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Full-Scale Application of Up-flow High Rate Anaerobic Reactor with Substrate Modification and Effluent Recirculation for Sugarcane Vinasse Degradation and Biogas Generation

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ABSTRACT

This study was aimed at studying the potential of biogas (methane) production from vinasse wastewater in real full-scale application using a two-stage sequencing Up-flow High Rate Anaerobic Reactor (UHRAR), with effluent recirculation and substrate modification. A batch experiment was initially conducted prior to the full-scale application experiment. The batch experiment was done with experimental condition variable: undiluted sample (pH 6) and diluted samples (pH: 5; 6 and 7), while pH and methane production were observed for 50 days. Full-scale application was carried out in two-stage UHRAR reactors with volume 60 m^3, HRT 40 d and OLR 60.1–104 kg COD/m^3·d. The observation lasted for 32 d. The result from the batch experiment showed that the diluted samples achieved higher COD degradation and methane generation than the undiluted sample. The optimum condition occurred at pH 7, with theoretical methane yield of 7.5–10.64 L CH_4 per kg COD. In turn, in full scale application, at day 32, COD removal was 71% (69.1 kg COD/d removed), with methane production was 36.72 m^3 CH_4/d. Methane production per COD removed was 0.53 m^3 CH_4/kg COD·d. Substrate modification and effluent recirculation could improve the substrate biodegradability, maintain microbial diversity and enrich nutrients in the reactor.

Keywords: anaerobic digestion; biogas; $CaCO_3$ addition; recirculation; vinasse wastewater

INTRODUCTION

The Indonesian sugar and ethanol industries have developed rapidly in recent years. Indonesia Biofuel Association indicated that ethanol production could reach 180 million liters per year, with average domestic consumption of 100 million L per year. Sugarcane has high potential energy, 40% transformed into alcohol, and 31% remain in the by-products as bagasse (26%) and vinasse (5%) (Del Nery et al., 2018). Vinasse is the by-product of the alcohol distillation process. The production of vinasse in a traditional alcohol factory is around 8–20 L per Liter of ethanol produced (Cabrera-Díaz et al., 2017; Joppert et al., 2017). Vinasse is characterized by a high concentration of organic matter (10–65 g BOD/l), nutrient salts (potassium (K) and sulfate), low pH (3.5–5.0), high temperature (80–90°C), and permanent dark color (brown to black) (Cabrera-Díaz et al., 2017; Joppert et al., 2017; Marafon et al., 2020). The water and soil pollution resulting from sugarcane vinasse wastewater disposal is a challenging issue that comes from the biofuel ethanol industries (Harihastuti and Marlena, 2018).

Due to its high organic concentration and flowrate, sugarcane vinasse cannot be effectively treated by using conventional methods. A full-scale application that involves integrating several technologies should be applied to remove organic

pollutants to the level that will comply with the effluent stream standard. Integration technology for treating vinasse wastewater should be started by converting organics into biogas to reduce the organic concentration and utilize the biogas (methane) for energy (Parsaee et al., 2019). Biogas production from vinasse has been studied extensively from laboratory scale to a full-scale application with mixed results (Christofoletti et al., 2013; Moraes et al., 2015; Reis and Hu, 2017). Harihastuti et al. (2020a) scanned the potential of vinasse methane production on a laboratory scale and found that 51.7% of COD could be reduced and converted into methane during 42 days retention time. The methane production was 0.058 L, converted from 180.95 g COD degradation. The methane production in the study was low because the pH in the reactor was also low (pH was 5.1–5.7), not optimum for the methanogenic activity. Low pH also encourages the Sulfur Reducing Bacteria (SRB) to grow and compete with methanogenic bacteria for carbon sources, thus hindering the methane generation. Carbon dioxide gas was also abundantly detected in the reactor headspace (> 50%).

In an anaerobic system, the methane production rate is aligned with the substrate biodegradability potential. For a substrate with low biodegradability, such as vinasse wastewater, substrate pretreatment/modification is crucial to improve the potential of methane production (Mahajan et al., 2020). Substrate modification is performed via pH adjustment and alkalinity enhancement. In turn, pH was adjusted to 6.5 to 7.0 by adding some alkalinity (lime/calcium carbonate/$CaCO_3$) to ensure the growth of methanogenic bacteria (Hwang et al., 2004). The dilution of the substrate should also be conducted to reduce the suspension and solid and to improve the solubility/accessibility of substrate to microorganisms, thus enhancing biodegradability (Li et al., 2007). Effluent recirculation to dilute the substrate/influent is also significant to reduce the utilization of freshwater. Effluent recirculation is also beneficial to ensure the return of acidogenic bacteria into the anaerobic reactor, and improve the acidogenesis process vital for the formation of Volatile Fatty Acid (acetate, propionate, and butyrate). The building of VFA, specifically acetate, would allow the growth of methanogens. Beside substrate modification, anaerobic reactor modification is also used to enhance the methane production. Up-flow Anaerobic Filter reactors have proven robust to treat some wastewater sources and required shorter retention time (Drtil et al., 2002; Yuliasni et al., 2017).

There were not many studies about the biogas potential from vinasse, particularly about the full-scale application. Souza et al. (2018) studied the performance of full-scale UASB for treating vinasse wastewater with Organic Loading rate (OLR) of 25-30 kg COD/m^3.d, with volume 75 m^3 and HRT 10 h. It could remove 72% COD with methane generation of 10 Nm gas/m^3.d. However, this study and many others were operated in low to medium OLR (Fuess et al., 2017a). In reality, the vinasse wastewater could have a very high organic loading rate, between 80–150 kg COD/m^3.d, which makes it very difficult to treat.

