Comparative Analysis and Improvement of the 2-CSTRs Anaerobic Digestion System on Biofuel Production

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Abstract: A 2-CSTRs anaerobic digestion system using molasses wastewater as a biochemical substrate for fermentative bioenergy production and substrate degradation under various ethanol recovery rates was developed. The effects of ethanol recovery rates (0%-80%) and organic loading rates (OLRs; 8, 16 and 24 kgCODm⁻³ d⁻¹) on bioenergy production rate (ε_T) and bioenergy production yield were examined, respectively. Since the system produced a significant amount of biofuels (i.e. hydrogen, methane and ethanol), the process performance in terms of energy conversion derived from the combination of three biofuels was calculated according to production rates and combustion heat values. Recycle and reflux are the two reuse approaches for effluents in the system. A maximum HPR of 1.34 (± 0.7) mol d⁻¹ and a maximum EPR of 0.45 (± 0.4) mol d⁻¹ were observed at the OLR of 24 kgCOD m⁻³ d⁻¹. Linear regression of the collection between HPR and EPR can be expressed as $y=0.3182x+0.0114$ ($r^2=0.9853$). The highest MPR of 0.45 (± 0.07) mol d⁻¹ was obtained at the original OLR of 24 kgCOD m⁻³ d⁻¹, with an ethanol recover rate of 0%. Recycle resulted to a higher ε_T and energy yield than reflux with the ethanol recover rate of 0% and 20%, respectively. This was equal to the ethanol recover rate of 50%, but lower than the ethanol recover rate of 80%; indicating that recycle would be more feasible for lower ethanol recover rates, and reflux may be more suitable with other kinds of ethanol recover rates.

Keywords: continuous stirred tank reactor; two-phase anaerobic digestion system; substrate degradation rate; ethanol recovery rate; reuse approaches.

1. Introduction

With the development of the modern industry and the enormous consumption of energy sources, it is inevitable to look at cooperating in developing alternative non-conventional and renewable sources of energy ^[1]. Hydrogen, methane and ethanol have attracted a great deal of attention due to the high caloric value, recyclability and non-polluting nature of these sources ^[2]. As one of the main research objects of the technology area, bioenergy (biohydrogen, biomethane and bioethanol) production has gained wide attention. However, these biological methods have limitations. Take biohydrogen production as an example. Photobiological processes are limited by many practical and fundamental limitations ^[3], and dark bacterial hydrogen fermentations have relatively low yields of hydrogen obtained with typically 10-30% of the substrate converted to hydrogen ^[4]. Therefore, improving bioenergy production yield and efficiency has been an area of emphases in the field of bioenergy production.

Anaerobic digestion technology is a bioenergy production method that not only allows bioenergy to be obtained, but also provides the extra benefit of treating organic waste ^[5,6]. Anaerobic digestion involves a commensal of two major types of bacteria flora: acidogenic bacteria, mainly hydrolyzes the substrates into hydrogen, carbon dioxide and short-chain volatile fatty acids (SCFAs); and methanogenic bacteria, converts metabolites from the acidogenic bacteria to methane ^[6]. For optimizing the growth of each type of bacteria, in 1971, Pohland and Ghosh have proposed a hierarchical split-phase anaerobic digestion theory ^[7]. The two basic processes can partially be separated into a series of separate bioreactors ^[8]. The first reactor is for

