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Biological Fermentative Methane Production from Brown Sugar Wastewater in a Two-Phase Anaerobic System

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Abstract

In this study, a two-phase anaerobic digestion system was established to combine the bioenergy recovery and chemical oxygen demand (COD) removal. The synthetic brown sugar wastewater was used as a substrate. Six system organic loading rates (OLRs) from 12 to 32 kg/(m³·d) were analyzed. Results showed that the highest CH_4 production rate (18.5 L/d) were obtained at OLR= 24 kg/(m³·d). The total energy recovery rate was calculated to assess the overall efficiency of energy recovery capacity. The highest energy recovery rate was 728.67 kJ/d, occurred at OLR=24 kg/(m³·d). Meanwhile, the total COD removal was very high, up to 69.4%. Therefore, the system had a great contribution to energy recovery from brown sugar wastewater.

Keywords: CSTR-UASB; OLR; Hydrogen production; Methane production; Energy recovery

Introduction

With the rapid development of industry and an increase in the standards of living, water pollution is becoming a general phenomenon [1]. Over the years, the widespread use of fossil fuels like coal and oil has already caused serious pollution in the global environment [2], and has even posed a threat to the survival of mankind itself [3]. At the same time, fossil fuels are a non-renewable energy source which can be depleted because of overexploitation [4]. Therefore, the question of how to degrade pollutants quickly, change waste into valuable commodities, and achieve the sustainable development of energy resources has become one of the most urgent problems facing the field of contemporary environmental science [5].

The exploitation and application of new alternative clean energies represent the general trend [6]. As efficient, clean, and environment-friendly sources of energy, hydrogen and methane have aroused people's extensive attention [7]. Continuous flow CSTR-UASB two-phase anaerobic systems have the advantage of high mass transfer efficiency, fast degradation rate of organic compounds and strong ability to produce hydrogen and methane. It can achieve both the removal of pollutants as well as the recycling of new energy which is of great industrial value. Biohydrogen production from wastewater through fermentation is carried out by anaerobic acidogenic bacteria with highly diverse fermentation characteristics [8] and hydrogen production capabilities [9]. After hydrogen production, the effluent contains high content of organic acids. Anaerobic digestion for methane production is an ideal way to utilize metabolites (volatile fatty acids (VFA), and alcohols) from hydrogen production process for additional energy production [10]. The two-phase process separates and enriches acidogens and methanogens in different reactors that may improve the process stability and efficiency compared to traditional one-phase methane production process. Although hydrogen and methane production from waste under lower OLR has been reported [11], performance of the two-phase process under higher OLR was seldom investigated. Moreover, there are few reports on brown sugar wastewater by treatment of a two-phase anaerobic system [12,13]. Investigation on the process performance of two-phase CSTR-UASB under higher OLR may accelerate its application.

In this study, using brown sugar wastewater as the carbon substrate, the performance of continuous H_2 and CH_4 production rates were investigated at different OLRs for CSTR-UASB two-phase anaerobic system.

Materials and Methods

Experimental set-up

This experiment utilized a continuous-flow CSTR-UASB two-phase anaerobic system, with the effective volume of CSTR being 7.0 L and the total volume being 15.8 L. The reactor was equipped with a stirring device which ensured the complete and continuous mixture of microorganisms and water at a stirring speed of 120 r·min^{-1}. Anaerobic conditions in the reactor were ensured through the liquid seal on the shaft; the total volume of UASB was 21.2 L while its effective volume was 9.8 L. There were gas-liquid-solid three-phase separators located in both reactors which had an integrated structure of reaction and settling zone. The reactor walls were wound with resistance wires and a temperature control system maintained a reactor temperature of (35 ± 1)°C in order to ensure high microorganism activity. The continuity of the experiment was maintained by using a peristaltic pump providing water into the reactor at a constant speed. The peristaltic pump could change the influent flow rate and then change hydraulic retention time (HRT) by adjusting its revolution speed. The structure of CSTR-UASB two-phase anaerobic system was as shown in Figure 1. The characteristics of substrate used in this study were shown in Table 1.