Therefore, the aim of this study was to test the potential of biogas (methane) production from vinasse wastewater in a real full-scale application using a two-stage sequencing Up-flow High Rate Anaerobic Reactor (UHRAR), combined with effluent recirculation and substrate modification. The initial substrate/influent will be modified by adding lime to enhance the alkalinity and dilute the influent with water to improve dissolved organic matters that are more accessible to microorganisms. Effluent recirculation was applied to enhance the acidogenesis process and supply the system with more nutrients. This study explored the degree of organic degradation, the amount of biogas produced (the quantity and quality), the optimum retention time achieved, and the quality of vinasse effluent after treatment.

MATERIAL AND METHODS

Experimental set up

The experiment was divided into 2 phases. The first phase was a batch experiment conducted in the laboratory. The batch experiment aimed to study the optimum pH for methane generation. The batch experiment was similar to the Biochemical Methane Potential (BMP) test. The second phase was a full-scale continuous running application.

Batch experiment

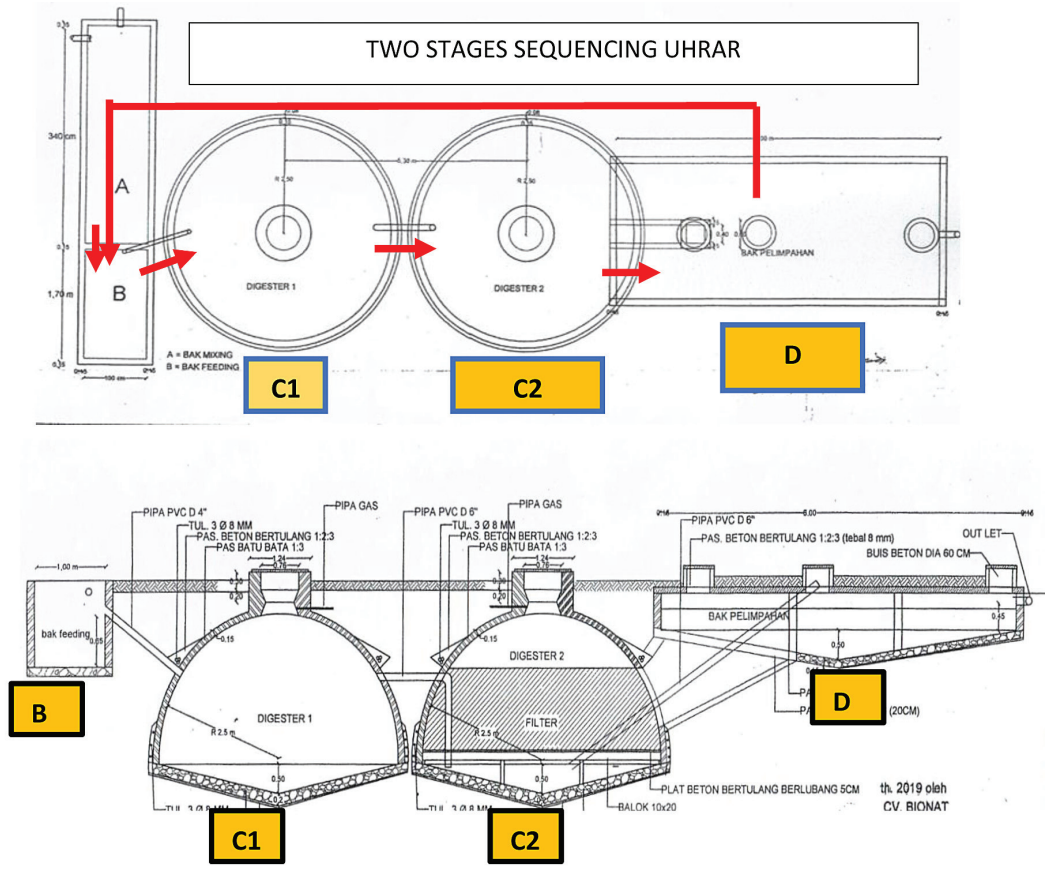
Three anaerobic bottles, with volume 1 L, were used. To every bottle, 300 ml mix cultures sludge and 700 ml vinasse wastewater were

added. Besides, nutrients in the form of urea and phosphorous were added with the ratio BOD: N: P = 100: 2.5: 0.5. Three samples were diluted with water, with ratio water: vinasse = 4:1, and the pH was adjusted to 5, 6 and 7 (the samples were identified as pH 5, pH 6, and pH 7). One sample was undiluted but pH was adjusted to 6. Lime was used to adjust the pH. The experiment lasted for 50 days.

Full-scale continuous running experiment

Full-scale application was taken place in the traditional ethanol industry in Polokerto, Sukoharjo Central Java. Seeding was conducted by adding 20% of microbial seeds into the reactor, about 6 m^3 in each dome. Microbial seeds were sludge derived from anaerobic digester, taken from a WWTP of the ethanol industry. Macronutrients were added with the ratio BOD: N: P = 100: 2.5: 0.5. After seeding, both UHRAR dome 1 and 2 (figure 1, C1 and C2) was filled with a mixture of water and vinasse with the ratio 4:1; pH was adjusted to 5-6 using lime until both domes were full and ran to the overflow chamber (Figure 1, Part D). The reactor was left for another seven days. After seven days, the reactor was operated with continuous running. In full-scale operation, vinasse wastewater with characteristic of Q = 1.5 m^3/d, COD 60,990 – 104,000 mg/L and OLR = 60.1 – 104 kg COD/m^3.d was pumped to the reactor every day for the duration of 34 days.

