PERFORMANCE AND KINETIC STUDY OF THE ANAEROBIC CO-DIGESTION OF COCOA HUSK AND DIGESTED COW MANURE WITH HIGH ORGANIC LOADING RATE

PERFORMANSI DAN STUDI KINETIKA PADA PROSES ANAEROBIK CO-DIGESI KULIT KAKAO DAN LIMBAH MANURE SAPI DENGAN LAJU PEMASUKAN BAHAN ORGANIK YANG TINGGI

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ABSTRACT
Biogas is a clean and cheap renewable energy that can be used for generating electricity. One of the current methods applied to enhance biogas production was through anaerobic co-digestion. The current study revealed that anaerobic digestion of cow manure co-digested with cocoa husk produced higher biogas production (348.3 mL/day) than anaerobic digestion of cow manure alone (26.5 mL/day). Even if a high organic loading rate (OLR) (4.173 kg VS/m^3.day) was applied to the reactor of anaerobic co-digestion, no inhibition was found at which pH was stable at 7.08. The results suggested that high OLR and short HRT (10 days) did not inhibit biogas production.

ABSTRAK
Biogas adalah energi terbarukan yang bersih dan murah yang dapat digunakan untuk menghasilkan listrik. Salah satu metode saat ini yang telah diterapkan untuk meningkatkan produksi biogas adalah melalui anaerobic co-digesi. Penelitian saat ini mengungkapkan bahwa pencernaan anaerobik dari kotoran sapi yang dicerna bersama dengan kulit coklat dapat menghasilkan produksi biogas yang lebih tinggi (348,3 mL / hari) dibandingkan dengan pencernaan anaerobik dari kotoran sapi saja (26,5 mL / hari). Meskipun OLR yang tinggi (4,173 kg VS / m3.day) diaplikasikan pada reaktor anaerobic codigesi, tidak ada hambatan yang ditemukan di mana pada penelitian ini pH tetap stabil pada 7,08. Hasil penelitian ini menunjukkan bahwa OLR tinggi dan HRT singkat (10 hari) tidak menghambat produksi biogas.

INTRODUCTION

Anaerobic digestion is an established technology that has been widely used as well as applied for biologically treating wastewater in the wastewater treatment plants *(Monroy et al, 2000; Gomec C.Y., 2010).* The application of anaerobic digestion process is not only used for treating and/or managing the organic liquid and solid wastes but also can be used for generating renewable energy (i.e. biogas) *(Lastella et al, 2002; Gontupil et al, 2012).* In the process of anaerobic digestion, complex organic materials are biologically decomposed and converted into biogas (methane) as the end-product. The process involves different types of microbial consortia *(Narihiro et al, 2007; Riviere et al, 2009; Barta et al., 2010).*

In order to achieve the complete conversion of the organic materials into biogas, the process of anaerobic digestion should be controlled and maintained in accordance to the optimal operating conditions. Some parameters that highly affected the process of anaerobic digestion to generate biogas include pH, alkalinity and/or buffer capacity, temperature, hydraulic retention time (HRT) and organic loading rate (OLR) *(Maharaj et al., 2001; El-Mashad et al., 2004; Gustin et al., 2011; Liu et al., 2012).* As the application of anaerobic digestion for the purpose of generating renewable energy and converting organic wastes becomes more attractive to the researchers, current study would be more focused on finding out the significant approaches and better strategies for preventing the process failure of anaerobic digestion, and/or optimizing the production of biogas as the end-product.

Some studies had revealed that anaerobic digestion process operated in the continuous as well as semi-continuous modes highly depended on the HRT *(Salminen and Rintala, 2002; Dareioti and Kornaros, 2014).* HRT is known as a measure of the average length of time at which the anaerobic culture remains in the reactor. Therefore, the steady state condition of the anaerobic digestion process would be highly dependent on the flow rate of the influent as well as effluent of the anaerobic reactor *(Sialve et al., 2009).* Further, HRT applied would affect the microbial growth rate in the anaerobic reactor in which insufficient retention time applied to the process of anaerobic digestion would lower the concentration of biomass in the digester *(Zhang et al., 2006).* This suggested that short HRT would slow down the process of anaerobic digestion to form methane due to the biomass washout *(Chen et al., 2008; Sialve et al., 2009).*

