained from this study were consistent to their findings. H₂-Yield₉₀₃ and H₂-yield₉₅₄₈ also reached their maximum on Day 141 at 47.11 and 64.06 mL·g⁻¹·d⁻¹, respectively. Because of the fluctuations, this was the longest stage (39 days) to reach the steady state.

On Day 168, when the influent COD concentration was raised to about 16 g·L⁻¹, hydrogen production briefly spiked, then plummeted. At this point in the experiment, the OLR became too high for the system to stay stable. As a result, H₂-% dropped to below 10%, and H₂-VPR dropped under 20 mL·L⁻¹·d⁻¹. H₂-yield₉₀₃ and H₂-yield₉₅₄₈ were under 5 mL·g⁻¹·d⁻¹ by the end of the steady state, due to the huge amount of organics added that overloaded the system. Xing and Zhao (2009) reported that at higher ORLs, lactate-type fermentation became dominant. Lactic acid has been shown to have inhibitory effects on hydrogen production (Noike et al., 2002). Unfortunately, lactic acid concentration was not measured, but it was likely that lactic acid buildup was a contributor to the decline in hydrogen production.

Day 200 came with a COD increase to almost 19 g·L⁻¹. Hydrogen production further declined for the next nine days. Afterwards, the excess organics clogged the pumps, and the two-phase system was no longer operational.

Table 3 showed the hydrogen production metrics during the steady states. Each hydrogen production metric increased during each OLR stage from 1148.6 to 8613.6 mg·COD·L⁻¹·d⁻¹. For all metrics except H₂-%, the most dramatic increase was between 6890.8 and 8613.6 mg·COD·L⁻¹·d⁻¹. From 8613.6 to 10335.9 mg·COD·L⁻¹·d⁻¹,
there was a sharp decline in all parameter values, reflecting the reactor’s decline due to the excess organics.

In literature, it is often reported that hydrogen production is more efficient at higher experimental OLRs. A report by Shen et al. (2009) studied the hydrogen production potential with various OLRs (from 4 to 30 g COD L$^{-1}$ d$^{-1}$). They found that, as the OLR increased stage-by-stage, H$_2$-VPR always increased, and the H$_2$-yield per mole of glucose increased overall. Similarly, Dareioti and Kornaros (2015) reported that H$_2$-VPR and H$_2$-% increased steadily as the OLR was increased from 17.16 to 171.60 g COD L$^{-1}$ d$^{-1}$, and the H$_2$-yield per mole of carbohydrates consumed experienced a dramatic overall increase over the experimental OLR range. In this experiment, the optimal hydrogen production occurred at the relatively high value of 8613.6 mg COD L$^{-1}$ d$^{-1}$.

### 3.2. Methane production

Methane production rose to significant levels much more quickly than hydrogen (Fig. 3). Methane concentration in the UASB (CH$_4$-%) was around 30% by Day 10 and rose to 65% by Day 42 (Fig. 3a). This observation agreed with the results from other studies, where methane levels of 63.5% (Farhat et al., 2018) and 53.9 to 72.7% (Salem et al., 2009). Between Day 80 and 128, CH$_4$-% and CH$_4$-yield TVS increased from 88.47 to 171.60 g COD L$^{-1}$ d$^{-1}$, and the H$_2$-yield per mole of carbohydrates consumed experienced a dramatic overall increase over the experimental OLR range. In this experiment, the optimal hydrogen production occurred at the relatively high value of 8613.6 mg COD L$^{-1}$ d$^{-1}$.

### Table 3

<table>
<thead>
<tr>
<th>OLR (mg COD L$^{-1}$ d$^{-1}$)</th>
<th>1148.6</th>
<th>3445.1</th>
<th>5167.9</th>
<th>6890.8</th>
<th>8613.6</th>
<th>10335.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_4$-% (%)</td>
<td>9.41 ± 0.17</td>
<td>17.94 ± 0.36</td>
<td>22.25 ± 0.51</td>
<td>27.11 ± 0.48</td>
<td>31.86 ± 0.68</td>
<td>7.55 ± 0.09</td>
</tr>
<tr>
<td>CH$_4$-VPR (ml L$^{-1}$ d$^{-1}$)</td>
<td>17.05 ± 0.29</td>
<td>56.63 ± 0.44</td>
<td>90.40 ± 1.11</td>
<td>152.09 ± 1.85</td>
<td>294.06 ± 3.06</td>
<td>16.35 ± 0.29</td>
</tr>
<tr>
<td>CH$_4$-yield COD (ml g$^{-1}$)</td>
<td>14.85 ± 0.25</td>
<td>16.44 ± 0.13</td>
<td>17.49 ± 0.21</td>
<td>22.07 ± 0.27</td>
<td>34.14 ± 0.26</td>
<td>15.8 ± 0.03</td>
</tr>
<tr>
<td>CH$_4$-yield TVS (ml g$^{-1}$)</td>
<td>19.72 ± 0.37</td>
<td>21.73 ± 0.16</td>
<td>28.81 ± 0.58</td>
<td>29.97 ± 0.48</td>
<td>45.46 ± 0.71</td>
<td>2.12 ± 0.04</td>
</tr>
<tr>
<td>CH$_4$-% (%)</td>
<td>63.90 ± 0.24</td>
<td>74.97 ± 0.48</td>
<td>81.02 ± 0.83</td>
<td>82.66 ± 0.33</td>
<td>28.65 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>CH$_4$-VPR (ml L$^{-1}$ d$^{-1}$)</td>
<td>100.32 ± 0.57</td>
<td>291.82 ± 33.80</td>
<td>348.83 ± 4.30</td>
<td>497.94 ± 10.01</td>
<td>82.11 ± 1.70</td>
<td></td>
</tr>
<tr>
<td>CH$_4$-yield COD (ml g$^{-1}$)</td>
<td>307.30 ± 3.93</td>
<td>187.68 ± 17.21</td>
<td>185.06 ± 28.26</td>
<td>131.19 ± 3.85</td>
<td>126.32 ± 2.65</td>
<td></td>
</tr>
<tr>
<td>CH$_4$-yield TVS (ml g$^{-1}$)</td>
<td>193.22 ± 1.49</td>
<td>128.13 ± 4.40</td>
<td>179.28 ± 26.69</td>
<td>176.57 ± 1.67</td>
<td>261.42 ± 8.41</td>
<td></td>
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</table>