Analytical methods

The biogas composition including hydrogen and methane was measured using a gas chromatograph (GC, 6809 N Network GC System, Agilent Technologies, Waldron, Germany) equipped with a thermal conductivity detector (TCD). The column (2 m×5 mm) was

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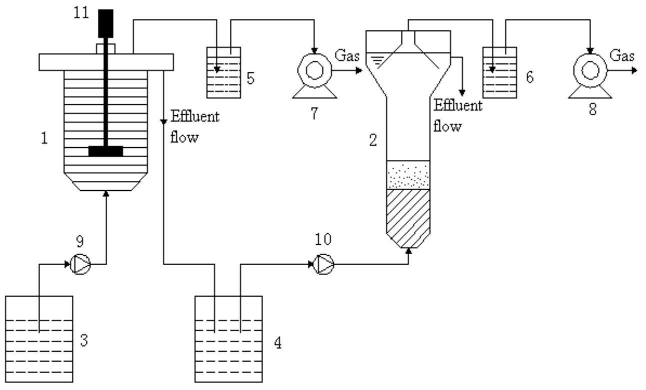

1. CSTR reactor 2.UASB reactor 3(4). Waste water box 5(6). Water lock

7(8). Biogas meter 9(10). Feed pump 11. Agitator

Figure 1: Schematic diagram of CSTR-UASB two-phase anaerobic system.

Parameters	Values	Parameters	Values
Volatile suspended solid (VSS)	1.3 g/L	SO_4^{2-}	1.5 g/L
Total nitrogen (TN)	2.5 g/L	PO_4^{3-}	0.3 g/L
Chemical oxygen demand (COD)	30 g/L	pH	6.3
Total organic carbon (TOC)	10.8 g/L		

Table 1: The characteristics of wastewater used in this study.

filled with porapak Q (50-80 meshes), Nitrogen was used as carrier gas with a flow rate of 40 mL/min.

Volatile fatty acids (VFA) and ethanol in liquid samples were measured by using a gas chromatograph (GC, 6890N Network GC System, Agilent Technologies, Waldbrown, Germany) equipped with a flame ionization detector (FID). The column (Zm) was packed with supporter of GDX-103 (60-80 meshes). The temperatures of the injection port, the oven, and the detector were adjusted to 220°C, 190°C, and 220°C, respectively. The carrier gas was nitrogen at a flow rate of 30 mL/min.

CODs of the samples were measured according to Standard Methods [14]. The pH and ORP were measured by pH meter (PHS-25). A wet gas meter (LML-1) was utilized to measure biogas yield.

The sludge cultivation and operational control parameters

The sludge acclimation and operational control of CSTR reactor: The inoculated sludge of the reactor adopted sludges from a secondary sedimentation tank in a sewage treatment plant in Harbin. It could remove imprities and large particulate matters by precipitation, washing and filtering. Brown sugar water with 10000 mg/L COD was used. The COD: N: P was maintained at a ratio of 1000:5:1 [15] by adding a certain amount of NH_4Cl and KH_2PO_4 in order to supply microorganisms with adequate nitrogen and phosphorus and then cultivated with intermittent aeration for 20 days. During this process, aeration was stopped for 1 h daily so that we could remove the supernatant fluid and add clear water. The mature sludge after domestication was yellow-brown granule with good settlement ability.

The acclimated sludge was then transferred to the CSTR reactors and started with continuous-flow approach under the conditions of HRT of 6 h, temperature of (35 ± 1) °C, influent pH of 7.00 ± 0.1, OLR of 12 kg/(m³·d), suspended solid (SS) of 12.81 g/L, volatile suspended solid (VSS) of 8.35 g/L and VSS/SS (biological activity) of 0.65 in inoculated sludge. After about 30 days, the reactor reached a steady state. At this point, the hydrogen production was about 3.5 L/d and the hydrogen content was around 43%. The liquid end products are shown in Table 2, of which the content of ethanol and acetate accounted for 71.5%, mainly for ethanol fermentation.