Some studies revealing that the optimum HRT for optimizing the process of anaerobic digestion operated in continuous as well as semi-continuous system was in the range 20-25 days *(Bouallagui et al., 2005; Demirer and Chen, 2005; Chandra et al., 2012).* Within those HRT range the production of anaerobic digestion could be optimized due to the fact that the anaerobic microbes would have sufficient time for completely converting the complex organic materials into biogas. The maximum production of methane gas was achieved when the continuous process of anaerobic co-digestion was operated at 20 days of HRT *(Kaosol and Sohgrathok, 2012).* Study conducted by *Onthong and Juntarachat (2017)* revealed that the highest biogas yield obtained from the process of anaerobic co-digestion of cow manure with bagasse and soybean residue, could be reached by applying 25 days of HRT. However, the authors also found that anaerobic co-digestion of cow manure with papaya peels had the highest biogas yield at 15 days of HRT. This suggested that the different types of substrate loaded into the anaerobic digester would have a different optimum HRT for optimizing the production of biogas *(Willeghems and Buysse, 2016).*

Beside the HRT, OLR is also a vital parameter that would affect the performance of anaerobic digestion. The production of biogas as well as biogas yield would significantly decrease once OLR was increased *(Elango et al., 2007).* High OLR could also contribute to the acid accumulation in the reactor which could increase the proton concentration and pH drop. This occurred as high OLR applied would stimulate acidogenic bacteria to consume and convert all soluble organic matters into volatile fatty acids (VFA) *(Elango et al., 2007). Manyi-Loh et al. (2013)* mentioned that the rate of VFA formation is higher than the rate of its conversion into methane gas, and thereby the VFA would be accumulated in the digester and could inhibit the whole process of anaerobic digestion.

To understand the performance of anaerobic digestion process, kinetic model could be used to evaluate the fermentation pathways as well as the behaviour of microorganisms in the anaerobic digester *(Kafle et al., 2014).* Kinetic models are developed to assess whether the process of anaerobic digestion could run properly for generating biogas as the end-product *(Mähnert and Linke, 2009)*. A simple kinetic model that had been developed for assessing the behaviour of microbial activity in the fermentation process was based on the Monod equation *(Fernández et al., 2010).* As the Monod model is only suitable for the single culture, the use of this model on the assessment of anaerobic digestion process would be infeasible *(Momoh et al., 2013).* This is due to the fact that the process of anaerobic digestion is normally inoculated with the mixed microbial consortia.

Study on the kinetic assessment of anaerobic co-digestion of digested cow manure and lignocellulosic biomass (i.e. bagasse), revealed that the specific growth rate obtained was higher than the dilution rate applied representing the presence of cell mass in the reactor (*Darwin et al., 2017*). Some other previous studies also added that some parameters such as, volatile solids (VS) and chemical oxygen demand (COD) could be used as the input parameters on the kinetic models to assess the microbial activity during the process of anaerobic digestion *(Fernández et al., 2010; Momoh et al., 2013)*. Kinetic assessment on the process of anaerobic digestion inoculated with microbial mixed cultures could generate various bioconversion stages which could stimulate the dynamic change of biochemical pathways (*Momoh et al., 2013*). This is due to the fact that the anaerobic digestion inoculated with mixed cultures might have different types of microbial population involved *(Momoh et al., 2013).*

The purpose of the study reported here was to investigate the effects of high organic loading rate and short hydraulic retention time on the biogas production from the single stage anaerobic co-digestion of cocoa husk and digested cow manure. Parameters involved in the process of anaerobic digestion operated in semi-continuous system were also evaluated through kinetic model assessment.

MATERIALS AND METHODS

Preparation of substrates

Cocoa husk used for this experiment was collected from cocoa plantation located in Lamtamot Village, Seulawah Valley-District of Aceh Besar. The cocoa husk obtained was dried to reach moisture content of ± 7 % (wet basis). The dried cocoa husk was milled using a laboratory mill to an average particle size of 35 Mesh. The percentage of total solids of cocoa husk used as a co-substrate was about 93.22 ± 0.08 %. The digested cow manure used for the experiment was taken from the anaerobic wastewater treatment plant located in the Cow Farming at Sibreh, the District of Aceh Besar. The sludge was stored in the fridge at the temperature of ± 5ºC prior to using it. For characterization of substrates, cocoa husk and sludge used was analyzed for solid concentration. Samples of cocoa husk and sludge were dried at the temperature of 105ºC, and followed with burning the samples at the temperature of 550ºC in the furnace for total solid and volatile solid analysis, respectively. Total solids (TS) and volatile solids (VS) analysis was carried out in accordance with the Standard Methods *(APHA, 2012).*