The operational procedure for continue running was: 1.5 m^3 raw vinasse wastewater was filled in the mixing chamber (Figure 1A). In the mixing chamber, pH was adjusted into 5–6 by adding a lime solution. The wastewater was streamed into the feeding chamber (Figure 1B). From feeding chamber, the wastewater runs to UHRAR 1 and 2 (Figure 1, C1, and



Legends:
A – mixing chamber (V = 3 m^3)
B – feeding chamber (V = 3 m^3)
C1 – UHRAR dome 1 (without filter) (V = 30 m^3)
C2 – UHRAR dome 2 (with filters) (V = 30 m^3)
D – overflow chamber

Figure 1. Design of two stages sequencing UHRAR

Table 1. Characteristic of Vinasse wastewater influent

Parameter	Unit	Influent Concentration		
		Day 23	Day 25	Day 32
pH	-	5.7	6.2	3.4
COD	mg/L	60,990	104,000	95,800
BOD_5	mg/L	3,553	32,990	33,299
TSS	mg/L	3,080	5,612	5,756
TKN (Total Kjeidahl Nitrogen)	mg/L	224	322	196
P. Total	mg/l,	4.57	<0.001	< 0.001
MLVSS	mg/L	3,990	9,300	7,300
MLSS	mg/L	1,250	4,080	2,020
Sulphate (SO_4^{2-})	mg/L	<0.26	<0.6	< 0.6
Sulfide (S^-)	mq/L	8.55	6.55	12.85
Total Alkalinity	mg/L	9,150	12,685	10,150
Nitrate (NO_3)	mg/L	<0.001	22.51	8.252
Nitrite (NO_2)	mg/L	< 0.001	< 0.001	< 0.001
Total Plate Count	Coloni/mL	4.4×10^6	1.3×10^8	3.7×10^7
Temperature (^{0}C)	^{0}C	37.1	65.0	52.0

C2). From UHRAR, wastewater overflowed to the overflow chamber (D). On day 24 to 32, 25% of the effluent in the overflow chamber was circulated back to the feeding chamber (Figure 1, from D to B).

The vinasse influent wastewater characteristic during full-scale application in the continuous running was measured. The quality parameters of vinasse wastewater influent are presented in Table 1.

Method

The vinasse wastewater quality parameters were analyzed by using the analytical method: Total COD, BOD_5, Total Suspended Solid, Total Nitrogen Kjeidahl, MLVSS, MLSS, Nitrate, Nitrite, Sulfate, sulfide and phosphate using APHA AWWA 22rd, 2012, temperature (SNI 06-6989.23-2005) and pH (SNI 6989.11.2019). TPC (Total Plate Count) were measured using SNI 3554:2015 point 3.28.1. VFA was measured using Gas Chromatography (Shimadzu, GC 2100 plus), column RTX-wax. Temperature SPL:250, Column: 150, Detector FID: 250, RT 7.5 min, and split ratio 43.2. Gases (methane, CO_2, and CO) were measured using Gas Chromatography (HP 5890 A), with Thermal Conductivity Detector (TCD), diameter column: 183×0.32 cm. H_2S gas was measured using Ion Science PhoCheck 1000 Portable Handheld PID VOC Gas Detector. Gas Flowrate was measured using the Wet Gas Meter (Shinagawa) 5L/rev.

RESULTS AND DISCUSSION

Batch experiment

Batch experiment (BMP test) was carried out under two conditions, namely: one sample was undiluted and 3 samples were diluted. The undiluted sample had pH adjusted to pH 6 by adding lime. Three samples were diluted with water, with ratio water: vinasse = 4:1, and pH was adjusted from 3 to 5, 6 and 7. The result was presented in Table 2.

Table 2 depicted the COD degradation profile and pH variation from the undiluted and diluted samples. Figure 2 showed that on day 11, at least 50% COD removal was achieved on the diluted samples. In contrast, on the undiluted sample, only 32% COD removal occurred. On diluted samples, maximum COD removals were 68% (pH 7); 65.7% (pH 6) and 70.1% (pH 5) respectively. In turn, on the undiluted sample, only 33.5% of COD removal was achieved. The diluted samples had higher organic removal efficiency due to the increase of a dissolved organic fraction of the samples. Dissolved organic matters was more available to the microorganisms and easier to be converted into smaller molecules (hydrolysis stage) that later could be converted into VFA (acidogenesis), acetate (acetogenesis) and methane and carbon dioxide (methanogenesis), as final products (Gharsallah, 1994; Park et al., 2018).

The pH and alkalinity have a significant role in organic conversion into methane.

Table 2. COD degradation profile versus pH

Day	pH 6 (undiluted)			pH 7			pH 6			pH 5		
	pH	COD (mg/L)	Cumulative % removal	pH	COD (mg/L)	Cumulative % removal	pH	COD (mg/L)	Cumulative % removal	pH	COD (mg/L)	Cumulative % removal
0	6	168,516	0	7.0	34,421	0	6.0	38,499	0	5.0	43,356	0
11	6.4	113,844	32.4	6.0	17,556	49.0	6.1	19,026	50.6	5.8	19,799	54.3
18	6	126,877	24.7	6.7	11,787	65.8	7	18,243	52.6	7.0	22,072	49.1
21	5.9	117,868	30.1	6.9	10,961	68.2	6.9	13,213	65.7	7.1	17,042	60.7
22	5,9	117,231	30.4	7.0	16,328	52.6	7.0	19,564	49.2	7.1	20,993	51.6
25	5.9	113,469	32.7	7.0	14,673	57.4	7.1	16,328	57.6	7.2	18,510	57.3
27	5.8	112,026	33.5	7.1	12,480	63.7	7.2	13,872	64.0	7,2	15,838	63.5
29	5.7	119,236	29.2	7.0	12,592	63.4	7.2	13,639	64.6	7.3	15,865	63.4
32	5.9	116,678	30.8	7.1	12,667	63.2	7.4	13,694	64.4	7.4	14,748	66.0
43	6	122,494	27.3	7.3	12,748	63.0	7.6	13,925	63.8	7.6	14,748	66.0
50	5.9	124,260	26.3	7.5	12,960	62.3	7.6	13,920	63.8	7.7	12,960	70.1

Methanogenic bacteria have strict pH for optimum growth, around 6.7–7.5 (Chen et al., 2008; Jung et al., 2000; Lukitawesa et al., 2018), and alkalinity has a role, as a pH buffer, to balance the pH in the anaerobic in the acidogenesis and acetogenesis phase. Thus, the pH and alkalinity have to be in sufficient concentration by adding limestone ($CaCO_3$) (Fuess et al., 2017a). Table 2 showed that in the undiluted sample (pH 6 undiluted), the pH was continually dropping from 6 into 5.9, an indication of acidogenesis/acetogenesis phase. In the diluted samples, the pH escalated into 7.4 (pH optimum for methanogenic activity).