The sludge acclimation and operational control of UASB reactor: It adopted the same sludge which the CSTR used as the inoculated sludge of the reactor and experienced an identical impurity removal process. Using the effluent of CSTR reactor (liquid fermentation products) as the reaction substrate, the OLR stood at approximately 7.2 kg/ (m³·d). At the same time, a small amount of NH_4Cl and KH_2PO_4 were added to adjust appropriate nutrition and phosphorus levels in order to maintain COD: N: P in a 200:5:1 proportion. It began to produce methane after about 50 days when the sludge acclimation had completed in the reactor. At that time, SS and VSS in the reactor were 16.28 and 10.36 g/L, respectively. Under the conditions of HRT of 8 h, temperature of (35 ± 1)°C and influent pH of 7.80 ± 0.2, the reactor could operate steadily after another 20 d, producing a methane yield of approximately 5.0 L and a methane content of around 68%.

The control parameters and running status are shown in Table 1 when two reactors reached equilibrium.

After being stabilized, CSTR-UASB two-phase reactors kept HRT a constant. It was from the continuous increase of OLR and the adjustment of the influent pH that the effects of OLR on CSTR-UASB anaerobic system can be observed and studied. The running process in which OLR increased from 12 to 32 kg/(m³·d) was divided into six stages, each stage increased 4 kg/(m³·d) and response (run) time was 6 days (Figure 2).

Results and Discussion

Bio-hydrogen and methane production

As can be seen from Figure 3, while OLR increased from 12 to 32 kg/(m³·d), the bio-hydrogen production in CSTR reactor presented a basic trend of sustained growth while the hydrogen content fluctuated between 30% and 50% whereas the methane production in the UASB reactor first increased and then decreased. On the first day that OLR reached 16 kg/(m³·d), the methane yield witnessed a sudden rise from

Parameter	CSTR	UASB
HRT(h)	6	8
Temperature (℃)	35 ± 1	35 ± 1
Influent pH	7.1	7.8
Effluent pH	5.0	6.8
Influent COD (mg/L)	3000	1800
Effluent COD (mg/L)	1800	1150
SS (g/L)	13.85	17.61
VSS (g/L)	9.62	11.53
Ethanol (mg/L)	283.5	0
Acetate (mg/L)	64.7	178.6
Propionate (mg/L)	44.6	30.1
Butyrate (mg/L)	87.6	129.5
Valerate (mg/L)	6.3	0

Table 2: Various control parameters and running status after the CSTR and UASB reaching equilibrium.

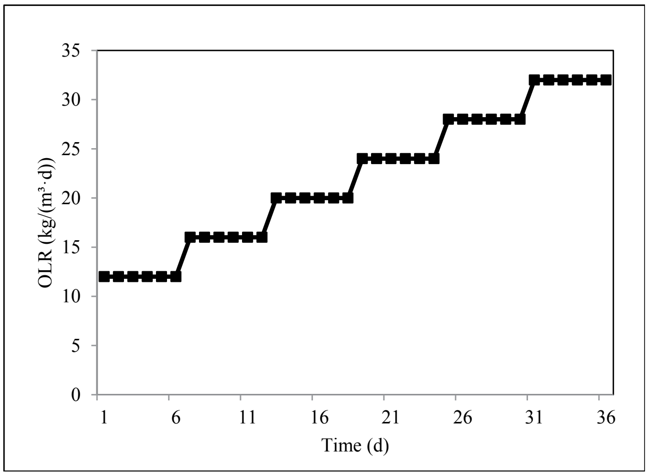

Figure 2: Variation of OLR at different days.

