EFFECT OF ORGANIC LOADING RATE ON COPRODUCTION OF HYDROGEN AND METHANE FROM MOLASSES WASTEWATER AND ENERGY CONVERSION BY TWO-PHASE ANAEROBIC FERMENTATION

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Abstract. Effect of organic loading rate on the operational performance of reactors and energy conversion efficiency by two-phase anaerobic fermentation was proposed and investigated using molasses wastewater. Hydrogen and methane production rate were determined in the hydrogenic up-flow anaerobic sludge blanket (UASB) reactor and the methanogenic UASB reactor. At analyzed optimum system organic loading rate (OLR) of 20 g COD/L $_{reactor}$·d, hydrogen was efficiently produced from the hydrogenic reactor with the highest production rate of 2.0±0.21 L/L $_{reactor}$·d and in the methanogenic reactor, methane was produced from residual organic matters and volatile fatty acids (VFAs) with a production rate of 1.9±0.3 L/L $_{reactor}$·d. Finally, the energy conversion efficiency was increased from 12.1% (hydrogen only production) to 91.2% (hydrogen and methane coproduction). The results of this study indicated that the hydrogenic reactor presented relatively low energy conversion efficiency while the methanogenic reactor presented a high one.
Keywords: *organic matter, upflow anaerobic sludge blanket, hydrogenic reactor, volatile fatty acids, methanogenic reactor*

Introduction

Hydrogen (H_2) as a renewable energy carrier can replace fossil fuels and address issues of energy shortage and environmental emissions (Lee and Chung, 2010). Hydrogen is being focused on as a promising alternative to fossil fuels because it does not discharge contaminants (Panda et al., 2016). Also, hydrogen is a promising future energy carrier because the sole byproduct of H_2 combustion is pure water that generates 2.75 times more energy (122 kJ/g) than hydrocarbon fuels (Scott, 2004). Furthermore, biological hydrogen production seems to be more attractive because organic waste materials can be used as substrate and the hydrogen-producing system can be operated under low temperature and pressure conditions (Sivagurunathan et al., 2016). The calorific value of hydrogen is three times greater than that of petrol, 3.9 times that of ethanol and 4.5 times that of coal (Fayaz et al., 2012). Among the various hydrogen production methods, hydrogen production from organic wastes by anaerobic fermentation seems to be the most promising and environmentally friendly (Siddiqui et al., 2011). Anaerobic fermentation has unique characteristics like high ecological adaptability, simple reaction conditions and low nutrient requirement

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(Puyol et al., 2017) and it can use microbes through manipulating the organic matter in the biomass to extract hydrogen in an anaerobic environment (Han et al., 2012). The advantages of the two-stage fermentation process are high-energy recovery and process stability (Nualsri et al., 2016). Anaerobic digestion entails two-stage processes involving the sequential action of acid-forming and methane-forming bacteria. In the first stage, acid-forming bacteria (facultative and anaerobic bacteria) convert the complex organic compounds into simpler organics (volatile fatty acids-VFAs) and also carbon dioxide and hydrogen gases. In the second stage, the organic acids and hydrogen are converted into methane and carbon dioxide by methanogens. The efficiency of an anaerobic treatment depends on both the acidogenic and methanogenic phases (Shi et al., 2017). Hydrogen is mainly produced in the acidogenic stage of anaerobic digestion during the fermentation of organic wastes. Several factors affect the fermentative hydrogen production, including inoculum, substrate characteristics, reactor type, nitrogen and phosphate contents, temperature and pH, among others (Wang and Wan, 2009). However, the low theoretical energy efficiency of about 33.5% is the main obstacle to hydrogen production by anaerobic fermentation which significantly limits its development and industrial application (Chen et al., 2012). How to increase energy conversion efficiency from organic wastes is a challenging topic. It was found in previous studies (Carucci et al., 2005; Luo et al., 2010) that the effluents of the anaerobic fermentation process including mainly volatile fatty acids (VFAs) and alcohols can be reutilized to recover the thermal enthalpies and further produce methane, thereby dramatically increasing the energy conversion efficiency. Among the various anaerobic wastewater treatment technologies, upflow anaerobic sludge blanket (UASB) reactors have achieved considerable success and these reactors have been applied to treat a wide range of effluents such as sugar, pulp and paper, dairy, chemical, potato starch, bean balancing, soft drinks, fish processing, noodle processing, yeast production, slaughterhouse, and coffee processing industries (Metcalf and Eddy, 2003; Yetilmezsoy and Sakar, 2008; Farghaly and Tawfik, 2017). Therefore, the upfow anaerobic sludge blanket (UASB) reactor has been recognized as an essential wastewater treatment technology among anaerobic treatment methods.