Figure 2 depicted the theoretical cumulative methane yields from the undiluted and diluted samples during 43 days observation. Theoretically, methane generation is calculated as 0.35–0.4 L CH_4 per kg COD removal (Djalma Nunes Ferraz Júnior et al., 2016; Harihastuti et al., 2020b). Theoretical methane yield was calculated based on the amount of kg COD removed from day 0 to day 43 multiplied by 0.35–0.4 L CH_4. On the basis of that calculation, the theoretical methane yield in this study was 7.51–10.64 L CH_4 per kg COD for diluted vinasse wastewater with pH adjustment (OLR = 38–40 kg COD/d, with COD degradation of 63–66% during 43 days). The maximum methane yield was 16.11 L CH_4 per kg COD (OLR = 168 kg COD/d, average COD degradation was 24–33%). Even though theoretically the undiluted sample has a higher methane yield than the diluted sample, under real conditions, the pH in the undiluted sample keeps dropping into acidic condition. Acidic pH is unfavorable for the methanogenic activity. The COD removal in the undiluted sample has also remained low. Those conditions are an indication of an unbalance system that sooner could deteriorate the reactor performance.

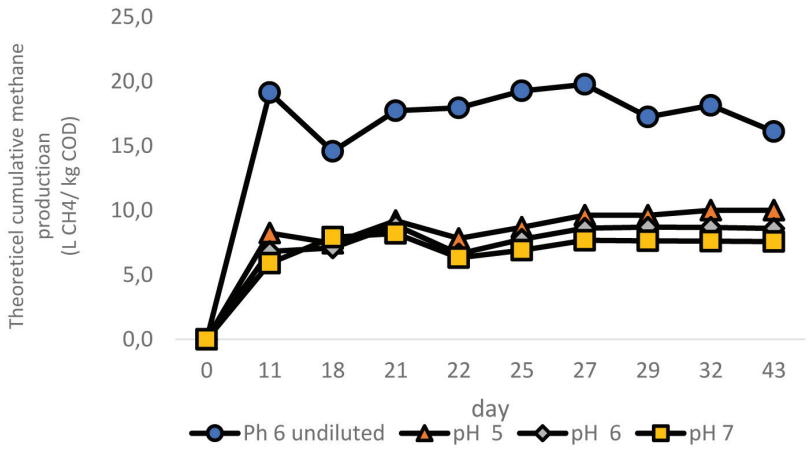


Figure 2. Theoretical methane accumulation from the batch experiment

Thus, based on the results of the batch experiment, for full-scale application, vinasse influent was diluted with water, pH adjusted to 5-6, and effluent recirculated. Effluent recirculation was done to return the hydrolysis-acidogenic bacteria in the effluent back to the reactor, to enhance the hydrolysis-acidification process. Microbial diversity of mixed culture in anaerobic digester has to be maintained in order to ensure hydrolysis, acidogenesis, acetogenesis, and methanogenesis reactions happened (Ma et al., 2020). Recirculation had also proven to give a stable performance and higher methane yield (Lukitawesa et al., 2018).

Full-Scale Application

The full-scale application was carried out in a two-stage Up-flow High Rate Anaerobic reactor (UHRAR), with a volume of 60 m^3 and hydraulic retention time of 40 days. The vinasse wastewater influent had COD of 60,990 – 104,000 mg/L (with OLR = 60.1 – 104 kg COD/m^3.d). The samples were taken periodically. Recirculation was applied on day 25 to day 32. On day 23, 25, and 32, COD and VFA from influent and effluent were measured and data was shown in Figures 3 and 4. Figure 3 showed the COD degradation profile of influent and effluent on day 23, 25, and 32. On day 23, the COD removal was only 52%, then increased to 81% on day 25. On day 32, the COD removal slightly decreased to 72%.

Figure 4 illustrated the VFA formation on day 23, 25 and 32. On day 23, butyrate was abundant in the influent with a concentration of 7385 mg/L, while the concentration of acetate and propionate were low. The VFA formation was altered on day 25 and 32. On day 25 and 32, the acetate concentration increased to 6459 mg/L and 4974 mg/L respectively, while the butyrate and propionate concentrations were low. Associating between figure 3 and figure 4, it could be concluded that the COD degradation performance was in line with the degree of acetate formation, and also the higher concentration of organic matter that converted into acetate, the higher methane production. The COD removal starts to increase and reached 81% and 71% when acetates were at high concentration, at 6.459 mg/L and 4974 mg/L on day 23 and 32, respectively. Acetate was a favorable substrate for almost any type of microorganisms, especially for methanogenic bacteria (Lalov et al., 2001).

The higher concentration of butyrate at day 23 indicated that the system was still in the acidogenesis stage. As described by Fuess et al. (2020), vinasse could be converted into butyrate via acidogenesis pathway in dark fermentation, if there are abundant of acidogenesis microorganisms and biohydrogen production in the reactor. The system was shifted into acetate production when recirculation was applied at days 25 and 32 (Figure 4, day 25, and 32). According to Degueurce et al. (2016), the effluent recirculation in anaerobic digester promotes the modification of the biological community, enhanced nutrients availability, and improves the pH buffering capacity, thus improving and stabilizing the biogas production. As depicted in Table 1, on day 23, the number of microorganisms (expressed as MLVSS and total plate count/ TPC) was also lower than day 25 and day 32.