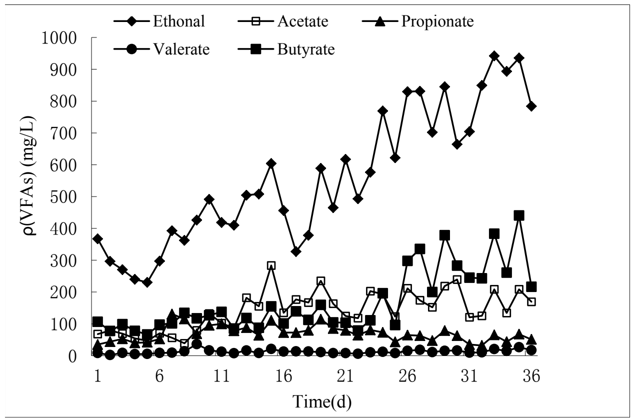

Figure 4: Variation of liquid fermentation products in operation process of CSTR reactor.

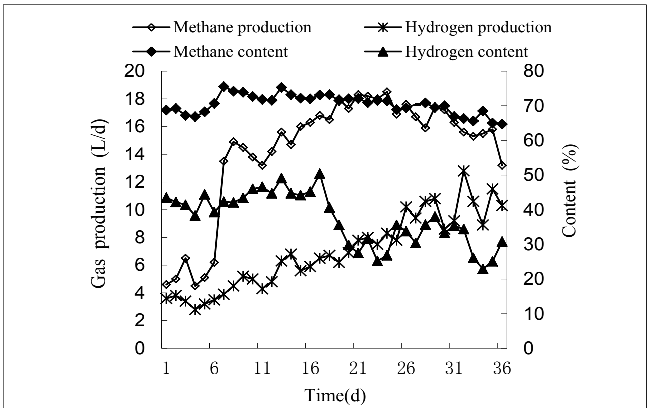

Figure 3: Biohydrogen and methane production in operation process of CSTR-UASB two-phase anaerobic reactor.

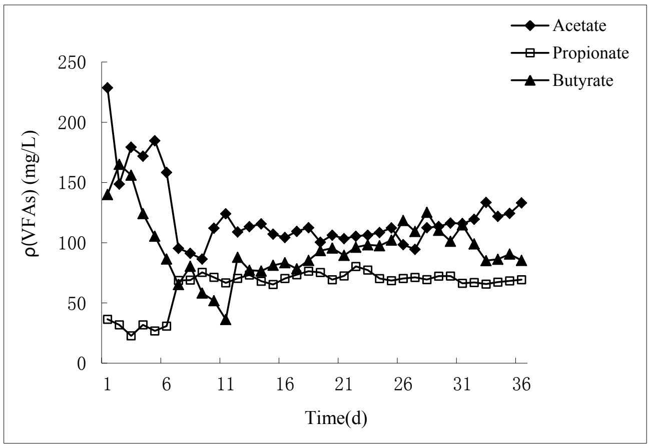

Figure 5: Variation of liquid fermentation products in operation process of UASB reactor.

6.2 to 13.5 L/d. At this point, ethanol and acetate contents in acidogenic phase were 392.7 and 56.1 mg/L, respectively. However, their contents changed into 0 and 95.4 mg/L in methanogenic phase. An explanation for this is that there might have still been a certain amount of acidogenic fermentation bacteria in methanogenic phase, which could take advantage of large amounts of ethanol of acidogenic phase, and ethanol was then oxidized to acetate rapidly. At the same time, methanogens had also begun to continuously use acetate for fermentation in order to produce methane. In the process of OLR gradually increasing from 16 kg/(m^3·d) to 24 kg/(m^3·d), the methane production yield sustained stable growth. When OLR continued to increase to 32 kg/(m^3·d), methane production began to decline and had a relatively large fluctuation. This phenomenon might be caused by methane-producing bacteria, which had a certain impact-resistance to the effluent OLR in acidogenic phase [16]. The maximum tolerance level was 24 kg/(m^3·d). Once this limit was exceeded, the activity of methanogenic bacteria for degrading organic matter would be inhibited and methane production would gradually decline.