Given these considerations, the present study aimed to evaluate the effect of OLRs on the hydrogen and methane production to achieve the optimum energy conversion efficiency using a two-phase anaerobic process which consisted of two up-flow anaerobic sludge blanket (UASB) reactor.

Materials and methods

Seed sludge and feeding

The seed sludge used in this study was the dewatering sludge obtained from a local municipal wastewater treatment plant (Harbin, China). For the hydrogenic UASB, the sludge was aerated for 30 days to inhibit the methane-producing bacteria activity. For the methanogenic UASB, the raw sludge was acclimatized in the reactor operated at pH 6.5, fed continuously with the molasses substrate at OLR of 4.0 g COD/L·d. After enough enrichment over 30 days, the methanogenic sludge began to be fed with the effluent of the hydrogenic UASB.

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Experimental setup

Coproduction of hydrogen and methane from molasses wastewater was carried in a two-phase anaerobic process which consisted of two up-flow anaerobic sludge blanket (UASB) reactor (*Figure 1*) with the same working volume of 10 L.

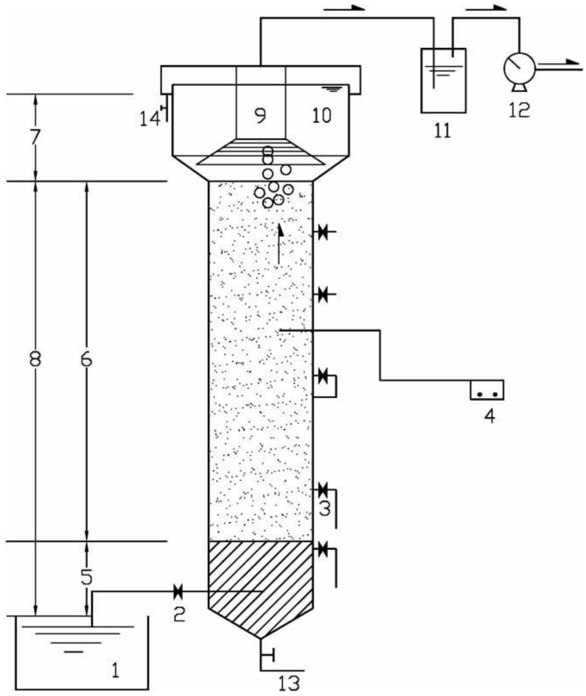

Figure 1. *Structure of the UASB reactor. 1. Raw water tank; 2.Peristaltic pump; 3. Sample connection; 4. Temperature controller; 5. Sludge bed; 6. Superposed layer; 7. Reaction zone; 8. Separating zone; 9. Three phase separation zone; 10. Precipitation area; 11. Water seal; 12. Wet gas flow meter. 13. Mud mouth; 14. Outlet*

The internal diameter was 10 cm and a height of 1.3 m for the two reactors. Operating temperature of the bioreactors was maintained through an electric jacket to 35˚C. The two reactors operated for 60 days, and the operational conditions (OLR and respective HRT) are presented in *Table 1*. The UASB reactor for hydrogen production (hydrogenic) was fed with molasses wastewater using a peristaltic pump while the UASB reactor for methane production was supplied with the effluent collected from the hydrogenic reactor. In this study, the steady-state condition was defined as the condition that the biogas varied within 5% for ten days (*Figure 2*).

Table 1. *operational conditions (OLR and respective HRT) of reactors*

Period (d)	**Hydrogenic UASB**		**Methanogenic UASB**	
	OLR (g COD/L $_{reactor}$·d)	**HRT** (h)	**OLR** (g COD/L $_{reactor}$·d)	**HRT**(h)
1-15	12	4	3.5	21
16-30	16	6	4.1	18
31-45	20	8	5.0	15
46-60	30	10	6.2	12

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Analytical methods

COD, pH, and alkalinity were monitored and measured daily according to Standard methods (Apha, 1995). Hydrogen and methane were analyzed using gas chromatography (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 meshes). Nitrogen was used as the carrier gas at a flow rate of 40 mL/ min.

Figure 2. *Physical photo of the UASB reactor*

Conversion efficiency of energy

The conversion efficiency of energy was calculated from hydrogen and methane according to the following equation:

$$R = (V_H \cdot \beta_H + V_M \cdot \beta_M) / (F \cdot W) \qquad \text{(Eq.1)}$$

where R represents the conversion efficiency of energy in the hydrogenic and methanogenic reactor, V_H and V_M represent the volume of hydrogen and methane (L/d), respectively. β_H and β_M represent the COD equivalent of hydrogen and methane, respectively and the hydrogen and methane COD equivalents (g O_2/L H_2 and CH_4) were 0.71 and 2.86, respectively. F represents the influent rate of the hydrogenic reactor (L/d). W represents the COD concentration of molasses wastewater.

