Research Article

Optimization of Hydraulic Retention Time and Organic Loading Rate in Anaerobic Digestion of Squeezed Pineapple Liquid Wastes for Biogas Production

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Abstract
Pineapple wastes are produced in huge amount during the industrial canning process of pineapple; in Thailand over 400,000 tons per annum of canned pineapple exported leaving behind the waste. Besides the pulps and peels as solid wastes, the squeezed pineapple liquid wastes (SPLW) extracted from solid wastes can also be used for anaerobic digestion. In the present study, the anaerobic digestion of liquid squeezed from industrial pineapple peels was carried out using a lab-scale hybrid reactor. The reactor was operated for over 170 days with the hydraulic retention time (HRT) of 20 days decreasing down to 5 days and simultaneous control of organic loading rate (OLR). Under controlled conditions in the hybrid reactor, pH was maintained at 6.5–7.6 by adding alkaline for anaerobic microbial activity. Results showed that the chemical oxygen demand (COD) removal efficiency was at ≥ 90% for all conditions. The biogas production (mL/day) increased thoroughly from longer HRT to shorter HRT, as same as methane production with the maximum values (HRT 5 days, OLR 5 g/COD/day with recirculation) of 55,130 and 30,322 mL/day, respectively. Moreover, the highest yields of biogas and methane were also investigated under similar conditions with the values of 0.504 and 0.277 L/gCOD, respectively. Interestingly, this optimization of both HRT and OLR of lab-scale anaerobic digestion process could be further practically applied to pilot or industrial scale in canned pineapple factories for biogas production.

Keywords: Pineapple wastes, HRT, OLR, COD removal, Biogas, Methane

1 Introduction

Thailand is one of the top exporters of tropical fruits, especially pineapples (*Ananas comosus* (L.) Merrill). The production of pineapple in the country was cumulated approximately 2 million metric tons from 2014 to 2018, with exported canned or processed pineapples valued at over 400 million USD in 2018 (Figure 1).

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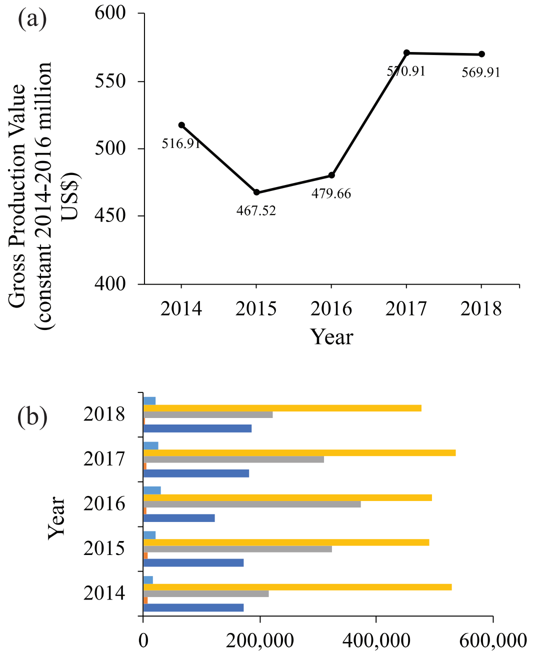

	2014	2015	2016	2017	2018
Viet Nam	16107	20942	29340	24943	20813
Thailand	528159	490851	494474	534740	477224
Philippines	214975	323810	372906	309763	221287
Malaysia	7961	7548	5807	5617	3726
Indonesia	171151	172286	123485	180732	185466

Export Quantity (Tonnes)

Figure 1: The report of pineapple production and export. (a) Value of pineapple production in Thailand. (b) Export quantity of canned pineapples in South-Eastern Asia (reported by FAO, 2020).

However, the report also showed the reduction in canned pineapples due to the lower prices caused by the oversupply in Thai market [1]. In contrast, the export volume of canned pineapples from Thailand remains highest in South-Eastern Asia region [2]. Consequently, the by-products or wastes from industrial processes could reach a massive amount per day and annum. Thus, the lowering of agribusiness wastes or adding value to them could be a key to improve the industrial profitability [3].