Experimental Procedure

Experiments were carried out in the Laboratory of Bioprocess and Postharvest Technology, Department of Agricultural Engineering, Syiah Kuala University. Some sample analysis measurements were conducted at the Institute for Research and Standardization of Industry, Banda Aceh. An anaerobic semi-continuous reactor was operated at the steady state condition in which the temperature was maintained under the mesophilic condition at the temperature of 34 ± 1ºC by using thermostatic water bath. The working volume of the digester was 3000 mL. The short hydraulic retention time applied in this experiment was 10 days. The loading rate applied in this experiment was 300 mL/day. High organic loading rate was applied in this experiment in which 30 g of cocoa husk was mixed with 300 mL of digested cow manure. This indicated that cocoa husk as a co-substrate was added at 10% of the total volume of the daily digested cow manure loaded. Prior to the start of the anaerobic digestion process, organic loading rate applied was firstly measured. The measurement of organic loading rate was based on the percentage of total solids and volatile solids of the anaerobic digestion culture. Based on the initial analysis, total solids and volatile solids of the culture obtained were 5.28% and 79.034%, respectively. As some parameters including the working volume of the anaerobic digester (3 litters) and loading rate (300 mL/day) had been obtained, organic loading rate (OLR) applied in this current experiment would be 4.173 kg VS/m^3.day.

The anaerobic digesters were equipped with the effluent and influent sample ports. Samples of the effluent were taken from the port for further analysis. To ensure the anaerobic digestion process operated in semi-continuous system running properly, pH of the samples including influent and effluent was measured periodically during the feeding and the discharging periods. No pH control was applied in this system, and thereby no alkaline and acid added to the reactors. Biogas production was measured using a gas meter based on water displacement method. The gas outlet located on the top of each reactor was connected using tubes to the port of the gas meter. The gas meter installed was calibrated periodically. Prior to the start of the feeding substrates, the culture was acclimated with the anaerobic digestion condition in order to avoid the failure of digestion process and to reach a steady state condition at pH between 6.8 and 7.2.

Analytical methods

The influent and effluent samples were analyzed for total solids (TS) and volatile solids (VS) contents, moisture content (MC), total dissolved solids (TDS), chemical oxygen demand (COD) and total kjeldahl nitrogen (TKN). All parameters measured were carried out based on the Standard Methods (APHA2012). To determine the strength and the amount of solid mixed and organic compounds present in the wastewater and sludge, the analysis of solid content and its removal after digestion process should be conducted *(Darwin et al., 2016a)*.VS reduction and COD removal were analyzed in order to know the efficiency of biodegradation during the anaerobic digestion process, which were based on the formula used by previous studies *(Darwin et al., 2016b)*. The rates of biogas production were measured as the volume of biogas produced per day. The biogas yield was measured based on the total biogas produced per gram volatile solids added *(Parawira et al., 2008; Darwin et al., 2016b)*.

RESULTS

The performance of anaerobic co-digestion with high organic loading rate

To evaluate the effects of high organic loading rate on the anaerobic co-digestion of cocoa husk with digested cow manure, the anaerobic reactors were operated under semi-continuous mode. As shown in Table 1, both single substrate digestion and co-digestion reactors had culture with pH neutral (7.0-7.06). The influent of anaerobic co-digestion of cocoa husk with digested cow manure had higher organic contents of COD and VS (11361 mg/l and 79.03%) than the COD and VS of the influent of anaerobic digestion of cow manure alone (1900 mg/l and 67%). This would indicate that the anaerobic digestion of digested cow manure using cocoa husk as a co-substrate would have the potential for enhancing the biogas production rather than the anaerobic digestion of cow manure alone.