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Results and discussion

Optimum system OLR for hydrogenic UASB

The UASB reactor for hydrogen production operated during 60 days under different OLR and HRT. Organic loading rate (OLR) is an essential parameter in studying bioreactors (Venetsaneas et al., 2009). To optimize a system for hydrogen production, it is necessary to define either a range of OLRs that the operation can handle effectively or an optimal OLR for a maximum hydrogen production rate. The pH variation at different OLRs and the hydrogen production and COD removal at different OLRs are respectively presented in *Figure 3* and *Figure 4*.

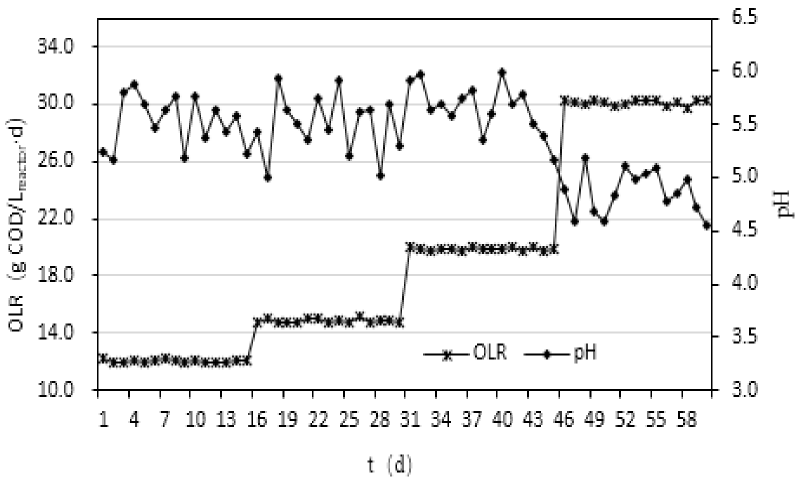

Figure 3. *The pH variation at different OLRs*

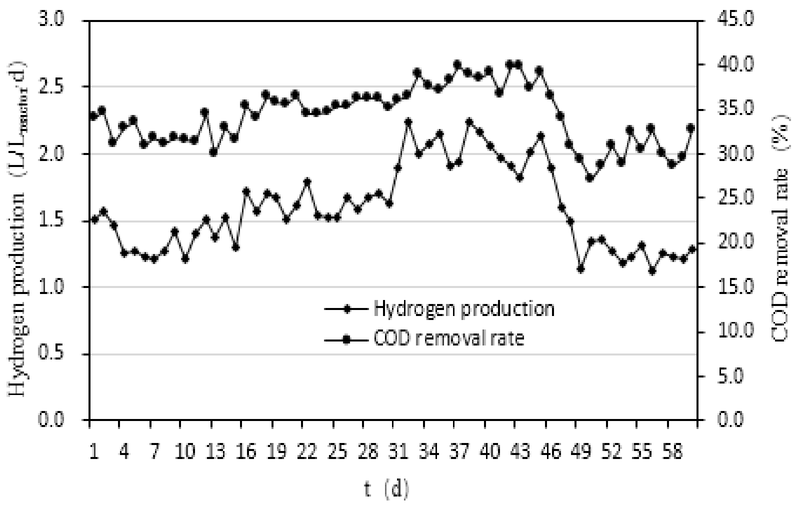

Figure 4. *The hydrogen production and COD removal at different OLRs*

The pH parameter was critical for maintaining the reliability of the hydrogenic fermentation process, and the VAFs (Ethanol, acetic acid, propionic acid and butyric acid) production resulted in a reduction of hydrogenase activity significantly. *Figure 3* shows that the hydrogenic system pH was within 4.5-6.0 range.

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As indicated in *Figure 4*, the maximum hydrogen production was achieved to be 2.0±0.21 L/L $_{reactor}$·d which stated the most exuberant metabolic activities of hydrogenic microorganisms at the OLR of 20 g COD/L $_{reactor}$·d. At the same time, the COD removal rate presented by hydrogenic reactor was up to the highest level of 38.1±0.55%. The higher OLR showed a negative influence on the hydrogenic microorganisms through decreasing hydrogen production and COD removal efficiency, which was explained by the reduction of pH value caused by VFAs accumulation. These results are similar to those obtained by Krishnan et al. (2017) who observed that increased OLR is considered to indicate vigorous biomass concentration conditions that could bring toxicity to the system. The observed trends were supported by the work of Zhang et al. (2015), they found that the anaerobic digestion of food waste at OLR of 3 gVS/L/d caused VFA accumulation. Therefore, the OLR of 20 g COD/L $_{reactor}$·d was determined to be the suitable operating condition for a hydrogenic reactor with the maximum hydrogen production from molasses wastewater and COD removal efficiency. Zahedi et al. (2018) examined the effect of the increase in organic loading rates (OLRs), by reducing the solids retention time (SRT) from 20 d to 5 d, in single-phase mesophilic anaerobic co-digestion of sewage sludge with glycerine (1% v/v). They confirmed that anaerobic co-digestion of these biowastes under steady-state conditions could achieve an 85 ± 5% reduction in volatile fatty acids (VFA) at SRTs between 20 and 9 d, with a methane production yield of around 0.8 l CH_4/L/d. Decreases in the SRT not only allow the sludge stability and biogas production to be maintained but also lead to an increase in the waste that could be treated and lower operating costs.