Pulps and peels are the primary pineapple wastes; as they are solids they can be processed as lignocellulosic biomass substrate for bioenergy production in the downstream process [4]–[6]. The biogas production rates from single [7] and mixed [8] solid pineapple wastes have been widely studied by optimizing the key parameters and stages of digestion process, which could generate a desirable biogas. In contrast, lignocellulosic solid wastes obviously demonstrate a more difficult and complexed to be degradable compared with liquid wastes, which leading to limitation in hydrolysis process [9]. Thus, the liquid wastes could provide a better time- and cost-effective process. Generally, the liquor obtained from solid wastes or squeezed pineapple liquid wastes (SPLW) pressing step are processed as pineapple syrup and applied in several food products. However, a large amount of the liquor is also left as waste to be treated in the wastewater treatment. This SPLW could be simultaneously used as the desirable organic carbon biomass to produce bioenergy, particularly bioethanol and biogas [10], [11]. The sugar compositions of pineapple liquid waste primarily consists of sucrose, glucose and fructose [12]. Interestingly, the biogas production as waste treatment demonstrates the benefits over other biofuels such as bioethanol and biodiesel, which could effectively reduce chemical oxygen demand (COD) in organic wastewater streams in agro-food industry [13]. Hence, the combination of wastewater treatment and biogas production promises to be an impactful option for pineapple processing industry.

The conversion of biomass into energy and digestate in the agricultural waste treatment is mostly achieved by the anaerobic digestion (AD) process, which involves three stages of hydrolysis: acidogenesis, acetogenesis and methanogenesis. Hydrogen, carbon dioxide (CO_2) and acetic acid (CH_3COOH) are the major biogas forms and are generally produced during acidogenesis and acetogenesis stages, followed by the methanogenesis stage which produced methane [14]. As much as over 150 m^3/mg FM (Fresh Matter) of biogas could be produced from vegetable, fruit and legume wastes with 55% CH_4 in biogas by AD process [15]. Several methods for enhancement of biogas yields in AD have been widely reported for different types of waste materials and wastewater treatment processes, for example, a two-stage system, co-digestion system, using micronutrient boosters, employing the right reactor type, and pretreatment methods [16], [17]. On the other hand, the optimization of operational parameters in the AD process is also important, especially the hydraulic retention time (HRT) and organic loading rate (OLR), in order to balance the COD removal rate and microbial activities in the system [18], [19]. In addition, other related parameters must be simultaneously monitored to evaluate the reactor performance, such as pH, COD, total solids (TS), total volatile acids (TVA), and volatile suspended solids (VSS) [19].

In this study, the optimization of HRT and OLR were studied to improve the biogas production and the impacts on fermentation parameters. In general, HRT is applied in a long-term process due to the microbial activity of methanogenesis and the adaptation of appropriate conditions while changing in each fermentative phase. However, short-term HRT is more practical to use in industrial applications [20]. Consequently, the optimization of HRT for long- and short-term AD processes could improve the biogas yields and be beneficial for processed pineapple industry in environmental aspects.

2 Materials and Methods

2.1 *Substrate, inoculum and biochemical compositions*

The inoculum for the experiment was collected from anaerobic pond at Samroiyod Co., Ltd. (Prachuap Khirikhan Province, Thailand), a company which processes tropical fruits for export, and kept in a tank under room temperature without additional feeding. The substrate was the SPLW during the processing (also provided by Samroiyod Co., Ltd.). Before the experiment, the collected and frozen SPLW were thawed before use. Then, COD in sample was analyzed for further calculation of influent volume, which are related to each period of controlled HRT (Total volume in fermenter (L)/ Influent feeding rate (L/d); days) and OLR (Influent feeding rate (L/d) × Input COD/ Total volume in fermenter (L); gCOD/L/day) in the bioreactor. The chemical composition of substrate, water, and wastewater were analyzed following the standard methods and/ or APHA, AWWA and WPCF (1995) protocols [21].

2.2 *Experimental design*

The reactor in this experiment was an acrylic-cylindrical hybrid vessel (inside diameter, 19 cm; height, 86 cm) with a total volume of 24.4 L including the packed-bed zone volume of 14.18 L (Density; ρ = 26 kg/m^3). Nylon fiber was used as a supporting media in packed-bed zone with surface area of 3.43 m^2 and 0.625 height ratio (height of packed zone to height of reactor; Hp/H) to the reactor. The 50 of packed nylon fibers were fixed and covered with a plastic net before being put into the packed-bed zone of the reactor. The direction of fluid flow in this hybrid reactor was in an upflow direction, where the gas valves are located on the top of the reactor for routinely collecting biogas and measuring the flow rate of biogas (Figure 2).