Recirculation also enhanced the level of biodegradability in the anaerobic system (Lin and Li, 2017)sequential batch SS-AD gradually reached steady state by 3 runs (30 days/run. Day 23 has lower BOD/COD than day 25 and 32. BOD/COD ratio at day 23 was 1: 17 whereas

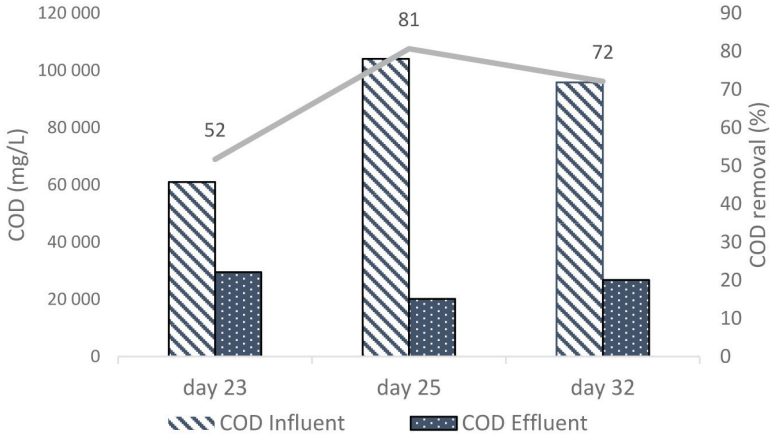


Figure 3. COD degradation in a full-scale application

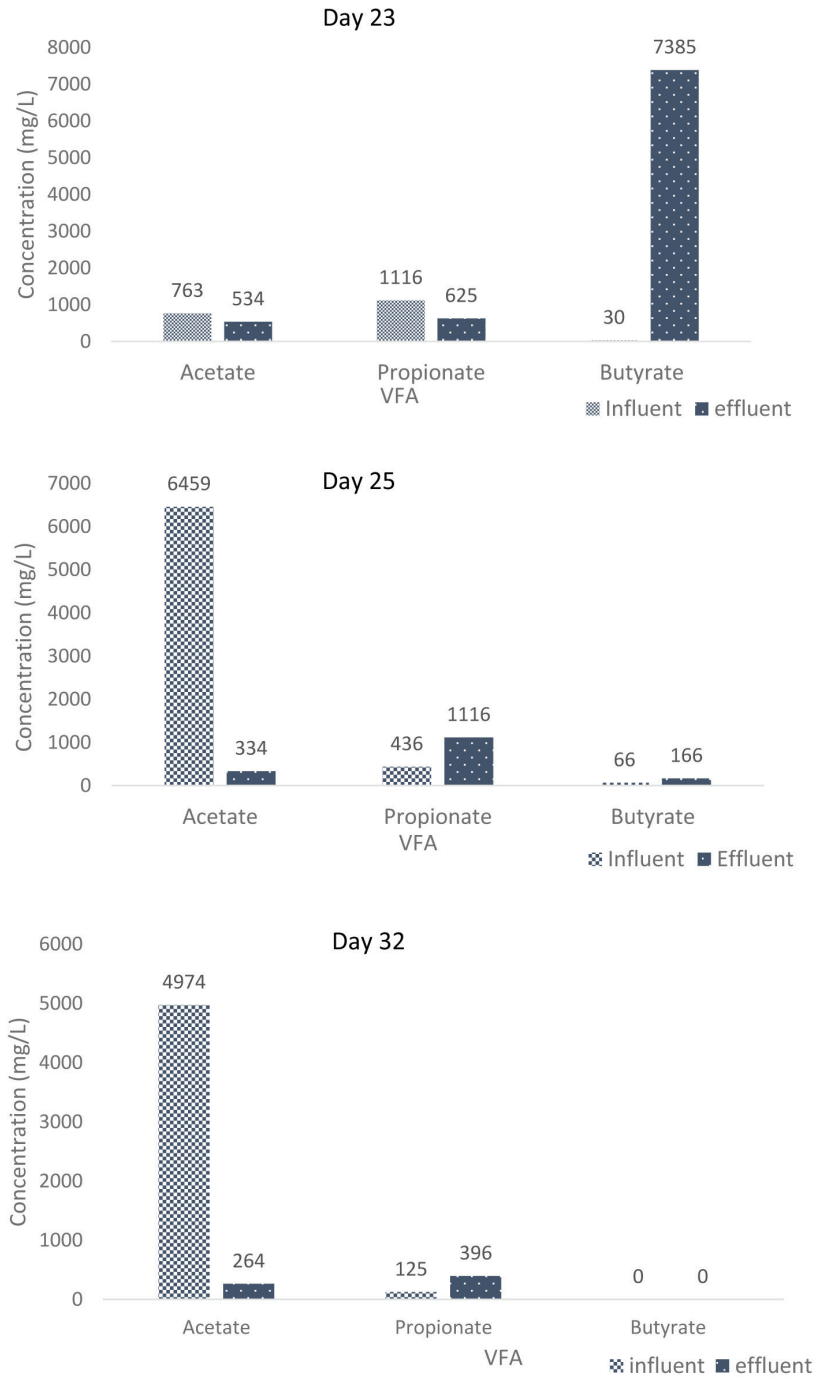


Figure 4 VFA formation on Day 23, 25 and 32

day 25 and 32 that have BOD/COD ratio = 1:3. BOD/COD ratio was an indication of the level of the sample biodegradability. The higher the BOD/COD ratio, the higher the biodegradability of the wastewater is. The addition of lime ($CaCO_3$) was proven to be not significantly enhance the buffering capacity of the system, as judging from the condition at day 23, even when $CaCO_3$ was added, the anaerobic system was still trapped in the acidogenesis stage. The anaerobic condition was able to shift into the acetogenesis stage when recirculation was applied at day 25 and 32. The reason is that lime was not properly dissolved in the wastewater and tends to be settled in the bottom of the reactor. Furthermore, the addition of alkaline chemicals, such as $CaCO_3$ or $NaHCO_3$, only slightly increased the pH; thus, it should be added in high dosage and tend to be costly for full-scale application (Fuess et al., 2017a). The better alternative is to use low dosages of chemicals coupled with the recirculation of the effluent (Fuess et al., 2017a).