Biase et al. (2018) tested the Bench-scale anaerobic moving bed biofilm reactors (AMBBR) at mesophilic conditions and different HRT of 24, 18, 12, 10, 8 and 6 h. They also studied temperatures of 15, 25, and 35˚C at a constant HRT of 18 h. During the HRT study, it was found that AMBBR could be operated with COD removal above 80% at all HRT and therefore OLR below 23 kg-COD $m^{-3}d^{-1}$ at 40% media fill ratio. The highest performance of 92% removal of sCOD was attained at 5.4 kg-COD $m^{-3}d^{-1}$. This corresponded to surface area loading rates (SALR) of 18 g-sCOD $m^{-2}d^{-1}$ with methane yields of 0.34 m^3-CH_4 kg-COD^{-1} removed at 35˚C. At OLR above 23 kg-COD $m^{-3}d^{-1}$, the performance decreased below 80% sCOD removal at 35˚C.

Amorim et al. (2018) evaluated the rapid startup of UASB reactors at 30˚C for the cassava wastewater treatment. The reactor was operated under eight different conditions with a hydraulic retention time (HRT) of 8 or 12 h and organic loading rates (OLR) of 12.0 or 15.5 g COD.$L^{-1}d^{-1}$. The UASB system with the best performance was that with the 8 h HRT and OLR of 12.0 g COD .$L^{-1}d^{-1}$, with COD removal rates ranging from 71 to 80 % and methane production of 0.260 L CH_4 g^{-1} $COD_{removed}$.

Moreover, Corsino et al. (2018) observed that under an OLR lower than 7 kg TCOD $m^{-3}d^{-1}$ the removal efficiency of total chemical oxygen demand (TCOD) was approximately 90% in the two aerobic granular sludge sequencing batch reactors (AGSBR) investigated. In contrast, at higher OLR a significant decrease in the removal efficiency (from 90% to less than 75%) was observed in the reactor R2.

Tritt and Kang (2017) reported that the reactor performance during their investigation showed a maximum 95% COD removal efficiency at an organic loading rate (OLR) of 1 kg COD/ m^3-d with its corresponding hydraulic retention time (HRT) of 7.5 d. At a higher OLR of 4.0 kg COD/ m^3-d, the COD removal efficiency of 75% was achieved with an HRT of 2 d. No significant difference in COD removal efficiencies was found between the reactors operated in both up-flow and down-flow modes.

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Methane production from hydrogenic effluent by methanogenic UASB

The UASB reactor for methane production was fed with the acidogenic effluent collected from the CSTR and operated during 60 days under different OLR and HRT. The OLR was gradually augmented by increasing the concentration of the influent and then by decreasing the HRT (*Table 1*). *Figure 5* reveals the profile of the methane production and COD removal rate of the methanogenic reactor during the operation.