The increase in methane production during this time was explained well by the increases in acetic and butyric acids at the same time. Similar to hydrogen production, methane production also reached its peak during the period from Day 129 to Day 168, with heavy fluctuations. CH$_4$-% reached its maximum of 88.80% on Day 140, and CH$_4$-VPR reached its maximum of 633.36 mL L$^{-1}$ d$^{-1}$ on Day 134. In the meantime, CH$_4$-yield TVS also peaked at 285.63 mL g$^{-1}$ d$^{-1}$ on Day 130. The decline of methane production on Day 169 was in tandem with the declining trend of hydrogen (Fig. 2a, b, and c). H$_2$ production occurred via acidogenesis (Zhou et al., 2018), so its decline caused VFA concentrations to fall. Therefore, methane production, which relied on VFAs (Schink, 1997), also declined. Besides, CH$_4$-%, CH$_4$-VPR, CH$_4$-yield COD, and CH$_4$-yield TVS all started to fall. By Day 199, these values fell by 54.48%, 395.98 mL L$^{-1}$ d$^{-1}$, 94.75 mL g$^{-1}$, and 219.60 mL g$^{-1}$, respectively. Methane production continued to decline for the remaining experimental period.

Table 3 showed the methane production metrics during each steady state. CH$_4$-% and CH$_4$-VPR both increased with every OLR increase from 1148.6 to 8613.6 mg COD L$^{-1}$ d$^{-1}$. However, CH$_4$-yield COD decreased with every OLR increase throughout the entire experiment, implying that as more and more COD was added to the system, methane production increased, but the efficiency of converting COD to methane decreased. CH$_4$-yield TVS did not have as clear of a correlation with OLR. Like CH$_4$-% and CH$_4$-VPR, CH$_4$-yield COD showed an overall increase from 1148.6 to 3445.1 mg COD L$^{-1}$ d$^{-1}$, but a significant decrease from 1148.6 to 219.60 mL g$^{-1}$ d$^{-1}$ (as did CH$_4$-yield TVS), indicating that the drastic COD variation during HRT transitioning from 24 h to 16 h in the experiment hindered the efficiency of converting organics into methane. All metrics drastically fell when OLR reached 10335.9 mg COD L$^{-1}$ d$^{-1}$. Aboudi et al. (2015), studying methane production from sugar beet byproducts and swine manure with varying OLRs, found a similar drastic decrease in methane production rate from over 25 L d$^{-1}$ to about 5 L d$^{-1}$ when OLR was increased from 11.2 to 12.8 g TVS L$^{-1}$ d$^{-1}$. The same study also reported similar trends to those of this study. The study showed that methane production rate increased with almost every OLR change from 4.2 to 11.2 g TVS L$^{-1}$ d$^{-1}$ and that the methane-TVS yield was more even across all OLRs up to 11.2 g TVS L$^{-1}$ d$^{-1}$ and did not show a strong increasing or decreasing trend, which was similar to the results achieved in this study.