The maximum COD degradation of 81% in this study was relatively higher when compared to a study conducted by Souza et al. (1992) using the UASB reactor. The COD removal was 71%, with lower OLR (25–30 kg COD/m^3.d) but faster HRT (10 h). However, using the UASB reactor was more complicated than using the UHRAR reactor, due to the long startup for granule formation and the need to be continuously fed. For full scale/ real application, UASB needs a very high skilled operator to keep the reactor performance stability. When compared to a study carried out by Fuess et al. (2017b), using a similar two-phase fixed-bed anaerobic reactor, this study was higher, with higher methane production.

Concentration analysis of the vinasse effluent

Table 3 presents the effluent concentrations that were taken during 32 days of observation, on day 23, day 25, and day 32. Overall, the pollutant concentrations were still high and needed further advance treatment technology to fulfill effluent stream standard regulation (Moraes et al., 2015). It also showed that the nutrient concentrations, TKN, and P, were also still very high. Due to still high content of nutrients (even after effluent recirculation), a suitable way to recover the nutrients is by converting it into organic fertilizer/compost (Madejón et al., 2001; Science, 2007). Hydrogen sulfide (H_2S) was also found in high concentration due to the fact that in the molasses fermentation process, sulfuric acid was added to inhibit the growth of undesirable (non-ethanol producing) bacteria that will outcompete the performance of ethanol fermenters (Fuess and Garcia, 2015). A high concentration of H_2S is an indication of lower methane production. In microbial mix cultures, Sulfate Reducing Bacteria (SRB) contributes to 80–85% of the total microorganism population. With abundant amount of SO_4^{2-} as an electron acceptor and acetate as an electron donor, SRB would definitely outcompete the methane-producing bacteria (MPB) by consuming acetate to convert into SO_4^{2} and H_2S (Dar et al., 2008), as both SRB and MPB utilize the same substrates. The analysis using H_2S gas detection also confirmed that all the biogas samples have very high H_2S concentration (> 150 mg/L). However, the H_2S concentration seems not to be high enough to inhibit the methanogenic activity. According to Yuan, et al., 2020 (Yuan et al., 2020), methanogenesis could be inhibited by high sulfide concentration at $COD/SO_4^{2-} < 10$, or H_2S could not be higher than 200 mg/L.

Biogas formation in Dome 1, Dome 2, and Dome (1 + 2)

The reactor was connected to the gas pipe to transfer the gas for further utilization. The gas production was measured by taking samples individually via dome 1 and dome 2, and also taking samples collectively via both dome one plus dome 2. The biogas production is presented in Table 4. Table 4 showed that the methane production increased along with time, reached maximum on day 25, and drop slightly on day 32. The methane production profile depicted in table 4 was in line with the COD removal profile presented in figure 4. The highest methane production occurred on day 25, with methane detected at dome 1 was 40.22% and for dome 2 was 43.49%. In turn, the total methane production from Dome 1 and Dome 2 could not be measured due to the high gas pressure in the sample due to the broken sample bottle. Higher gas pressure was suspected to be an indication of higher methane content as well (confirmed by measurement of Qgas = 52 L/min). Nevertheless, analyzing methane production along with time, depicted in from table 4, methane production could be as high as 51%. This value was slightly lower than result in BMP/ batch experiment (Table 2), which could achieve a maximum of 63–66% methane production.

The COD and methane data measured on day 32 were used to measure the methane production (m^3 CH_4/kg COD.d), based on Figure 3, Table 4 and measurement of Qgas = 50 L/min). On day 32, the COD removal was 69.1 kg COD/d, whereas the methane production was 36.72 m^3 CH_4/d. Thus, the methane production per COD removed was 0.53 m^3 CH_4/kg COD.d. The methane production in this study was 1.5 times higher than the study conducted by Fuess et al., 2017 (Fuess et al., 2017b). In Fuess et al., 2017, using thermophilic two phases anaerobic digestion with OLR of 25 kg COD/m^3.d, the maximum COD removal was up to 73.9%, with methane production was 0.301 m^3 CH_4/kg COD removed. This study used Up-flow High Rate Anaerobic Reactor (UHRAR) with OLR 95 kg COD/m^3.d, COD removal was up to 71%, with methane production reaching 0.53 m^3 CH_4/kg COD.d.

Table 4. CH_4, CO_2, and CO formation during 32 days observation

Days elapsed	Dome 1			Dome 2			Dome 1 + 2		
	CH4 (%)	CO_2 (%)	CO (ppm)	CH_4 (%)	CO_2 (%)	CO (ppm)	CH_4 (%)	CO_2 (%)	CO (ppm)
0	8.750	38.932	trace	not measured	not measured	not measured	not measured	not measured	not measured
10	22.84	7.59	trace	23.07	12.06	212	30.00	12.4	88
11	57.21	8.93	124	48.23	10.83	79	51.58	10.7	>1000
12	38.26	5.51	46.2	24.51	6.39	124	37.89	6.78	84
23	42.38	2.37	>1000	4.420	1.49	>1000	42.95	23.14	>1000
25	40.22	22.95	175	43.49	26.03	9	(*)	(*)	7.63
32	19.45	9.29	276	30.50	10.36	18	51.36	14.2	7.60

Table 4 also shows the formation of CO (Carbon Monoxides) as one of common trace components in the anaerobic system. As explained by Hickey and Switzenbaum (1990), CO was formed as acetate-catabolizing reactions of acetoclastic methanogens, when there are high concentrations of acetate, methane, and hydrogen in the system. The high concentration of H_2S gas was also detected due to the high concentration of sulfide (S^-) in the influent, coupled with the abundance of SRB, which consumes the same substrate as methanogen, leading to the formation of H_2S.