the acidogenic bacteria, while the second reactor is for methanogenic bacteria [8]. Hierarchical split-phase anaerobic digestion can be of some advantage in making the process more efficient to product renewable energy and more resistant to shock loading [9]. However, in earlier works on hierarchical split-phase anaerobic digestion, few measurements on simultaneous biofuel production have been reported in literature [10,11,12]. The fermentation pattern of the acidogenic phase is an important consideration for higher bioenergy production in a two-phase anaerobic digestion system. Fermentation types of the acidogenic phase mainly include the following: acetate-type, propionate-type, butyrate-type, and ethanol-type. For a long period, acetate-type fermentation has been depicted as the most common pathway in past surveys [13,14]. In the 1990s, the studies conducted by Ren *et al.* and H. Wang *et al.* revealed that ethanol-type fermentation is the optimal pathway for fermentative hydrogen production by organic waste water. Ethanol-type fermentation is able to preserve the balance of $\text{NADH}+\text{H}^+/\text{NAD}^+$, allowing the acidogenic fermentation process to be favorable for hydrogen production [15]. Since ethanol-type fermentation could produce a significant amount of biogas and liquid biofuels (i.e. hydrogen and ethanol), it is advisable that both hydrogen and ethanol can be recovered during the fermentation process, in order to realize additional economical benefits [16]. In previous studies, almost all researchers have focused on the recovery of only hydrogen or ethanol during the fermentation process, which resulted in low levels of energy production rate [4,17]. However, the production of hydrogen, ethanol and methane may be superior in producing biofuel. Previous works on continuous operation studies have revealed the mechanism and optimal parameters for biogas production, and insufficient information is available on biological energy production from molasses in a mixed microbial community culture [10,11,18].

In this study, energy production rate derived from the combination of biofuels was calculated according to combustion heat values. The performance of sequential biological energy production from molasses wastewater in the 2-CSTRs anaerobic digestion system at different organic loading rates (OLRs) was examined. The energy production rate (ϵ_T) and substrate degradation rate (SDR) of the system can be dug. Due to the beneficial impact on methane production and the dual recovery of two of the most important biofuels (hydrogen and ethanol), ethanol-type fermentation was employed by controlling a number of parameters such as OLR, oxidation reduction potential (ORP), hydraulic retention time (HRT) and temperature [19,20] in the acidogenic phase.

2. Material and Methods

2.1 Experimental Apparatus

Fig.1 Schematic diagram of 2-CSTRs anaerobic digestion system

The continuous stirred tank reactor (CSTR) was sealed to assure anaerobic condition. The reactor was made of hard PVC plastic with a gas-liquid-solid separating device installed inside the CSTR, in order to promote the retention of sludge. This was operated in continuous flow mode. Since the HRT of the hydrogen production phase was 1/4 of the methane production phase, the experimental anaerobic digestion system (Fig. 1) comprised of a 5 L CSTR for hydrogen-production reactor (HyCSTR) and a 20 L CSTR for the methane-production reactor (MeCSTR). Before feeding into the MeCSTR, the effluent of the HyCSTR would be recycled to recover ethanol in the metabolites of the acidogenic bacteria; and the MeCSTR effluent goes back to the MeCSTR for recycling. The temperatures of both reactors were automatically maintained at $(35 \pm 1)^{\circ}\text{C}$ using electric jackets around the reactors outside, and the substances were completely mixed by a constant speed stirred with a gear shift. The influent flow rates were controlled by feed pumps to regulate the HRTs and OLRs in the reactors. The generated biogas was collected with a water-lock and measured by a wet gas meter.

2.2 Sludge and Feeding

The seed sludge in the system was collected from a secondary settling tank in a local municipal wastewater treatment plant in Harbin. A 0.5 mm in diameter mesh was used to eliminate large particulate materials that could cause pump failure. The HyCSTR sludge was aerated and fed with molasses in batches and incubated at room temperature for 30 days to inhibit methanogen activity, and the MeCSTRs sludge was unaerated and fed with the same molasses. After cultivating, the sludge with suspended solids (SS) of 19.88 g/L and volatile suspended solids (VSS) of 12.37 g/L was inoculated into the HyCSTR; and the sludge with SS of 17.62 g/L and VSS of 11.03 g/L was inoculated into the MeCSTRs.

The molasses used in this study was collected from a local beet sugar refinery. Molasses that contain a high concentration of carbohydrates including sugars was diluted by water to a certain concentration. The COD: N: P was maintained at a ratio of 100:5:1 by adding synthetic fertilizer, in order to supply microorganisms with adequate nitrogen and phosphorus ^[21].