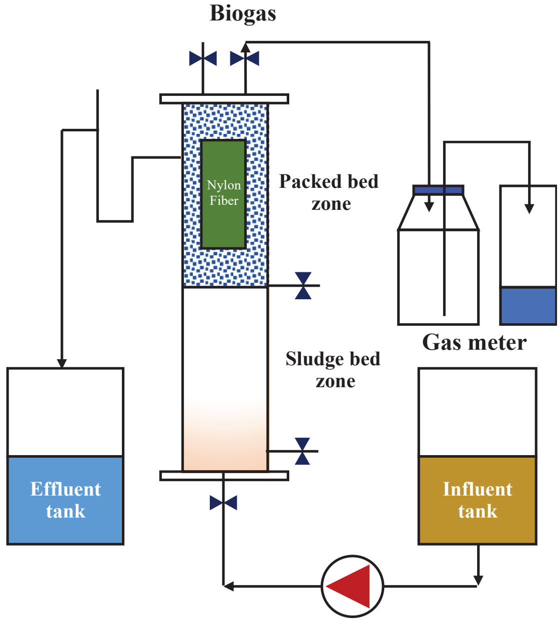

Figure 2: The scheme of hybrid reactor used in this study (modified from Suraraksa and Nopharatana [22]).

The peel squeezed liquid concentration was controlled in terms of initial COD concentration into 10 g/L and the concentration of inoculum used was 7 gVSS/L. The pre-culture was performed in 1 L Duran bottle under controlled pH by adding sodium bicarbonate for 1 month. The biogas production, COD removal (%) and pH shifts had been preliminarily investigated as the long-term HRT, i.e. HRT of 20 and 10 days, in a hybrid reactor as range of Day 1–54 and 55–174, respectively. Following that, the short-term HRT system, i.e. HRT 7 and 5, was operated for further 188 days, in which HRT 7 (OLR 2), HRT 5 (OLR 2.8), HRT 5 (OLR 5) and HRT 5 (OLR 5 with effluent recirculation) were operated from Day 1–70, 71–151, 152–173 and 174–193, respectively.

2.3 *Analytical methods*

The operation of reactor was set to feed the influent daily into the vessel by using a magnetic pump. Before feeding the fresh squeezed liquid into the reactor, the treated wastewater from the system was collected for analyses of key parameters (Table 1). The performance of the reactor was regularly monitored through pH (Eutech Instrument, Korea), total COD, TVA/Alkaline ratio, and COD removal (%) in the system following the methods shown in Table 1. The samplings of wastewater then were collected in duplicate for further analysis.

Table 1: Parameters and analytical methods used in this study

Parameters	Analytical Methods	Frequency
pH levels	APHA, AWWA and WPCF, 1995 No. 2540	2–3 days/time
Total COD	APHA, AWWA and WPCF, 1995 No. 2310	2–3 days/time
Total volatile acids	APHA, AWWA and WPCF, 1995 No. 2310	2–3 days/time
Alkalinity	APHA, AWWA and WPCF, 1995 No. 5220	2–3 days/time
Biogas production	Gas Meter	Everyday
Biogas composition	Gas Chromatography (Agilent GC 6980)	2–3 days/time

The determination of biogas composition was carried out by collecting the gas from the valves on the top of the reactor using U-tube and then analyzing with a 2 m Porapak Q column in a gas chromatography (GC) (Agilent GC 6980) system with thermal conductivity detector (TCD). Helium was used as a carrier gas at a flow rate of 5.3 mL/min. The temperatures of the oven, injector and detector were 60, 120 and 250°C, respectively.

The biogas yields per COD removed was calculated by following equation:

Biogas yield (L/ gCOD removed)

$$= \frac{\text{Biogas production rate (L/day)} \times \%CH_4}{Q\ (So - S)}$$

where Q = Input influent rate (L/day)
So = Average COD of input influent per day (mg/L)
S = Average COD of output effluent per day (mg/L)

3 Results and Discussion

3.1 *Composition of the SPLW*

Different sources and composition of biomass from agricultural and processed crops wastes could alter the biogas and methane yields due to their distinctive chemical composition [23]. Fruit wastes are majorly composed of carbohydrates and lipids in pulps, peels and bagasse. The presence of high sugar content mostly found in fruit juice could afford a desirable digestibility to the AD process and allow the high production rates for methane [23], [24]. Thus, the SPLW composition can be analyzed for the suitable treatment and condition for biogas production.