CONCLUSIONS

Substrate modification and effluent recirculation has been shown to increase the organic degradation and methane generation of vinasse wastewater with high OLR. Substrate modification by adding $CaCO_3$ was able to enhance alkalinity, and thus stabilize the pH. In turn, effluent recirculation was proven to increase substrate biodegradability by enhancing the solid organic matter solubility. On the basis of this result, full-scale application with high OLR, using UHRAR combined with substrate modification and effluent recirculation, is a promising technology for biogas generation from vinasse wastewater.

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REFERENCES

1. Cabrera-Díaz, A., Pereda-Reyes, I., Oliva-Merencio, D., Lebrero, R., Zaiat, M. 2017. Anaerobic Digestion of Sugarcane Vinasse Through a Methanogenic UASB Reactor Followed by a Packed Bed Reactor. Appl. Biochem. Biotechnol, 183, 1127–1145. https://doi.org/10.1007/s12010-017-2488-2
2. Chen, Y., Cheng, J.J., Creamer, K.S. 2008. Inhibition of anaerobic digestion process: A review. Bioresour. Technol, 99, 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057
3. Christofoletti, C.A., Escher, J.P., Correia, J.E., Marinho, J.F.U., Fontanetti, C.S., 2013. Sugarcane vinasse: Environmental implications of its use. Waste Manag, 33, 2752–2761. https://doi.org/10.1016/j.wasman.2013.09.005
4. Dar, S.A., Kleerebezem, R., Stams, A.J.M., Kuenen, J.G., Muyzer, G., 2008. Competition and coexistence of sulfate-reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. Appl. Microbiol. Biotechnol, 78, 1045–1055. https://doi.org/10.1007/s00253-008-1391-8
5. Degueurce, A., Tomas, N., Le Roux, S., Martinez, J., Peu, P., 2016. Biotic and abiotic roles of leachate recirculation in batch mode solid-state anaerobic digestion of cattle manure. Bioresour. Technol, 200, 388–395. https://doi.org/10.1016/j.biortech.2015.10.060
6. Del Nery, V., Alves, I., Zamariolli Damianovic, M.H.R., Pires, E.C., 2018. Hydraulic and organic rates applied to pilot scale UASB reactor for sugar cane vinasse degradation and biogas generation. Biomass and Bioenergy, 119, 411–417. https://doi.org/10.1016/j.biombioe.2018.10.002
7. Djalma Nunes Ferraz Júnior, A., Koyama, M.H., de Araújo Júnior, M.M., Zaiat, M., 2016. Thermophilic anaerobic digestion of raw sugarcane vinasse. Renew. Energy, 89, 245–252. https://doi.org/10.1016/j.renene.2015.11.064