2.3 Operating conditions

After the sludge was inoculated into reactors, the HyCSTR was operated in continuous mode by supplying the designed synthetic wastewater at HRT of six hours with OLRs of 8, 16 and 24 $\text{kgCOD m}^{-3} \text{d}^{-1}$, respectively. MeCSTRs were operated in continuous mode by supplying the effluent of HyCSTRs at HRT of 24 hours. Before feeding these into the MeCSTR, the effluent of the HyCSTR would be recycled to recover ethanol in the metabolites of acidogenic bacteria; and the MeCSTR effluent was sent back to the MeCSTR for recycling. Ethanol recover rates were 0%, 20%, 50% and 80%, respectively.

2.4 Analytical Methods

COD, pH, ORP, biogas yield and its constituents were measured and monitored daily in this study. COD was determined using a chemical oxygen-consumption measurer (Model LH-3BA; Beijing Lianhua Tech Co. Ltd., Beijing, China), while pH and ORP were determined using a combo detector (Model EP 366; Guangzhou Baonuo Co. Ltd., Guangzhou, China).

Biogas yield was measured at room temperature using a wet gas meter (Model LML-1; Changchun Filter Co. Ltd., Changchun, China), while its constituents were analyzed using SC-II gas chromatography equipped with a tube for collecting the gas sample from exportation. The gas chromatography was equipped with a thermal conductivity detector (TCD) and a stainless steel column filled with 2 m of Porapak Q (60-80 meshes). Nitrogen was used as the carrier gas at a

flow rate of 70 ml/min. The operational temperature for the column oven and detector is 150°C. The sample was injected at a dose of 0.5 ml each time. The hydrogen yield could be calculated based on the percentage of hydrogen in the biogas.

Detection of SCFAs in the fermentation solution was analyzed by another gas chromatograph (GC112; Shanghai Anal. Inst. Co.) using a flame ionization detector (FID). A 2-m stainless steel column was packed with the supporter GDX-103 (60-80 meshes). The temperatures of the injection port, oven and detector was 220°C, 190°C, and 220°C, respectively. The carrier gas was also nitrogen at a flow rate of 30 ml/min.

3. Results and Discussion

3.1 Hydrogen and ethanol production

Hydrogen production rate (HPR, mol d⁻¹) and ethanol production rate (EPR, mol d⁻¹) have generally been regarded as important indices to evaluate these biohydrogen producing processes. Figure 2 reveals the profile of HPR, hydrogen content (HC, %) EPR, and ethanol content (EC, %) with increasing OLRs. OLR significantly affected the production rate and content of hydrogen and ethanol from HyCSTR. The maximum HPR of 1.34 (±0.7) mold⁻¹ and the maximum EPR of 0.45 (±0.4) mol d⁻¹ were observed at a OLR of 24 kgCOD m⁻³ d⁻¹. Furthermore, the maximum HC of 26.17 (±3.62)% and the maximum EC of 32.43 (±3.97)% were obtained at an OLR of 16 kgCOD m⁻³ d⁻¹. The collection between HPR and EPR can be observed from Figure 3, showing that HPR and EPR were generally proportionately correlated, irrespective of the different OLRs. Linear regression was expressed as $y=0.3182x+0.0114$ ($r^2=0.9853$).

Fig.2 Performance of HyCSTR with respect to HPR, HC, EPR and EC

Fig.3 Collection between HPR and ethanol production rate

The performance of HyCSTR was evaluated for substrate degradation potential as COD removal rate (η_{COD} , %) using Equation 1. In this equation, C_0 denotes the initial COD concentration (mg/L) of the influent and C_T denotes the COD concentration (mg/L) of the outlet.

$$\eta_{\text{COD}} = [(C_0 - C_T) / C_0] \times 100 \quad (1)$$

The substrate degradation rate (SDR, $\text{kgCOD m}^{-3} \text{d}^{-1}$) was calculated to study the pattern of hydrolysis yield, according to Equation 2.

$$\text{SDR} = \text{OLR} \times \eta_{\text{COD}} \quad (2)$$

Table 1 HyCSTR performance during the whole process

HRT(h)	OLR ($\text{kgCOD m}^{-3} \text{d}^{-1}$)	pH	η_{COD} (%)	SDR ($\text{kgCOD m}^{-3} \text{d}^{-1}$)
6	8	4.72	21.37 (± 4.81)	1.71 (± 0.32)
6	16	4.43	25.14 (± 4.32)	4.24 (± 0.39)
6	24	4.21	20.59 (± 3.97)	5.42 (± 0.79)