Table 1

Influent data of anaerobic digestion process

Analysis	Unit	Cow manure	Cocoa husk co-digested with cow manure
COD	mg/L	1900	11361.08
TS	%	1.3	5.28
VS	%	67	79.03
pH	-	7.0	7.06
TKN	mg/L	321	769.92
TDS	mg/L	1600	1510

Results showed that anaerobic co-digestion of digested cow manure and cocoa husk produced biogas at thirteen times higher than biogas produced from the anaerobic digestion of digested cow manure alone (Fig. 1). High organic loading rate (OLR) and short HRT applied to the anaerobic co-digestion process did not give any significant inhibition to the biogas production. Results also showed that pH culture of anaerobic co-digestion of cocoa husk and digested cow manure were quite stable between 6.92 and 7.23 (Table 2), which was considered as the optimal pH range for biogas and/or methane production via anaerobic digestion *(Rajeshwari et al., 2000)*.

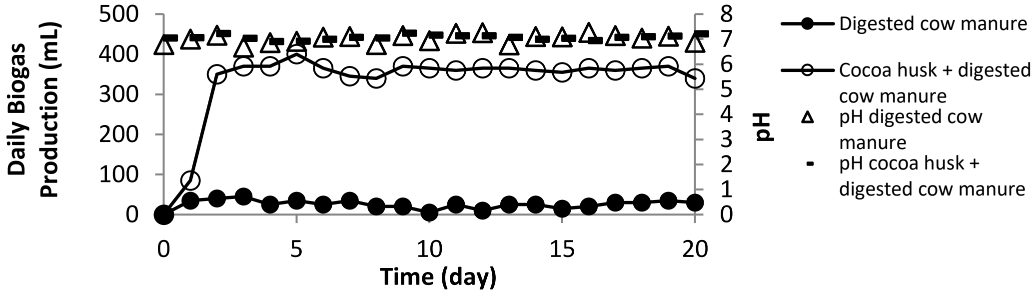

Fig. 1 - Daily biogas production of cocoa husk and digested cow manure under semi-continuous operation

Table 2

Effluent data of anaerobic digestion process

Analysis	Unit	Cow manure	Cocoa husk co-digested with cow manure
COD	mg/L	645	3225.22
TS	%	0.6	5.44
VS	%	40	77.12
pH	-	6.9	7.22
TKN	mg/L	212.2	752.8
TDS	mg/L	1620	3020

The current results were quite different from the previous study carried out by *Darwin et al. (2017)* on the anaerobic co-digestion of bagasse and digested cow manure, which revealed that the acid buildup occurred in the digester in which pH culture dropped from 7.0 to 5.0. The difference occurred as the characteristics of substrate used were quite different. The previous study used bagasse as a co-substrate that may have lower lignin content (≤ 5% lignin) in comparison to cocoa husk (14% lignin) that was used as a co-substrate for this current study *(Alemawor et al., 2009; Paixão et al., 2016).* This suggested that the different types and characteristics of substrate used for anaerobic digestion would affect the rate of hydrolysis as well as conversion of the organic materials into intermediates (e.g. organic acids) and/or end-product (e.g. biogas/methane) during the process of anaerobic digestion *(Taherzadeh and Karimi, 2008; Ge et al., 2010).*

Biodegradation efficiency assessment showed that high OLR (4.173 kg VS/m^3.day) and short HRT (10 day) applied to the process of anaerobic co-digestion of cocoa husk and digested cow manure did not inhibit the degradation process of organic matters to biogas. The degradation of organic materials in the process of anaerobic co-digestion of cocoa husk and digested cow manure was showed in a fairly high percentage of COD removal which was about 72% reduction. High organic conversion showed in COD removal, could lead to an increase of biogas production in the process of anaerobic co-digestion of cocoa husk and digested cow manure.

Results revealed that high OLR and short HRT applied did not affect the performance of anaerobic co-digestion of cocoa husk and digested cow manure to produce biogas. Overall, biogas generated from the anaerobic co-digestion of cocoa husk and digested cow manure was significantly higher than biogas produced from the anaerobic digestion of cow manure alone (Fig.1). As shown in Table 3, the yield and productivity of biogas generated from the anaerobic co-digestion of cocoa husk and digested cow manure were around 56mL/gVS added and 350 mL/day, respectively. Those results were twice than the yield and productivity of biogas generated from the anaerobic digestion of digested cow manure alone, which were 21 mL/gVS added and 27 mL/day, respectively. These results suggested that the short HRT and high OLR applied did not generate acid accumulation in which the proton concentration in the digester was quite low (pH 6.90 - 7.2).