Table 2: The chemical composition of SPLW

Chemical Composition	Concentration
Total solid	145,255 mg/L
Volatile solid	109,622 mg/L
Total sugar	321,645 mg/L
Reducing sugar	140,000 mg/L
Total nitrogen	0.75%
pH	3.9–4.0
Total COD	90,000–170,000 mg/L

The chemical composition of the SPLW is shown in Table 2. The amount of reducing sugar was high in liquid waste, which could reveal the potential for bacterial growth [25]. However, the pH level is relatively low, which could be detrimental for microbial growth and activity, especially methanogens that can be sensitive to alterative conditions [26]. The total COD indicates the available carbon in influent that will be further treated and converted into biogas, and implies that the higher initial COD amount could result in big reduction in final COD [27].

3.2 *Long-term HRT condition for preliminary optimization of biogas production*

To start up the biogas production, the long-term HRT was established with HRT at 20 and 10 in order to provide the sufficient time to convert organic materials into biogas [28]. The results showed that the highest biogas production at HRT 20 (OLR 0.5) and 10 (OLR 1.4) were 9,340 and 14,240 mL/day at 15 days and 171 days,

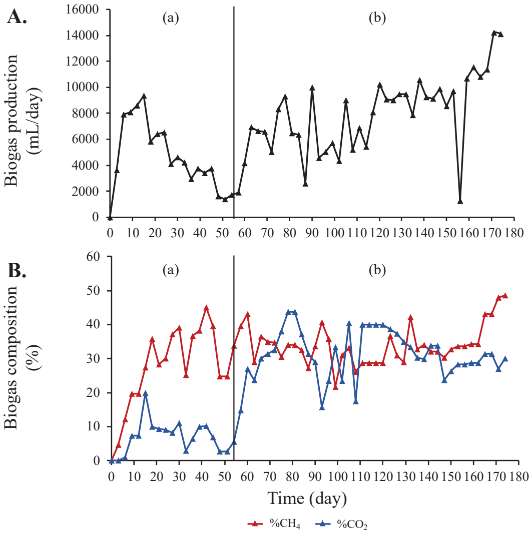

Figure 3: A. Biogas productivity (mL/day) and B. Biogas composition (%) in hybrid reactor (a) at HRT 20 day with OLR 0.5 gCOD/L/day and (b) at HRT 10 day with OLR 1.4 gCOD/L/day.

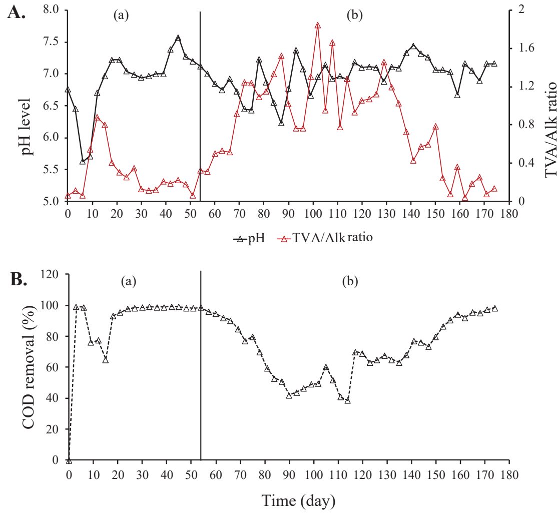

Figure 4: A. Changes of pH levels and TVA/Alk ratio, and B. COD removal (%) in hybrid reactor (a) at HRT 20 day with OLR 0.5 gCOD/L/day and (b) at HRT 10 day with OLR 1.4 gCOD/L/day.

respectively [Figure 3(A)]. In other words, the HRT 20 with OLR 0.5 resulted in a gradual decrease of biogas production after 15 days of the fermentation, suggesting that the carbon sources from the influent had been nearly depleted. However, when HRT 10 with OLR 1.4 (gCOD/L/day) was later immediately implemented, the biogas production continued to rise to the maximum point on Day 171. Thus, HRT 10 could provide higher efficiency on biogas production from SPLW fermentation than HRT 20 with OLR 0.5. Moreover, the biogas composition also showed the dramatic increase in methane content after 160 days of fermentation, which reached the maximum at 48.55% by the final day (Day 174) [Figure 3(B)]. Hence, the lower HRT with higher OLR could provide the for the increase in biogas production during SPLW fermentation, under the improved stability and performance of AD process.