Table 1 reveals the profile of SDR, η_{COD} and pH with increasing OLRs. The maximum SDR of $5.42 (\pm 0.79) \text{ kgCOD m}^{-3} \text{d}^{-1}$ was obtained at a OLR of $24 \text{ kgCOD m}^{-3} \text{d}^{-1}$, and the maximum η_{COD} of $25.14 (\pm 4.32)\%$ was obtained at an OLR of $16 \text{ kgCOD m}^{-3} \text{d}^{-1}$. pH was also inspected during the operation in HyCSTR. It has been generally accepted that enzyme activity, metabolic pathway, and dominant species can all be directly affected by the pH value [22, 26]. A pH around 5.5 has been reported as the optimal pH for biohydrogen production in treating glucose and rice winery wastewater on continuous operation studies [23]. In this study, the lowest η_{COD} occurred at a pH value of 4.2 (Table 1), and the highest η_{COD} , EC and HC (Fig. 2 and Table 1) occurred at a pH value of 4.4; which was consistent with existing studies. No methane was detected in the biogas during the whole process, indicating that there might be an attribute to the low operational pH that could effectively block methanogenesis. This might be the reason for the relatively low substrate degradation efficiency observed in the acidogenic process.

3.2 Methane production

After the inoculation of the anaerobic mixed consortia, the MeCSTR was operated with the

designed synthetic wastewater at an OLR of 8 kgCOD m⁻³ d⁻¹ initially by adjusting the feed pH to a seven for a period of 37 days. Subsequently, the reactor was operated with effluent, which was recycled to recover ethanol in the metabolites of acidogenic bacteria generated from HyCSTR as feed. The process performance of the MeCSTR versus ethanol recovery rate was determined based on methane recovery, COD removal, and the methanogenic activity of the activated sludge. These results are shown in Table 2. Experimental data illustrates that the methane production rate (MPR, mol/day) gradually increased with increasing original OLRs, and decreased with an increasing ethanol recover rate. The highest MPR of 0.45(±0.07) mol d⁻¹ was obtained at the original OLR of 24 kgCOD m⁻³ d⁻¹ with an ethanol recovery rate of 0%. In spite of the diversiform ethanol recover rate, global $\eta_{\text{COD-M}}$ was above 46%; which fluctuated at certain values in small range due to the stable function of the bacterial flora. However, when the ethanol recovery rate achieved 80%, $\eta_{\text{COD-M}}$ showed a downward trend. After feeding soluble fermentative metabolite bound substrate, consistent decrease in SCFAs concentration observed with the experimental period in MeCSTR. As seen in Table.2, reduction in COD concentration in concurrence with the biogas production suggested the fact that the residual carbon source composed with SCFAs generated from HyCSTR had participated in methanogenic metabolic process.

Table 2 Performance of MeCSTR with different ethanol recovery rates

Ethanol recover rate (%)	Original OLR (kgCOD m ⁻³ d ⁻¹)	MPR (mol d ⁻¹)	η (%)	VSS (g L ⁻¹)	VSS/SS (%)
0	8	0.182(±0.07)	50.1(±3.22)	13.15(±1.27)	63.22(±3.29)
	16	0.266(±0.09)	52.3(±4.17)	13.67(±2.09)	64.97(±4.11)
	24	0.448(±0.13)	52.1(±3.25)	15.52(±1.92)	65.87(±3.07)
20	8	0.157(±0.05)	46.6(±4.91)	12.32(±1.72)	65.02(±4.21)
	16	0.234(±0.11)	51.3(±5.11)	13.07(±2.07)	63.69(±5.13)
	24	0.373(±0.11)	53.3(±4.78)	14.97(±1.93)	63.07(±4.81)
50	8	0.129(±0.04)	44.3(±4.93)	12.94(±2.04)	59.24(±3.19)
	16	0.211(±0.07)	46.9(±4.72)	11.69(±2.01)	61.88(±4.35)
	24	0.254(±0.08)	49.3(±5.16)	12.33(±1.95)	59.42(±5.33)
80	8	0.079(±0.05)	24.7(±3.39)	9.54(±1.36)	52.42(±3.24)
	16	0.117(±0.08)	27.5(±3.53)	8.77(±1.29)	50.08(±4.98)
	24	0.137(±0.08)	20.9(±4.12)	10.02(±1.57)	51.59(±5.34)

The following equations are used for computing methanogenic fermentation balance consuming the hydrogen and SCFAs formed during the acidogenic process.