The current results are quite different from the study conducted by *Babaee and Shayegan (2011)* revealing that high organic loading rate applied in the process of anaerobic digestion could lower the production of biogas. The authors also mentioned that an increase of high organic loading rate could lead to the decrease of VS degradation and biogas yield. In their study, they applied a gradual increase of organic loading rate from 1.4 to 2.75 kg VS/m^3.day and resulted in a significant decrease of biogas production even if HRT applied was a normal retention time for operating anaerobic digestion which was 25 days. This is quite different from the current study in which high organic loading rate (4.173 kg VS/m^3.day) and short HRT (10 days) applied did not cause any significant inhibition for biogas production. The difference could be related to the substrate used for the anaerobic digestion in which the previous study (*Babaee and Shayegan, 2011)* used vegetable waste which was more biodegradable than the substrate used in this current study (cocoa husk). This is possible as the more biodegradable substrate loaded the easier the conversion would be *(Schiener et al., 1998)*. Therefore, high organic loading rate applied would generate acid buildup leading to an increase of proton (H^+) concentration in the anaerobic digester, and this condition would screw up the anaerobic digestion process completely *(Rincón et al., 2008; Darwin et al., 2018).*

Table 3

Biodegradation efficiency

Analysis	Unit	Cow manure	Cocoa husk co-digested with cow manure
COD removal	%	66.05	72
VS reduction	%	67.2	10.6
Total biogas accumulated	mL	530	6965
Biogas productivity	mL/day	26.5	348.3
Biogas yield	mL/gVS added	21	55.64

Kinetic assessment of anaerobic co-digestion of cocoa husk and digested cow manure

The process of anaerobic digestion highly depended on the microbial activity for succeeding the production of biogas as the end-product. Understanding the microbial activity during the anaerobic digestion process is essential to minimize the risk of process failure. Microbial activity in anaerobic digestion could be evaluated through the kinetic model analysis *(Linke, 2006).* The assessment included the kinetics on substrate consumption/uptake, the microbial growth and/or death rate.

In the process of anaerobic digestion, the limited substrate uptake could be described by assessing the first order reaction model *(Raj and Anjaneyulu, 2005; Vavilin et al., 2008)* as mentioned in Eq. (1):

$$\frac{-ds}{dt} = K'S \qquad (1)$$

As K' is the rate constant, the formula could be considered as the model of microbial exponential growth. The substrate concentration could be described by assessing the exponential growth of the biomass while the substrate is uptake. In this case, the influent substrate concentration (S_o, mg/l) is proportional to the effluent substrate concentration (*S,* mg/l) and the retention time applied (*t,* day).

$$S = S_o \exp(-K_s t) \qquad (2)$$

To evaluate whether the process of anaerobic co-digestion of cocoa husk with digested cow manure would follow the first order reaction describing the limited substrate uptake, the application of natural logarithm should be given into both sides of Eq. (2).

$$ln\left(\frac{S}{S_0}\right) = -K_s t \qquad (3)$$

Based on Eq. (3), the half –velocity constant, K_s(mg/l) would be expressed in Eq. (4).

$$K_s = \frac{-ln\left(\frac{S}{S_0}\right)}{t} \qquad (4)$$

Results revealed that a linear relationship between the half velocity constant and retention time was obtained indicating that the kinetics of the process of anaerobic co-digestion of cocoa husk with digested cow manure complied a first order reaction. As depicted in Fig.2, when $-ln\left(\frac{S}{S_o}\right)$ was plotted against the retention time, the linear curve was obtained with the regression coefficient of 0.999.

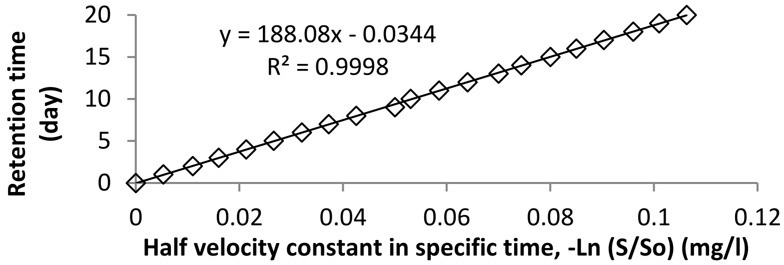

Fig. 2 - Kinetic plot of the first order reaction on the anaerobic co-digestion of cocoa husk with digested cow manure