The appropriate pH level for cultures is necessary for efficient anaerobic microbial fermentation, especially the methanogens, which need the optimal pH levels for their activity in the range of 6.5–7.8 [29]. Our results showed that the pH dramatically declined to 5.63 at the early phase of HRT 20 fermentation due to lack of alkaline addition, but it was later in a steady state at the optimal pH range (pH 6.23–7.58) [Figure 4(A)]. The lower pH at early stage of each HRT into the system could be explained that the acidogenic bacteria obtain a faster growth than methanogens leading to high organic acids accumulated in the system. The CO_2 was later produced as byproduct from acidogenesis and consequently used as substrate for methane production [Figure 3(B)], then the pH levels were stable from the middle stage of each HRT. The ratio of TVA/Alk represents the proper amount of alkaline supplement needed to maintain the pH level above 6.5 for suitable microbial activity [30]; the overall results revealed that pH levels were properly stabilized in both HRT 20 and 10 conditions [Figure 4(A)]. However, the TVA/Alk ratio indicated some unacceptable values during the middle of HRT 10 phase, which were above the normal range of 0.4–0.6 or below [31]. Thus, this could be assumed that the digester stability at HRT 10 with OLR 1.4 is low, as the optimal TVA/Alk ratio for methane production should be lower than 0.4 as shown in the later days of both HRT 20 and 10 conditions.

Besides the efficiency of biogas production, the COD removal efficiency is also an important factor for industrial application, which should be over 90% to signify an acceptable effluent quality [32]. In this study, the efficiency of COD removal (%) demonstrated the significant values (over 90% COD removal) from

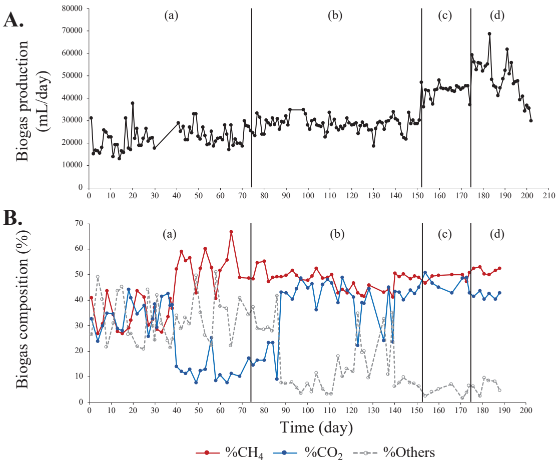

Figure 5: A. Biogas production (mL/day) and B. Biogas composition (%) in hybrid reactor under different conditions. (a) HRT 7 day with OLR 2 gCOD/L/day (b) HRT 5 day with OLR 2.8 gCOD/L/day (c) HRT 5 day with OLR 5 gCOD/L/day (d) HRT 5 day with OLR 5 gCOD/L/day (recirculation).

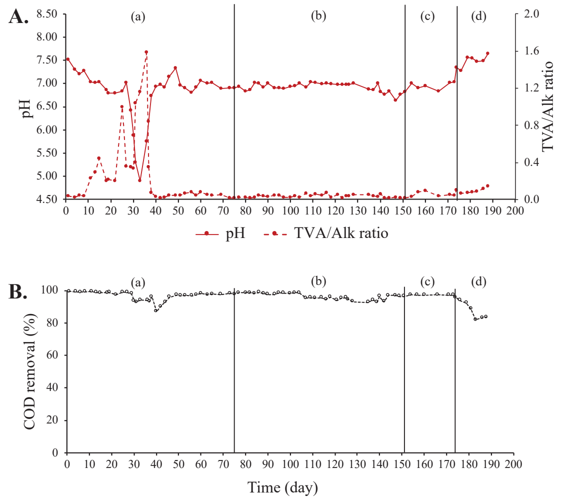

Figure 6: A. Changes of pH levels and TVA/Alk ratio, and B. COD removal (%) in hybrid reactor at (a) HRT 7 day with OLR 2 gCOD/L/day (b) HRT 5 day with OLR 2.8 gCOD/L/day (c) HRT 5 day with OLR 5 gCOD/L/day (d) HRT 5 day with OLR 5 gCOD/L/day (recirculation).