As depicted by these equations, the extractions of hydrogen and ethanol formed in the HyCSTR are essential for methane formation in the subsequent methanogenic step [23,29]. The lowest MPR and $\eta_{\text{COD-M}}$ obtained with an ethanol recovery rate of 80% in this study may be attributed to two reasons. The first one is due to the extraction of ethanol and hydrogen generated from HyCSTR. The second reason might be that the methanogen activity was inhibited by acidophilic conditions attributed to the ethanol-recovery process. Optimum pH range for methanogenus activity was

reported in a range of 6.0–7.5 [25-33], while the MeCSTR pH range was below 5.7. However, effluent pH revealed a gradual rise in pH values. This might be due to the metabolic process taken by the methanogen bacteria with respect to SCFAs in feeding. Shift of pH to basic conditions correlated well with the documented SCFAs consumption [34]. Samples during the course of experiments were analyzed for SCFAs composition viz., acetic acid (HAc) to butyrate (HBu), propionic acid (HPr) and ethanol (EtOH) to have understanding of the change in the metabolic pathway [35-39]. During methanogenic process in MeCSTR, composition of metabolites varied significantly. A marked variation in EtOH concentration was observed along with increase in HAc and HPr concentrations, and decrease in HBu (Table.3). The variation observed in soluble metabolites concentration suggested that of SCFAs was consumed under methanogenic microenvironment in the process of methane generation [40,41].

Table 3 Concentrations of SCFAs of the MeCSTR

Original OLR (kgCOD m ⁻³ d ⁻¹)	EtOH recover rate (%)	EtOH Proportion (%)		HAC proportion (%)		HBu proportion (%)		HPr proportion (%)	
		Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
8	0	28.27	25.21	42.77	58.76	22.49	8.39	6.47	7.64
	20		22.54		60.51		8.74		8.21
	50		16.33		65.45		9.65		8.57
	80		8.83		72.01		10.24		8.92
16	0	32.43	27.52	49.84	61.01	13.85	7.3	3.88	4.17
	20		24.79		63.99		6.6		4.62
	50		18.13		69.69		7.05		5.13
	80		10.07		77.03		7.92		4.98
24	0	31.62	26.35	52.13	62.96	12.14	6.22	4.11	4.47
	20		23.58		64.91		6.99		4.52
	50		17.11		70.39		7.47		5.03
	80		9.11		76.32		9.56		5.01

The compositional ratio of metabolites formed during hydrogen and methane generation were often regarded as a significant indicator in evaluating the metabolic pathway of the biochemical process [27,41,43]. HAc and EtOH were the major metabolites observed, which indicate a good pathway for hydrogen production in the HyCSTR [39,42,43]. On the contrary, since HAC and EtOH are the main carbon sources for the methanogenic stage, and visible reduction in HAC and EtOH concentration were observed during the methanogenic process in this study. However, the effluent of the system continued to contain a certain amount of HAC and EtOH due to the incomplete reaction that resulted from the characteristics of the microorganism. The content of SCFAs in the effluent of the whole system is shown in Table 4.