Some essential kinetic parameters that could be used for describing the performance of anaerobic digestion included the maximum specific growth rate of biomass (μ_{max}) and the maximum substrate uptake rate (*K*). Those parameters could be obtained by plotting the total substrate effluent (*St*) against a retention time (*T*) in which the models were expressed in Eq. (5) and (6) *(Darwin et al., 2017; Zainol, 2012).*

$$S_t = \frac{S_o - S}{S} \tag{5}$$

$$T = \frac{1}{\mu_{max}} + \frac{K}{\mu_{max}} \frac{S_o - S}{S} \tag{6}$$

Based on the data plot depicted in Fig. 3, the value of μ_{max} were an inverse of intercept (1/intercept), and *K* were the division of slope and intercept (slope/intercept) *(Zainol, 2012; Darwin et al., 2017).*

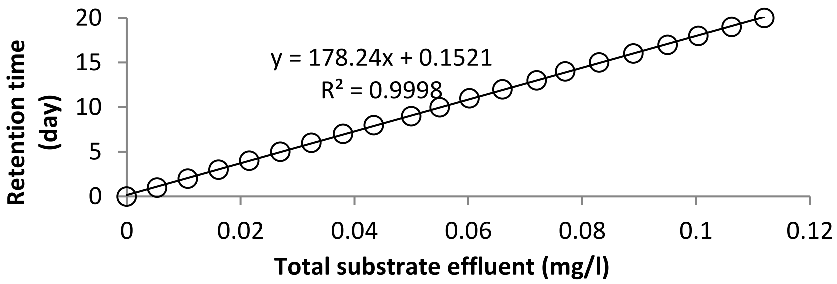

Fig. 3 - Determination of the maximum specific growth rate of biomass and the maximum rate of substrate utilization

Results of the current study revealed that the maximum specific growth rate of biomass (μ_{max}) and the maximum substrate uptake rate (*K*) during the process of anaerobic co-digestion of cocoa husk and digested cow manure obtained were 6.58 day $^{-1}$ and 1172.4 mg/mg, respectively.

As the valued of the maximum specific growth rate of biomass was higher than dilution rate (*D)* applied which was 0.1 day $^{-1}$. This suggested that the cell mass in the reactor still could retain and grow effectively in the anaerobic bioreactor even if short HRT (10 days) and 300 mL/day of flow rate were applied to the process of anaerobic co-digestion of cocoa husk and digested cow manure. This current study was in line with some previous studies *(Wirtz, 2002; Wick et al., 2002; Darwin et al., 2017)* revealing that the dilution rate applied should be lower than the value of μ_{max} in order to sustain the culture and biomass cells in the bioreactor, and also to prevent culture wash out during the process of anaerobic digestion.

Results showed that the value of the maximum specific growth rate in the process of anaerobic co-digestion of cocoa husk and digested cow manure obtained was quite high suggesting that the microbial cell mass present in the reactor was relatively small. This is due to the fact that the specific microbial growth rate was inversely proportional to the concentration of microbial cell mass during the process of anaerobic digestion *(Zainol, 2012; Darwin et al., 2017)*. The results revealed that high OLR and short HRT applied did not cause acid accumulation or drop of pH during the anaerobic co-digestion process, and thereby the production of biogas was still stable at around 348.3 ml/day (Table 2).

In order to evaluate the relationship between the substrate uptake rate (*U*) and the concentration of the substrate after the digestion process (*S*), the kinetic model would be expressed as depicted in Eq. (7) *(Viessman and Hammer, 1993).*

$$\frac{1}{U} = \frac{K_S}{KS} + \frac{1}{K} \tag{7}$$

K_s is the value of the half-velocity constant, which is related to the hydrolysed substrate (mass/volume) during the digestion process *(Faisal and Unno, 2001).* In this current study, K_s obtained was 22.04 mg/L. According to the data of the half-velocity constant K_s and the maximum substrate uptake rate (*K*) obtained, the performance of anaerobic co-digestion of cocoa husk and digested cow manure did not run effectively. This occurred as organic loading rate applied was quite high, which may lead to the inefficient conversion during the anaerobic digestion process.

An inefficient digestion process was also influenced by the short HRT. As some studies had revealed that an optimum HRT for operating anaerobic digestion process was 25 to 30 days *(Bougrier et al., 2008; Juntarachat, 2017).* This suggested that the HRT applied in this current study was about 2.5 times lower than a normal hydraulic retention time of anaerobic digestion process for biogas production, and thereby the anaerobic microbes did not have sufficient time for completely converting organic materials into biogas. As an optimum HRT for operating anaerobic digestion was 25 days, this indicated that the OLR that should be feasible for optimizing the process of anaerobic co-digestion of cocoa husk and digested cow manure to form biogas, was at around 1.67 kg VS/m^3.day.