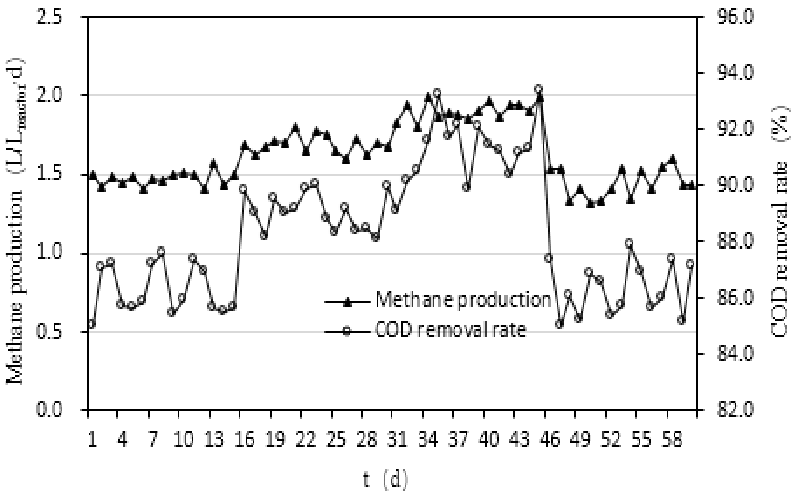

Figure 5. *The methane production and COD removal at different OLRs*

At the HRT 15 h, the OLR of 5.0 g COD/L reactor·d, the methanogenic UASB presented the optimal operation performance with the methane production of 1.9±0.3 L/L reactor·d. At HRT 12 h, the increased existence of activated sludge was detected in the methanogenic effluent caused by excessive flow velocity, so the methane production began to decrease remarkably down to 1.5±0.55 L/L reactor·d which showed that the reactor tends to go to lousy run statement. The COD removal rate has a similar variation curve with methane production, range between 85% and 93.5%, showing its high treatment efficiency and excellent process stability. In another study, Wang et al. (2009) found that increasing the HRT from 6 to 24 h, the COD of the effluent was gradually reduced from 257 to 124.4 mg/L, and the total removal rate of the COD increased from 88.1 to 95.6% in the anaerobic reactor treating starch wastewater. Besides, Colin et al. (2007) observed that the amount of biogas product (L-biogas/L.d) increased with the increase in organic loading rate. At the maximum of OLR of 18.11 g COD/L.d and HRT of 9.5 h, gas productivity of 3.7 L/ (L.d) was achieved in an anaerobic horizontal flow filter packed with bamboo peace treating starch wastewater. Moreover, Wu et al. (2018) reported in their study that the daily CH_4 production from the three treatments increased with the OLR. Results of Begum et al. (2018) disclosed that the range of methane yield in single and two-stage AD fluctuated between 0.21 and 0.34 L CH_4/(g COD removed) and 0.2 to 0.32 L CH4/(g COD removed) respectively with an overall increase of 21% in COD removal efficiencies can be achieved in two-stage AD.

Furthermore, Hu et al. (2018) related that the methane production rate increased with OLR increasing in thermophilic and mesophilic reactors. Their results indicated that

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thermophilic anaerobic reactors allow higher loading rate and yield higher methane production, substrate degradation, and pathogen destruction.

Shen et al. (2018) reported in their study that the volumetric methane production rate (VMPR) of experimental and control group revealed an increasing tendency with the increment of OLR. They attributed that phenomenon to the accumulation of VFAs in the system, which further inhibited the activity of methanogens and eventually resulted in the acidification of the AD system.

Arreola-Vargas et al. (2018) reported a linear trend between methane production and OLR with the highest volumetric methane production rate of 3.03 L CH_4 $d^{-1}L^{-1}$. Wickham et al. (2018) also reported that the increase in biogas production was proportional to the increase in organic loading rate (OLR) in their study.

Increased energy conversion efficiency by two-phase UASB

During hydrogenic fermentation, the most amounts of organic matters were converted into the VFAs, and the degraded COD was just removed in the form of hydrogen. So, the hydrogenic reactor presented relative low energy conversion efficiency varying between 8.0% and 12.1%, which is shown in *Figure 6*.

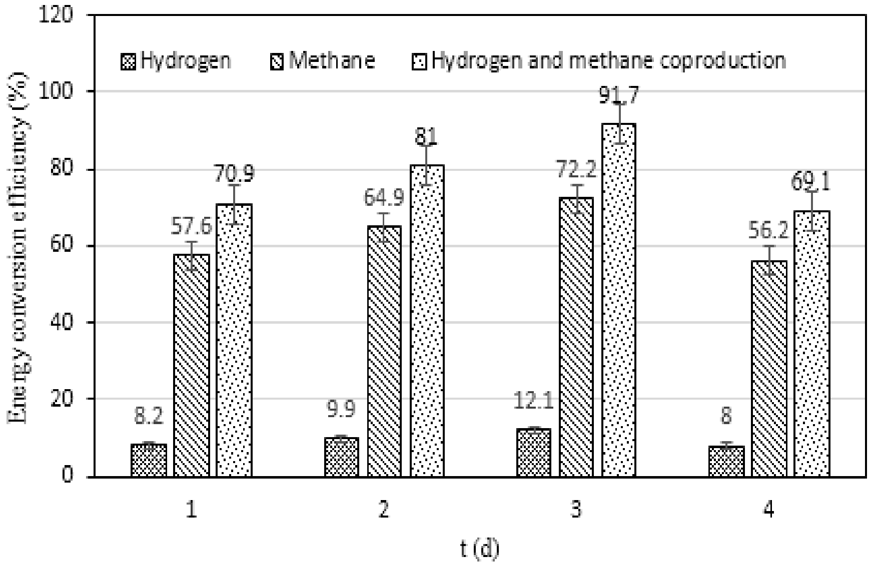

Figure 6. *The energy conversion efficiency for hydrogen and methane production*

At the operation condition of OLR 20 g COD/L $_{reactor}$·d for the hydrogenic reactor, the VFAs existed in hydrogenic effluent could be efficiently utilized by methanogenic microorganisms to produce the methane, which resulted in the high level of COD removal efficiency. The energy conversion efficiency of the methanogenic reactor reached up to the maximum value of 72.2% at OLR 5 g COD/L $_{reactor}$·d. The total energy conversion efficiency was also improved from 12.1% to 91.7% by two-phase anaerobic fermentation system. In the same way, Corona and Razo-Flores (2018) studied continuous H_2 and CH_4 production in a two-stage process to increase energy recovery from agave bagasse enzymatic-hydrolysate. The two-stage ongoing process significantly increased energy conversion efficiency (56%).