Table 4 Content of SCFAs of the system effluent

EtOH recover rate (%)	Original OLR (kgCOD m ⁻³ d ⁻¹)	EtOH (mg L ⁻¹)	HAC (mg L ⁻¹)
0	8	117.43(±13.29)	273.71(±21.07)
	16	241.46(±27.62)	543.17(±49.82)
	24	377.34(±21.17)	901.68(±72.59)
20	8	99.31(±7.66)	266.54(±27.62)
	16	204.20(±23.52)	527.05(±38.19)
	24	319.12(±29.81)	878.46(±43.33)
50	8	67.10(±5.98)	268.96(±21.98)
	16	137.97(±9.09)	530.22(±41.24)
	24	215.62(±16.88)	887.20(±52.17)
80	8	43.61(±5.24)	355.44(±19.83)
	16	89.68(±8.33)	685.53(±39.52)
	24	140.15(±9.21)	1174.46(±88.53)

3.3 Bioenergy production of the system

Considering the input and output ratio of energy, it was not necessary to recovery total residual ethanol. In this study, if the content of ethanol was less than 100 mg/L, there was no need for ethanol recovery. If the content of ethanol was more than 250 mg/L, the recovery rate was 60%. In other cases, the recovery rate was 40%. ε_T derived from the combination of three biofuels was calculated according to their production rate and combustion heat values (Q , kJ/mol), which are shown in the following equations.

$$\varepsilon_H = \text{HPR} \times Q_H \quad (3)$$

$$\varepsilon_{\text{EtOH}} = \text{EPR} \times Q_{\text{EtOH}} \quad (4)$$

$$\varepsilon_M = \text{MPR} \times Q_M \quad (5)$$

$$\varepsilon_T = \varepsilon_H + \varepsilon_{\text{EtOH}} + \varepsilon_M \quad (6)$$

In addition, $Q_H=286\text{kJ/mol}$, $Q_{\text{EtOH}}=1366\text{kJ/mol}$, and $Q_M=890\text{kJ/mol}$.

Degradation yield of the organic substrate by the whole system was established with regard to OLR and the ethanol recovery rate during the steady state period of each reactor. SDR and η_{COD} are reported in Table 5, and performance of bioenergy production rate (ε_T , kJ/day) with different experiment conditions in the 2-CSTRs anaerobic digestion system can be observed in Table 6.

The degradation yield of the organic substrate is based on microbiological activity, metabolic pathway, duration of the metabolic process, and occasionally, the area where the duration of the metabolic process is the highest and could not have the best ability for degradation yield. The experimental data revealed that the lowest SDR and η_{COD} was obtained when the system had no reuse approaches. However, the highest SDR and η_{COD} do not necessarily occur in recycle or reflux (Table 5). Generally, the reflux process can obtain a higher degradation yield of organic substrate, because it can degrade more kinds of organic matter, and the recycle process is just for ethanol. In practice, the accumulation of soluble microbial products (SMP) occurred in the system, and this caused the lower degrading efficiency.

Table 5 SDR and η_{COD} of 2-CSTRs system with different reuse approaches

Recovery rate (%)	OLR (kgCOD m ⁻³ d ⁻¹)	No reuse		Recycle		Reflux	
		SDR (kgCOD m ⁻³ d ⁻¹)	η_{COD} (%)	SDR (kgCOD m ⁻³ d ⁻¹)	η_{COD} (%)	SDR (kgCOD m ⁻³ d ⁻¹)	η_{COD} (%)
0	8	4.75	59.38(±3.11)	5.21	65.13(±2.21)	5.02	62.75(±2.53)
	16	9.58	59.88(±2.97)	11.08	69.25(±2.17)	10.16	63.51(±2.99)
	24	14.58	60.75(±3.62)	16.76	69.84(±2.17)	15.14	63.08(±2.76)
20	8	5.14	64.25(±2.88)	5.14	64.25(±2.45)	5.31	66.38(±2.82)
	16	10.96	68.5(±2.95)	11.38	71.13(±2.97)	11.25	70.31(±3.17)
	24	16.14	67.25(±3.24)	17.15	71.46(±3.07)	16.65	69.38(±3.04)
50	8	5.7	71.25(±3.79)	5.41	67.63(±2.99)	5.62	70.25(±2.83)
	16	12.51	71.19(±4.52)	11.82	73.75(±3.89)	11.61	72.56(±4.05)
	24	16.96	70.67(±4.03)	17.78	72.54(±3.25)	17.78	74.08(±3.74)
80	8	5.08	63.55(±3.28)	5.08	63.52(±3.88)	5.33	66.25(±3.62)
	16	11.04	69.02(±3.92)	11.09	69.2(±3.71)	11.44	71.55(±3.58)
	24	15.88	66.17(±4.01)	16.07	67.41(±3.64)	16.07	66.96(±3.93)