In anaerobic digestion, the specific substrate uptake rate could be related to some kinetic parameters including the biomass yield, mean cell residence time and the endogenous decay coefficient. The relationship among those parameters could be expressed in kinetic model as shown in Eq. (8).

$$\frac{1}{\theta} = YU - K_d \tag{8}$$

where θ is mean cell residence time (day), *Y* is the biomass yield (mg/mg), and K_d is the endogenous decay coefficient (day^{-1}) *(Taherzadeh M.J.; Karimi K., 2008)*.

By plotting the substrate uptake rate (*U*) against the inverse of cell residence timeθ^{-1}, some kinetic parameters including the biomass yield and the endogenous decay coefficient would be obtained (Fig. 4).

The results of this current study were quite different from the previous study conducted by *Darwin et al. (2017)* in which the biomass yield was quite low, and the endogenous decay coefficient obtained was fairly high indicating the short HRT (10 days) and high OLR (3.47 kg VS/m^3.day) applied were not feasible for the process of anaerobic co-digestion of bagasse and digested cow manure. Short HRT and high OLR applied in the previous study also contributed to the drop of pH during the anaerobic digestion process. An acid accumulation and high proton concentration inhibited the growth as well as the activity of the anaerobic microbes, and thereby lower the biomass yield.

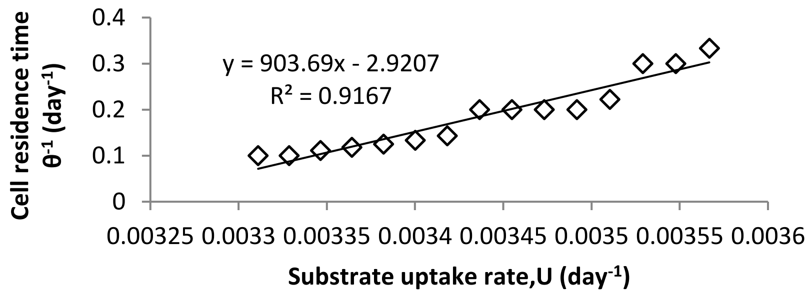

Fig. 4 - Plot for determination of the biomass yield and the endogenous decay coefficient

CONCLUSIONS

High organic loading rate and short HRT applied to the process of anaerobic co-digestion of cocoa husk and digested cow manure did not inhibit the biogas production in which pH was stable at between 6.8 and 7.2 suggesting that acid was not accumulated in the reactor. The production of biogas was stable at around 348.3 mL/day, and the biogas yield obtained was about 55.64 69 mL/gVS added. The kinetic study revealed that the maximum specific growth rate $\mu_{\max}$obtained from the anaerobic co-digestion process of cocoa husk and digested cow manure was quite high at about 6.58 day $^{-1}$. Pre-treatment of cocoa husk would be potential to be applied in order to enhance biogas production as well as its yield.

Results showed that the biomass yield and endogenous decay coefficient obtained from the process of anaerobic co-digestion of cocoa husk and digested cow manure were 903.6 mg/mg and 2.92 day^{-1}, respectively. As the yield of biomass was quite high, and the endogenous decay coefficient obtained was fairly low, this suggested that anaerobic microbes still could grow during the anaerobic digestion. This indicated that high OLR and short HRT applied to the anaerobic process did not significantly affect the microbial activity. As depicted in Fig.1, the production of biogas generated from the anaerobic co-digestion of cocoa husk and digested cow manure was still quite high, or about 10 times higher than biogas produced from the anaerobic digestion of cow manure alone.

Further, the reason why the different outcome appeared between the previous and the current studies possibly related to the co-substrate usage. As the current study using cocoa husk as the co-substrate, the hardness of the substrate may lead to the slow hydrolysis process to generate cellulose and release sugar, and thereby acid buildup did not occur during the digestion process. Further, in this current study short HRT and high OLR applied did not significantly affect the pH drop. As pH was stable at 7, the process of anaerobic digestion could run stably, and thereby could optimize biogas production, and enhance the yield of biomass.

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