Days 18 to 66 with the highest efficiency at Day 42 (99.26%), and 97.35% at Day 171 as the maximum biogas production point [Figure 4(B)]. Thus, the low OLR, i.e. 0.5 and 1.4, were suitable rates of influent feeding leading to satisfied COD removal efficiency to the system as stated by previous report [33].

3.3 *Short-term HRT condition for enhancement of biogas production*

Under optimized conditions, the shorter HRT is reported to demonstrate more economic impacts to practical uses, which provides lower digester volume and lower cost [28]. The decrease in HRT together with consequent increase in OLR resulted in the increase of biogas production reaching to the maximum of 68,625 mL/day on Day 183 under HRT 5 with OLR 5 (recirculation) [Figure 5(A)]. Thus, the enhanced OLR up to certain point could influence the microbial community and increase the biogas yield [28], [34]. On the other hand, the methane production declined after the HRT reduction with OLR increase, suggesting the fermentation may have become unstable possibly due to an irreversible acidification [35]. Consequently, the highest biogas composition that was obtained was methane 66.64% under HRT 7 with OLR 2 (65 days), which higher than previous study (51 and 41%) in biogas production from fresh and dried solid pineapple, respectively [36]. Moreover, the highest production of other gases (50.88%) and carbon dioxide (50.76%) were investigated under HRT 7 with OLR 2 (58 days) and HRT 5 with OLR 5 (154 days), respectively [Figure 5(B)].

As mentioned earlier, the lower OLR rates could provide better COD removal efficiency. Although the highest efficiency was observed under HRT 20 with OLR 0.5, over 90% COD removal was also achieved in all shorter OLR conditions [Figure 6(B)]. Thus, the optimized HRT and OLR were successfully achieved in the reactor. Additionally, the overall biogas and methane yields (Table 3) were greater under shorter HRT with higher OLR (especially with recirculation), consequently in 0.504 and 0.277 L/gCOD removed, respectively, and also indicated in similar range of methane yield as previous two-stage AD study [37]. Similarly, the lower OLR conditions also affect to better COD removal efficiency but lower in biogas production rate (by the determination methods in Table 3) in recent study [8]. This could be suggested that the effluent recirculation could allow solution to alleviate the overloading limitation in the system [28]. Moreover, the effluent recirculation could enhance biogas production up to the steady state due to the effects of dilution and pH adjustment under high OLR condition [Figure 6(A)] [38], [39], which could provide a suitable condition for biogas and methane production in hybrid reactor operation.

Table 3: The comparison between long- and short-term HRT

HRT[a], OLR[b]	Biogas[c]	CH_4[c]	Biogas Production Rate[d]	Gas Yield[e]		COD Removal (%)
				Biogas	CH_4	
20, 0.5	6,000	2,119	0.267	0.270	0.100	98.80
10, 1.4	12,120	5,262	0.534	0.390	0.170	95.54
7, 2	19,754	10,351	0.870	0.446	0.270	97.55
5, 2.8	29,203	14,455	1.280	0.476	0.234	96.40
5, 5	44,926	22,469	1.980	0.407	0.202	97.20
5, 5*	55,130	30,322	2.420	0.504	0.277	96.33

Unit: [a] day, [b] gCOD/L/day, [c] mL/day, [d] L/L/day, [e] L/gCOD removed
* recirculation
Note: that all values represent as the maximum values.

4 Conclusions

The liquid squeezed from pineapple pulps and peels, which mostly composed of sugar was suitable for further microbial biogas fermentation through anaerobic digestion. Though steady state still not reach in this condition, the highest biogas production was observed at short-term HRT 5 with recirculation for both biogas and methane production at 55,130 and 30,322 mL/day, respectively. Additionally, this condition (HRT 5, OLR 5 with recirculation) also provided the highest biogas production rate, which increased from HRT 20 for approximately 10-fold. Moreover, the COD removal efficiency were over 90% for all fermentation conditions, which implies to a good quality of effluent. Hence, this study could be beneficial to local pineapple processing industry as well as other similar industry resulting in better environmental prospects.

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