Table 6 Bioenergy production of 2-CSTRs system with different reuse approaches

Recovery rate (%)	OLR (kgCOD m ⁻³ d ⁻¹)	No reuse		Recycle		Reflux	
		ε_T (kJ d ⁻¹)	Energy yield (kJ kgCOD ⁻¹)	ε_T (kJ/day)	Energy yield (kJ kgCOD ⁻¹)	ε_T (kJ d ⁻¹)	Energy yield (kJ kgCOD ⁻¹)
0	8	269.81	2840.02	297.69	2856.99	283.15	2820.24
	16	471.55	2461.09	557.59	2516.19	508.04	2500.18
	24	782.25	2682.59	916.71	2734.82	812.51	2683.31
20	8	286.62	2788.13	310.21	3017.63	291.07	2740.77
	16	511.64	2334.12	560.15	2461.17	523.21	2325.37
	24	837.89	2595.69	951.61	2774.37	869.93	2612.41
50	8	320.31	2809.65	320.31	2960.27	325.64	2897.16
	16	594.03	2374.22	626.81	2655.96	611.83	2634.92
	24	915.57	2699.21	966.79	2776.55	936.04	2632.28

80	8	334.41	3291.36	334.41	3291.36	359.32	3389.84
	16	613.23	2777.31	613.23	2764.78	663.07	2898.03
	24	995.03	3132.97	1028.33	3177.77	1035.08	3220.54

As shown in Table 2, the highest average MPR was obtained with an ethanol recover rate of 0%. However, ε_T gradually increased with an increasing ethanol recover rate, because Q_{EtOH} was significantly higher than Q_M (Table 6). As shown in Table 6, recycle provided a higher ε_T than reflux, with an ethanol recover rate of 0% and 20%, respectively; which is equal to the ethanol recovery rate of 50%, but lower with the ethanol recover rate of 80%. Moreover, similar trends were found in energy yield. This result indicated that recycle would be more feasible with a lower ethanol recover rate. In other cases, for energy production, effluent reflux may be a more suitable for the reuse application of the effluent.

4. Conclusion

The integration process facilitates the utilization of a carbon source along with hydrogen, ethanol and methane generated from the 2-CSTRs anaerobic digestion system as biofuels with additional substrate degradation. The performance of the system was evaluated with variable ethanol recovery rates of 0%, 20%, 50% and 80%, respectively, at variable OLRs ranging between 8-24 kgCODm⁻³d⁻¹. The maximum HPR of 1.34 (± 0.7) mol d⁻¹ and the maximum EPR of 0.45 (± 0.4) mol d⁻¹ were observed at an OLR of 24 kgCOD m⁻³ d⁻¹. In addition, the maximum HC of 26.17 (± 3.62)% and the maximum EC of 32.43 (± 3.97)% were obtained at an OLR of 16 kgCOD m⁻³ d⁻¹. Linear regression of the collection between HPR and EPR can be expressed as $y = 0.3182x + 0.0114$ ($r^2 = 0.9853$). The highest MPR of 0.45 (± 0.07) mol d⁻¹ was obtained at the original OLR of 24 kgCOD m⁻³ d⁻¹, with an ethanol recover rate of 0%. Recycle and reflux are the two reuse approaches for effluents in the system for producing greater bioenergy. Recycle provided a higher ε_T and energy yield than reflux, with an ethanol recovery rate of 0% and 20%, respectively. This was equal to the ethanol recovery rate of 50%, but lower than the ethanol recovery rate of 80%. This indicates that recycle would be more feasible with lower ethanol recovery rates, and reflux may be more suitable with other kinds of ethanol recovery rates. This study provides a protocol for the use of the 2-CSTRs anaerobic digestion system for biofuel production and substrate degradation. This could effectively improve the efficiency of the anaerobic digestion system.

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