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Comparison of hydrogen and methane production with other two-stage anaerobic digestion processes

Several researchers have studied the two-stage anaerobic digestion processes as shown in *Table 2*. Among the presented works, the two stages CSTR-UASB achieved the highest maximum hydrogen rate of 6.0 L/L/d and maximum methane rate of 6.4 L/L/d using agave bagasse (Corona and Razo-Flores, 2018).

Table 2. *Comparison of hydrogen and methane production with other two-stage anaerobic digestion processes*

Substrate	Reactor type	Temp.	HRT	Maximum HPR (L/L/d)	Maximum MPR (L/L/d)	Reference
Cheese whey	CSTR PBR	35 °C 35 °C	24 h 4.4 d	2.51	5.04	(Antonopoulou et al., 2008)
Olive pulp	CSTR CSTR	35 °C 35 °C	7.5 h 10 d	0.46	1.13	(Koutrouli et al., 2009)
Municipality Biowaste	CSTR CSTR	55 °C 55 °C	5 h 13 d	0.85	1.75	(Cavinato et al., 2011)
Mixture wastewater	CSTR CSTR	37 °C 37 °C	0.75 d 25 d	1.72	0.33	(Dareioti and Kornaros, 2014)
Molasses	CSTR UASB	35 °C 35 °C	5 h 15 h	3.06	2.01	(Wang et al., 2013)
Molasses	PBR PBR	35 °C 35 °C	6 h 6 d	2.8	1.94	(Park et al., 2010)
Pelletized Grass	CSTR CSTR	35 °C 35 °C	18 h 270 h	0.65	1.4	(Massanet-Nicolau et al., 2015)
Agave bagasse	CSTR UASB	35 ° C 22–25 °C	6 h 14 h	6.0	6.4	(Corona and Razo-Flores, 2018)
Molasses	UASB UASB	35 °C 35 °C	8h 15h	2.0	1.9	This study

NR: data not reported; CSTR: continuous stirred-tank reactor; PBR: Packed-bed reactor; HPR: hydrogen product rate; MPR: methane product rate

Conclusion

The hydrogen and methane coproduction system containing two UASB reactors in this study were established at optimum OLR of 20 g COD/L reactor·d and 6.2 g COD/L reactor·d, respectively, for maximum biogas production from molasses wastewater. The maximum hydrogen production rate was reached at the OLR of 20 g COD/L reactor·d while the maximum methane production rate was reached at the OLR of 5.0 g COD/L reactor·d. Experimental data illustrated that the two-phase anaerobic fermentation process was a promising mean to recover energy from organic wastewater and provided the essential operation parameters guideline for the future industrial application. The feasibility of the two-phase anaerobic fermentation would need to be further studied.

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REFERENCES

[1] Amorim, M. C. C., de S. e Silva, P. T., Gavazza, S., Sobrinho, M. A. M. (2018): Viability of rapid startup and operation of UASB reactors for the treatment of cassava wastewater in the semi-arid region of northeastern Brazil. – The Canadian Journal of Chemical Engineering 96(5): 1036-1044.

[2] Antonopoulou, G., Stamatelatou, K., Venetsaneas, N., Kornaros, M., Lyberatos, G. (2008): Biohydrogen and methane production from cheese whey in a two-stage anaerobic process. – Industrial & Engineering Chemistry Research 47(15): 5227-5233.

[3] Apha, A. (1995): WPCF, Standard methods for the examination of water and wastewater. – American Public Health Association, Washington, DC.

[4] Arreola-Vargas, J., Snell-Castro, R., Rojo-Liera, N. M., González-Álvarez, V., Méndez-Acosta, H. O. (2018): Effect of the organic loading rate on the performance and microbial populations during the anaerobic treatment of tequila vinasses in a pilot-scale packed bed reactor. – Journal of Chemical Technology & Biotechnology 93(2): 591-599.

[5] Begum, S., Anupoju, G. R., Sridhar, S., Bhargava, S. K., Jegatheesan, V., Eshtiaghi, N. (2018): Evaluation of single and two stage anaerobic digestion of landfill leachate: Effect of pH and initial organic loading rate on volatile fatty acid (VFA) and biogas production. – Bioresource technology 251: 364-373.

[6] Carucci, G., Carrasco, F., Trifoni, K., Majone, M., Beccari, M. (2005): Anaerobic digestion of food industry wastes: effect of codigestion on methane yield. – Journal of Environmental Engineering 131(7): 1037-1045.