Variation in liquid fermentation products

Figures 4 and 5 show the variation of liquid fermentation products in the acidogenic and methanogenic phases. As the influent OLR continued to increase, the total content of liquid fermentation products in acidogenic phase also increased, from 486.4 mg/L at the initial OLR of 12 kg/(m^3·d) to 1376.4 mg/L at an OLR 32 kg/(m^3·d). When compared to other liquid end products, the upward trend of ethanol was more evident, while other volatile acids fluctuated. The ORP of the CSTR reactor changed between -430 and -320 mV and UASB varied from -460 to -380 mV. In the entire process of operation, the total content of liquid fermentation products in various stages in CSTR represented 486.4´730.5´860.4´975.5´1274.7´1376.4 mg/L while the content of ethanol and acetate were 348.2´499.4´646.1´757.7´935.4´1012.5 mg/L, which accounted for 71.6%´68.4%´75.1%´77.7%´73.4%´73.6% of the total, respectively. It can be suggested that the acidogenic phase was mainly maintaining ethanol-type fermentation.

Compared with the acidogenic phase, there was no ethanol or valerate in the liquid fermentation products of the UASB. When the initial OLR was 12 kg/(m^3·d), the acetate content of the methanogenic phase was quite high and the average level was 178.6 mg/L. When the OLR increased to 16 kg/(m^3·d), acetate content rapidly decreased to 103 mg/L, subsequently stabilizing at around 110 mg/L. This change might

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be due to the existence of a large amount of active microorganisms in methanogenic phase [17], which could make full use of ethanol for fermentation in acidogenic phase, resulting in the immediate formation of acetate. With the OLR increasing gradually, microorganisms in methanogenic phase continuously used this acetate for further fermentation. Thus, acetate content would not be too high. The butyrate in methanogenic phase showed a downward trend during the influent OLR of 12 and 16 kg/(m^3·d). On the 11th day, the butyrate content reduced to the minimum value of 36.3 mg/L and then leveled out at 100 mg/L with slight fluctuation. This process might be the result of the combined effects of hydrogen-producing acetogens and methanogenus [18]. In addition, since valerate can easily convert to propionate and the conversion rate of propionate is slow, propionate would accumulate to some extent [19]. As a result, the timely adjustment of pH was necessary in order to ensure a higher rate of two-phase anaerobic fermentation of microorganisms.

Variations in COD removal

Figure 6 shows the variation of COD removal for two-phase anaerobic system in operation process. As the figure shows, the maximum COD removal in acidogenic and methanogenic phases was 49.7% and 54.9%, respectively. The total COD removal varied from 60.8% to 72%. When the OLR reached 28 kg/ (m^3·d), the total removal was at a maximum, up to 72%. At that time, the energy recovery rate was 712.82 kJ/d (Table 3). An explanation for this is that the anaerobic active micro-organisms had better resistance of high OLR on brown sugar wastewater after being specially domesticated and had better removal effects on COD. In a CSTR-UASB two-phase anaerobic system, the COD removal mainly existed in two areas: Initial organic matter was converted into intermediate products by hydrogen-producing acetogens; intermediate products were further converted into methane by methanogenus [20]. After two stages of degradation, COD removal improved significantly.

Energy recovery rate

Since our two-phase anaerobic system produced a large amount of H_2 and CH_4, the process performance in terms of energy recovery derived from the combination of the two biofuels was calculated according to their combustion heat values. As shown in Table 2, the energy recovery rate tended to increase as OLR increased from 12 to 24 kg/ (m^3·d), which is quite obvious because both H_2 and CH_4 production rates increased with increasing OLR. The system achieved the maximum energy recovery rate of 728.67 kJ/d at OLR of 24 kg/ (m^3·d), this difference could be attributed to the variation in bacterial population and structure [21]. The optimum rates for energy recovery in the comparable process differed significantly among previous studies (Table 4). The substrate used in this study was more complex compared to other studies. Thus, the CSTR-UASB two-phase anaerobic system may be effective bioreators when applied to energy recovery from brown sugar wastewater. However, further research would be needed to find optimal conditions for higher energy recovery rate. In the future it could be possible to make full use of energy from organic wastewater through CSTR-UASB two-phase anaerobic system.