### 3.3. COD removal efficiency

The COD removal efficiency of the integrated two-phase system, calculated as the average of the daily removal rates during each steady state, did not have a clear linear relationship with OLR. Beginning at 73.10 ± 0.03% at the lowest OLR of 1148.6 mg COD L$^{-1}$ d$^{-1}$, the COD removal rate decreased twice with the next two OLR increases, reaching its minimum value of 52.86 ± 2.24% at 5167.9 mg COD L$^{-1}$ d$^{-1}$. The maximum COD removal efficiency of the whole system was 75.54 ± 0.19%, occurring at the next OLR of 6890.8 mg COD L$^{-1}$ d$^{-1}$. When the OLR increased to 8613.6 mg COD L$^{-1}$ d$^{-1}$, hydrogen and methane production were boosted, but the removal efficiency dropped.
to $69.15 \pm 0.13\%$. Aboudi et al.’s study (2015) which shared similarities with this study in terms of methane production rate and yield, also reported similar results for removal of organics. They found that the OLR of maximum VS removal efficiency ($7.4 \text{ g VS L}^{-1} \text{ d}^{-1}$) was lower than the OLR of maximum methane production rate ($11.2 \text{ g VS L}^{-1} \text{ d}^{-1}$), a phenomenon that also occurred in this study. Several two-stage studies reported COD removal rates similar to those of this study. Wang et al. (2013), using sugar wastewater, achieved a maximal COD removal efficiency of $79.2 \pm 1.2\%$. Zhu et al. (2008), fermenting potato wastes, removed 64% of COD. Finally, Lay et al. (2019), using low-strength beverage wastewater, removed 78%.

### 3.4. Multi-study comparison

Several studies consisting of integrated two-phase systems for hydrogen and methane production under mesophilic conditions were compared with this study on the basis of hydrogen and methane production. Cooney et al. (2007), using a glucose-yeast-peptone medium, was able to produce approximately $711.4 \text{ mL L}^{-1} \text{ d}^{-1}$ of hydrogen gas (converting from mmol L$^{-1}$ d$^{-1}$ using the $22.4 \text{ mol L}^{-1}$ equivalence) and $121.9 \text{ mL}$ of methane gas. Because of the substrate containing high sugar, they were able to achieve a higher hydrogen production rate than in this study ($294.06 \pm 3.06 \text{ mL L}^{-1} \text{ d}^{-1}$), which utilized substrates that also contained complex organics such as lignocellulose. However, the methane production rate in their study was significantly lower than in this study ($497.94 \pm 10.01 \text{ mL L}^{-1} \text{ d}^{-1}$). It was known that higher hydrogen production often gave rise to lower methane production (Giordano et al., 2011). Salem et al. (2018) studied the effects of different methods of pretreating potato wastes on biogas yield, and only those results without pretreatment were compared with the results from this study since no pretreatment was applied to the substrates. Although their hydrogen and methane production rates ($1.01 \pm 0.31 \text{ L H}_2 \text{ L}^{-1} \text{ d}^{-1}$ and $0.62 \pm 0.20 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$, respectively) were higher than those of this study, the methane production rate was comparable. Giordano et al. (2011) studied the biogas production potential of several substrates, among which the one with durum wheat as the substrate was compared with the results in this study. For hydrogen gas, they produced $76 \pm 12 \text{ mL H}_2 \text{ g}^{-1} \text{ COD}$, which doubled the yield obtained from this study ($34.14 \pm 0.36 \text{ mL H}_2 \text{ g}^{-1} \text{ COD}$ at an OLR $8613.6 \text{ mg COD L}^{-1} \text{ d}^{-1}$). On the contrary, at an

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**Fig. 3.** Experimental data from the UASB throughout the experiment, i.e., (a) total volume of biogas produced daily and methane concentration in the biogas, (b) daily methane volumetric production rate, and (c) methane yield per gram COD and TVS.
OLR of 114.86 mg COD L\(^{-1}\) d\(^{-1}\), 270.30 ± 3.93 mL CH\(_4\) g\(^{-1}\) COD was obtained in this study, which was higher than their methane yield of 243 ± 11 mL CH\(_4\) g\(^{-1}\) COD. Finally, Wang et al. (2013) studied biogas production from sugar wastewater at low HRT, in which much higher hydrogen and methane per day (3.09 L H\(_2\) L\(^{-1}\) d\(^{-1}\) and 2.01 L CH\(_4\) L\(^{-1}\) d\(^{-1}\) respectively) were produced than in this study.

4. Conclusions

This study investigated a two-phase anaerobic digestion process (ASBR followed by UASB) for hydrogen and methane production using swine manure and brewery wastewater as substrates. Hydrogen production was lower than in other studies due possibly to the difficulty in digesting swine manure, while methane production was comparable. The best OLR for biogas production was 8613.6 mg COD L\(^{-1}\) d\(^{-1}\). The highest COD removal (75.54%) was achieved at the OLR of 6890.8 mg COD L\(^{-1}\) d\(^{-1}\). Increasing OLR to 8613.6 mg COD L\(^{-1}\) d\(^{-1}\) had insignificant impact on COD removal rates. In summary, swine manure and brewery wastewater were viable co-substrates for anaerobic digestion.

Funding

This work was supported by the USDA National Institute of Food and Agriculture, Hatch project (grant number 1003122); the China Scholarship Council (grant number 1508290052); and the National Natural Science Foundation of China (grant number 41271244). None of the above organizations had any involvement in the design, execution, or interpretation of this study, and they did not influence in any way the decision to submit this article.

Declaration of Competing Interest

None.

Acknowledgements

We thank the employees of the Arkansas Agricultural Experiment Station for their help with the experiment.

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