[7] Cavinato, C., Bolzonella, D., Fatone, F., Cecchi, F., Pavan, P. (2011): Optimization of two-phase thermophilic anaerobic digestion of biowaste for hydrogen and methane production through reject water recirculation. – Bioresource Technology 102(18): 8605-8611.

[8] Chen, C.-C., Sen, B., Chuang, Y.-S., Tsai, C.-J., Lay, C.-H. (2012): Effect of effluent recycle ratio in a continuous anaerobic biohydrogen production system. – Journal of Cleaner Production 32: 236-243.

[9] Colin, X., Farinet, J.-L., Rojas, O., Alazard, D. (2007): Anaerobic treatment of cassava starch extraction wastewater using a horizontal flow filter with bamboo as support. – Bioresource technology 98(8): 1602-1607.

[10] Corona, V. M., Razo-Flores, E. (2018): Continuous hydrogen and methane production from Agave tequilana bagasse hydrolysate by sequential process to maximize energy recovery efficiency. – Bioresource technology 249: 334-341.

[11] Corsino, S. F., Di Trapani, D., Torregrossa, M., Viviani, G. (2018): Aerobic granular sludge treating high strength citrus wastewater: Analysis of pH and organic loading rate effect on kinetics, performance and stability. – Journal of environmental management, 214: 23-35.

[12] Dareioti, M. A., Kornaros, M. (2014): Effect of hydraulic retention time (HRT) on the anaerobic co-digestion of agro-industrial wastes in a two-stage CSTR system. – Bioresource technology 167: 407-415.

[13] di Biase, A., Devlin, T., Kowalski, M., Oleszkiewicz, J. (2018): Performance and design considerations for an anaerobic moving bed biofilm reactor treating brewery wastewater: Impact of surface area loading rate and temperature. – Journal of environmental management 216: 392-398.

[14] Farghaly, A., Tawfik, A. (2017): Simultaneous hydrogen and methane production through multi-phase anaerobic digestion of paperboard mill wastewater under different operating conditions. – Applied biochemistry and biotechnology 181(1): 142-156.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1705_1116311174

[15] Fayaz, H., Saidur, R., Razali, N., Anuar, F., Saleman, A., Islam, M. (2012): An overview of hydrogen as a vehicle fuel. – Renewable and Sustainable Energy Reviews 16(8): 5511-5528.
[16] Han, W., Wang, B., Zhou, Y., Wang, D.-X., Wang, Y., Yue, L.-R., Li, Y.-F., Ren, N.-Q. (2012): Fermentative hydrogen production from molasses wastewater in a continuous mixed immobilized sludge reactor. – Bioresource technology 110: 219-223.
[17] Hu, Y., Kobayashi, T., Qi, W., Oshibe, H., Xu, K.-Q. (2018): Effect of temperature and organic loading rate on siphon-driven self-agitated anaerobic digestion performance for food waste treatment. – Waste Management 74: 150-157.
[18] Koutrouli, E. C., Kalfas, H., Gavala, H. N., Skiadas, I. V., Stamatelatou, K., Lyberatos, G. (2009): Hydrogen and methane production through two-stage mesophilic anaerobic digestion of olive pulp. – Bioresource Technology 100(15): 3718-3723.
[19] Krishnan, S., Singh, L., Sakinah, M., Thakur, S., Wahid, Z. A., Ghrayeb, O. A. (2017): Role of organic loading rate in bioenergy generation from palm oil mill effluent in a two-stage up-flow anaerobic sludge blanket continuous-stirred tank reactor. – Journal of cleaner production 142: 3044-3049.
[20] Lee, Y.-W., Chung, J. (2010): Bioproduction of hydrogen from food waste by pilot-scale combined hydrogen/methane fermentation. – International journal of hydrogen energy 35(21): 11746-11755.
[21] Luo, G., Xie, L., Zou, Z., Wang, W., Zhou, Q., Shim, H. (2010): Anaerobic treatment of cassava stillage for hydrogen and methane production in continuously stirred tank reactor (CSTR) under high organic loading rate (OLR). – International journal of hydrogen energy 35(21): 11733-11737.
[22] Massanet-Nicolau, J., Dinsdale, R., Guwy, A., Shipley, G. (2015): Utilising biohydrogen to increase methane production, energy yields and process efficiency via two stage anaerobic digestion of grass. – Bioresource technology 189: 379-383.
[23] Metcalf, E., Eddy, H. (2003): Wastewater engineering: treatment and reuse. – In: Techobanoglous, G., Burton, F. L., Stensel, H. D. (eds.) Wastewater Engineering, Treatment, Disposal and Reuse. Tata McGraw-Hill Publishing Company Limited, 4th edition. New Delhi, India.
[24] Nualsri, C., Kongjan, P., Reungsang, A. (2016): Direct integration of CSTR-UASB reactors for two-stage hydrogen and methane production from sugarcane syrup. – International Journal of Hydrogen Energy 41(40): 17884-17895.
[25] Panda, S. K., Mishra, S. S., Kayitesi, E., Ray, R. C. (2016): Microbial-processing of fruit and vegetable wastes for production of vital enzymes and organic acids: Biotechnology and scopes. – Environmental research 146: 161-172.
[26] Park, M. J., Jo, J. H., Park, D., Lee, D. S., Park, J. M. (2010): Comprehensive study on a two-stage anaerobic digestion process for the sequential production of hydrogen and methane from cost-effective molasses. – International journal of hydrogen energy 35(12): 6194-6202.
[27] Puyol, D., Batstone, D. J., Hülsen, T., Astals, S., Peces, M., Krömer, J. O. (2017): Resource recovery from wastewater by biological technologies: opportunities, challenges, and prospects. – Frontiers in microbiology 7: 2106.
[28] Scott, D. S. (2004): Hydrogen-the case for inevitability. – International journal of hydrogen energy 3(29): 225-227.
[29] Shen, F., Li, H., Wu, X., Wang, Y., Zhang, Q. (2018): Effect of organic loading rate on anaerobic co-digestion of rice straw and pig manure with or without biological pretreatment. – Bioresource technology 250: 155-162.
[30] Shi, X., Leong, K. Y., Ng, H. Y. (2017): Anaerobic treatment of pharmaceutical wastewater: a critical review. – Bioresource technology 245: 1238-1244.
[31] Siddiqui, Z., Horan, N., Salter, M. (2011): Energy optimisation from co-digested waste using a two-phase process to generate hydrogen and methane. – International journal of hydrogen energy 36(8): 4792-4799.