8. Drtil, M., Bod, I., Herdov, B., 2002. The use of upflow anaerobic filter and AnSBR for wastewater treatment at ambient temperature 36, 1084–1088.
9. Fuess, L.T., de Araújo Júnior, M.M., Garcia, M.L., Zaiat, M., 2017a. Designing full-scale biodigestion plants for the treatment of vinasse in sugarcane biorefineries: How phase separation and alkalinization impact biogas and electricity production costs? Chem. Eng. Res. Des, 119, 209–220. https://doi.org/10.1016/j.cherd.2017.01.023
10. Fuess, L.T., dos Santos, G.M., Delforno, T.P., de Souza Moraes, B., da Silva, A.J., 2020. Biochemical butyrate production via dark fermentation as an energetically efficient alternative management approach for vinasse in sugarcane biorefineries. Renew. Energy, 158, 3–12. https://doi.org/10.1016/j.renene.2020.05.063
11. Fuess, L.T., Garcia, M.L., 2015. Bioenergy from stillage anaerobic digestion to enhance the energy balance ratio of ethanol production. J. Environ. Manage, 162, 102–114. https://doi.org/10.1016/j.jenvman.2015.07.046
12. Fuess, L.T., Kiyuna, L.S.M., Ferraz, A.D.N., Persinoti, G.F., Squina, F.M., Garcia, M.L., Zaiat, M., 2017b. Thermophilic two-phase anaerobic digestion using an innovative fixed-bed reactor for enhanced organic matter removal and bioenergy recovery from sugarcane vinasse. Appl. Energy, 189, 480–491. https://doi.org/10.1016/j.apenergy.2016.12.071
13. Gharsallah, N., 1994. Influence of dilution and phase separation on the anaerobic digestion of olive mill wastewaters. Bioprocess Eng, 10, 29–34. https://doi.org/10.1007/BF00373532
14. Harihastuti, N., Marlena, B., 2018. Bioenergy Potential Based on Vinasse from Ethanol Industrial Waste to Green Energy Sustainability. E3S Web Conf, 31, 2017–2019. https://doi.org/10.1051/e3sconf/20183102015
15. Harihastuti, N., Marlena, B., Irnaning, N.H., Yuliasni, R., 2020. Decomposition process of stilage/vinasse organic compound from ethanol industry as renewable energy source, AIP Conf. Proc. 2197, 3–8. https://doi.org/10.1063/1.5140897
16. Hickey, R.F., Switzenbaum, M.S., 1990. Behavior of Carbon Monoxide as a Trace Component of Anaerobic Digester Gases and Methanogenesis from Acetate. Environ. Sci. Technol, 24, 1642–1648. https://doi.org/10.1021/es00081a003
17. Hwang, M.H., Jang, N.J., Hyun, S.H., Kim, I.S., 2004. Anaerobic bio-hydrogen production from ethanol fermentation: the role of pH. J. Biotechnol, 111, 297–309. https://doi.org/10.1016/j.jbiotec.2004.04.024
18. Joppert, C.L., dos Santos, M.M., Costa, H.K.M., dos Santos, E.M., Moreira Simões, J.R., 2017. Energetic shift of sugarcane bagasse using biogas produced from sugarcane vinasse in Brazilian ethanol plants. Biomass and Bioenergy 107, 63–73. https://doi.org/10.1016/j.biombioe.2017.09.011
19. Jung, J.Y., Lee, S.M., Shin, P.K., Chung, Y.C., 2000. Effect of pH on phase separated anaerobic digestion. Biotechnol. Bioprocess Eng, 5, 456–459. https://doi.org/10.1007/bf02931947
20. Lalov, I.G., Krysteva, M.A., Phelouzat, J.L., 2001. Improvement of biogas production from vinasse via covalently immobilized methanogens. Bioresour. Technol, 79, 83–85. https://doi.org/10.1016/S0960-8524(01)00045-1
21. Li, J., Li, B., Zhu, G., Ren, N., Bo, L., He, J., 2007. Hydrogen production from diluted molasses by anaerobic hydrogen producing bacteria in an anaerobic baffled reactor (ABR). Int. J. Hydrogen Energy, 32, 3274–3283. https://doi.org/10.1016/j.ijhydene.2007.04.023
22. Lin, L., Li, Y., 2017. Sequential batch thermophilic solid-state anaerobic digestion of lignocellulosic biomass via recirculating digestate as inoculum – Part I: Reactor performance. Bioresour. Technol, 236, 186–193. https://doi.org/10.1016/j.biortech.2017.03.136
23. Lukitawesa, Wikandari, R., Millati, R., Taherzadeh, M.J., Niklasson, C., 2018. Effect of effluent recirculation on biogas production using two-stage anaerobic digestion of citrus waste. Molecules, 23, 1–11. https://doi.org/10.3390/molecules23123380
24. Ma, X., Yu, M., Yang, M., Zhang, S., Gao, M., Wu, C., Wang, Q., 2020. Effect of liquid digestate recirculation on the ethanol-type two-phase semi-continuous anaerobic digestion system of food waste. Bioresour. Technol, 313, 123534. https://doi.org/10.1016/j.biortech.2020.123534
25. Madejón, E., López, R., Murillo, J.M., Cabrera, F., 2001. Agricultural use of three (sugar-beet) vinasse composts : effect on crops and chemical properties of a Cambisol soil in the Guadalquivir river valley (SW Spain), 84, 55–65.
26. Mahajan, R., Chandel, S., Puniya, A.K., Goel, G., 2020. Effect of pretreatments on cellulosic composition and morphology of pine needle for possible utilization as substrate for anaerobic digestion. Biomass and Bioenergy, 141, 105705. https://doi.org/https://doi.org/10.1016/j.biombioe.2020.105705
27. Marafon, A.C., Salomon, K.R., Amorim, E.L.C., Peiter, F.S., 2020. Use of sugarcane vinasse to biogas, bioenergy, and biofertilizer production, Sugarcane Biorefinery, Technology and Perspectives. Elsevier Inc. https://doi.org/10.1016/b978-0-12-814236-3.00010-x
28. Moraes, B.S., Zaiat, M., Bonomi, A., 2015. Anaerobic digestion of vinasse from sugarcane ethanol production in Brazil: Challenges and perspectives.

Renew. Sustain. Energy Rev. 44, 888–903. https://doi.org/10.1016/j.rser.2015.01.023

29. Park, J.H., Kumar, G., Yun, Y.M., Kwon, J.C., Kim, S.H., 2018. Effect of feeding mode and dilution on the performance and microbial community population in anaerobic digestion of food waste. Bioresour. Technol. 248, 134–140. https://doi.org/10.1016/j.biortech.2017.07.025
30. Parsaee, M., Kiani Deh Kiani, M., Karimi, K., 2019. A review of biogas production from sugarcane vinasse. Biomass and Bioenergy, 122, 117–125. https://doi.org/10.1016/j.biombioe.2019.01.034
31. Reis, C.E.R., Hu, B., 2017. Vinasse from sugarcane ethanol production: Better treatment or better utilization? Front. Energy Res. 5, 1–7. https://doi.org/10.3389/fenrg.2017.00007
32. Science, W., 2007. Co-composting of distillery and winery wastes with sewage sludge Co-composting of distillery and winery wastes with sewage sludge. https://doi.org/10.2166/wst.2007.488
33. Souza, M.E., Fuzaro, G., Polegato, A.R., 2018. Thermophilic Anaerobic Digestion Of Vinasse In Pilot Plant UASB Reactor, Wat Sci Tech, 25, 213–222.
34. Souza, M.E., Fuzaro, G., Polegato, A.R., 1992. Thermophilic anaerobic digestion of vinasse in pilot plant UASB reactor. Water Sci. Technol, 25, 213–222. https://doi.org/10.2166/wst.1992.0153
35. Yuan, Y., Cheng, H., Chen, F., Zhang, Y., Xu, X., Huang, C., Chen, C., Liu, W., Ding, C., Li, Z., Chen, T., Wang, A., 2020. Enhanced methane production by alleviating sulfide inhibition with a microbial electrolysis coupled anaerobic digestion reactor. Environ. Int. 136, 105503. https://doi.org/10.1016/j.envint.2020.105503
36. Yuliasni, R., Setyaningsih, N.I., Handayani, N.I., Budiarto, A., 2017. The performance of combined technology Upflow anaerobic reactor (UAR)-activated sludge (AS) for treating batik wastewater. Adv. Sci. Lett, 23, 2246–2250. https://doi.org/10.1166/asl.2017.8725