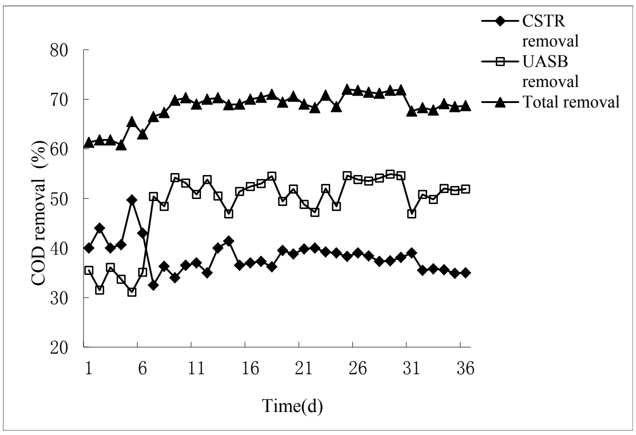

Figure 6: Variation of COD removal in operation process of CSTR-UASB two-phase anaerobic reactor.

Conclusions

This experiment used a CSTR-UASB two-phase anaerobic system with artificial brown sugar water as a fermentation substrate to combine the bioenergy recovery and COD removal. As we studied the process of the influent OLR increasing from 12 to 32 kg/(m^3·d), results showed that the two-phase anaerobic system operated at OLR=24 kg/ (m^3·d) exhibited the best energy recovery rate of 712.82 kJ/d. Meanwhile, the COD removal was up to 69.4%, which meant that the system had good effects on the degradation of brown sugar wastewater as well as energy recovery capacity.

OLR (kg/ m^3·d)	COD (mg/L)	H_2 production rate (mol/d)	CH_4 production rate (mol/d)	Energy recovery rate (kJ/d)
12	3000	0.15	0.24	228.54
16	4000	0.21	0.63	504.63
20	5000	0.28	0.71	636.47
24	6000	0.33	0.81	728.67
28	7000	0.43	0.76	712.82
32	8000	0.47	0.68	658.42

(A energy recovery rate= H_2 production rate (mol/d) ×242kJ/mol H_2 + CH_4 production rate (mol/d) ×801kJ/mol CH_4)

Table 3: Performance of $H_2$and CH_4 production rate as well as energy recovery rate with OLR increasing.

Substrate	Reactor HR / MR	Culture condition: Temperature	pH	COD	Maximum energy recovery rate	Reference
Molasses	PBR	35°C	5.5	28g/L	99.62 kJ/L/d	Park et al. [22]
	PBR	35°C	7.0			
Glucose	CSTR	35°C	5.5	3.39g/L	27.77 kJ/L/d	Michael et al. [23]
	CSTR	35°C	7.0			
Cheese whey	CSTR	35°C	N.C	61g/L	201.48 kJ/L/d	Georgia et al. [24]
	PABR	35°C	N.C			
Sucrose	CSTR	35°C	5.2	10~20g/L	189.37 kJ/L/d	Kyazze et al. [25]
	UAF	35°C	N.C			
Olive pulp	CSTR	55°C	N.C	——	9.29 kJ/L/d	Gavala et al. [26]
	CSTR	55°C	N.C			
Food waste	LBR	37°C	N.C	13.1g/L	103.79 kJ/L/d	Han et al. [27]
	UASB	37°C	N.C			
Brown Sugar	CSTR	35°C	5.0	3~8g/L	77.6 kJ/L/d	This study
	UASB	35°C	6.8			

HR: Hydrogenic reactor; MR: Methanogenic reactor; UAF: Up-flow anaerobic filter; LBR: Leaching-bed reactor; PABR: Periodic anaerobic baffled reactor; PBR: Packed-bed reactor; N.C. means Non-control.

Table 4: Comparison of previous performance of two-stage system for energy recovery capacity.

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