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DOI: http://dx.doi.org/10.15666/aeer/1705_1116311174

[32] Sivagurunathan, P., Kumar, G., Bakonyi, P., Kim, S.-H., Kobayashi, T., Xu, K. Q., Lakner, G., Tóth, G., Nemestóthy, N., Bélafi-Bakó, K. (2016): A critical review on issues and overcoming strategies for the enhancement of dark fermentative hydrogen production in continuous systems. – International journal of hydrogen energy 41(6): 3820-3836.

[33] Tritt, W. P., Kang, H. (2017): Slaughterhouse wastewater treatment in a bamboo ring anaerobic fixed-bed reactor. – Environmental Engineering Research 23(1): 70-75.

[34] Venetsaneas, N., Antonopoulou, G., Stamatelatou, K., Kornaros, M., Lyberatos, G. (2009): Using cheese whey for hydrogen and methane generation in a two-stage continuous process with alternative pH controlling approaches. – Bioresource technology 100(15): 3713-3717.

[35] Wang, J., Wan, W. (2009): Factors influencing fermentative hydrogen production: a review. – International journal of hydrogen energy 34(2): 799-811.

[36] Wang, R.-M., Wang, Y., Ma, G.-P., He, Y.-F., Zhao, Y.-Q. (2009): Efficiency of porous burnt-coke carrier on treatment of potato starch wastewater with an anaerobic–aerobic bioreactor. – Chemical Engineering Journal 148(1): 35-40.

[37] Wang, B., Li, Y., Wang, D., Liu, R., Wei, Z., Ren, N. (2013): Simultaneous coproduction of hydrogen and methane from sugary wastewater by an “ACSTRH–UASBMet” system. – International Journal of Hydrogen Energy 38(19): 7774-7779.

[38] Wickham, R., Xie, S., Galway, B., Bustamante, H., Nghiem, L. D. (2018): Anaerobic digestion of soft drink beverage waste and sewage sludge. – Bioresource technology 262: 141-147.

[39] Wu, C., Huang, Q., Yu, M., Ren, Y., Wang, Q., Sakai, K. (2018): Effects of digestate recirculation on a two-stage anaerobic digestion system, particularly focusing on metabolite correlation analysis. – Bioresource technology 251: 40-48.

[40] Yetilmezsoy, K., Sakar, S. (2008): Development of empirical models for performance evaluation of UASB reactors treating poultry manure wastewater under different operational conditions. – Journal of Hazardous materials 153(1-2): 532-543.

[41] Zahedi, S., Rivero, M., Solera, R., Perez, M. (2018): Mesophilic anaerobic co-digestion of sewage sludge with glycerine: Effect of solids retention time. – Fuel 215: 285-289.

[42] Zhang, W., Zhang, L., Li, A. (2015): Anaerobic co-digestion of food waste with MSW incineration plant fresh leachate: process performance and synergistic effects. – Chemical Engineering Journal 259: 795-805.

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DOI: http://dx.doi.org/10.15666/aeer/1705_1116311174