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Sustainable anaerobic digestion of euphorbiaceae waste for biogas production: Effects of feedstock variation

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Abstract. Anaerobic digestion (AD) refers to the biological process which can convert organic substrates to biogas in the absence of oxygen. The aim of this study was to determine the capability of feedstock to produce biogas and to quantify the biogas yield from different feedstocks. A co-digestion approach was carried out in a continuous stirred tank reactor operated under mesophilic conditions and at a constant organic loading rate of 0.0756 g COD/ L.day, with a hydraulic retention time of 25 days. For comparison, mono-digestion was also included in the experimental work. 2 L working volumes were used throughout the experimental work. The seed culture was obtained from composting as substrate digestion. When the feedstock was added to seeding, the biogas started to emit after three days of retention time. The highest volume of biogas was observed when the seeding volume used for 1000mL. However, the lowest volume of biogas yield was obtained from both co-digestion reactors, with a value of 340 mL. For methane yield, the highest methane production rate was 0.16 L CH_4/mg. The COD with yield was at 8.6% and the lowest was at 0.5%. The highest quantity of methane was obtained from a reactor of Euphorbiaceae peel with added seeding, while the lowest methane yield came from a reactor of Euphorbiaceae stems with added seeding. In this study, sodium bicarbonate ($NaHCO_3$) was used as a buffering solution to correct the pH in the reactor if the reactor condition was found to be in a souring or acidic condition.

Keywords: anaerobic digestion; euphorbiaceae; composting tea; COD; CH_4; sustainable; CSTR

1. Introduction

Anaerobic digestion is a series of biological processes in which microorganisms break down biodegradable material in the absence of oxygen. One of the end products is biogas, which can be used to generate electricity and heat. Biogas can also be processed into renewable natural gas, used for transportation fuel and the slurry can be used as a bio fertilizer while simultaneously treating or

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stabilizing the waste streams. A few types of reactor may be used in the production of biogas; the preferred type being single stage anaerobic digestion. This is because multi-stage reactors are more complicated and too costly to operate. Multi-stage reactors offer separate reactors for hydrolysis and methanogenesis. Anaerobic digestion can be carried out in either batch, semi-continuous, or continuous fermentation systems at psychroplic, mesophilic, or thermophilic temperatures. However, there will be a difference in terms of biodegradation. The VFA concentration will increase in thermophilic conditions because there is a limitation for the substrate to be degraded but in mesophilic conditions complete degradation will take place (Komemoto *et al.* 2009, Montañés *et al.* 2015).

Co-digestion or co-fermentation is a substrate treatment method in which different substrates are mixed and treated together. Implementing the co-digestion process can help improve the reactor or digester performance. By adding the nutrient or microbes, the fermentation performance will increase at the same time, thereby improving the physicochemical parameters of the reactor. Co-digestion is preferred for improving yields of anaerobic digestion due to its numeral benefits (Kwietniewska & Tys 2014). Much is known about the basic metabolism of the different types of anaerobic digestion processes, but little is known about the microbes responsible for these processes. For the growth and survival of the specific groups of microorganisms, several macro and micronutrients are necessary. The rate of biogas production is significantly affected by the addition of enzymes or microbes (Weiland 2010). It is, therefore, assumed that increases in cellulose activity might be beneficial for the biodegradation of algal cell walls.

Organic materials can be used as feedstock such as industrial wastewater, food waste, sewage sludge, and farming waste (biomass) in order to produce biogas (Kamaruddin *et al.* 2015). Biomass refers to any organic matter such as wood, crops, seaweed and animal waste that can be used as an energy source. Biomass is the oldest source of energy because, thousands of years ago the heat from the burning of wood was used to cook with. There are five types of biomass: wood and agriculture, solid waste, landfill gas and biogas, ethanol, and also biodiesel (Kamaruddin *et al.* 2017). However, plants or agricultural waste are also rich in lignocellulose and actually represent the most promising renewable organic feedstock for biogas production, as their production does not compete for food sources and arable land (Sawatdeenarunat *et al.* 2015).

The production of biogas is generated from the conversion of organic substances under anaerobic conditions, i.e., conditions without oxygen. The anaerobic process have been used for centuries. The quantity and quality of biogas production depends on the characteristic and types of the feed materials (Oslaj *et al.* 2010)(Singhal *et al.* 2012). The amount of yield produced from any type of biomass also depends on the C/N ratio, concentration, temperature, and also the pH (Dioha *et al.* 2013). In Malaysia, biogas production was introduced into palm oil production, this sector being vital for the economy of Malaysia. There are many types of biomass which can be used as feedstock to produce biogas such as rice straw, wheat straw, Euphorbiaceae peel, and Euphorbiaceae tubers. Renewable energy from methane-rich biogas can help in reducing greenhouse gases while at the same time slowing down climate change as methane replaces the usage of fossil fuels to generate energy and heat (Jekayinfa, 2013). Of course, the primary energy demand is highly dependent upon the types and price of fuels used as well as the technology utilized in providing the energy to the various sectors which impacts directly or indirectly on energy supply and demand (Tan *et al.* 2013).

Euphorbiaceae (Manihot esculenta) is relatively easy to grow and is one of the fastest-growing staple foods in the world compared to the other plants. Africa is the largest producer of Euphorbiaceae, and accounts for 52.4% of world production. Global production of

Euphorbiaceaeis approximately 262.6 million Mt/annum (Bayitse *et al.* 2014). More than half of the countries producing Euphorbiaceae are located in Africa and 70% of this production depends on imported energy (Okudoh *et al.* 2014). In West African countries, waste such as Euphorbiaceae root tubers and Euphorbiaceae peel are typically dumped in landfills or burned. Only a small percentage of the waste is washed and dried to feed animals (Bayitse *et al.* 2014). In the 21st century, awareness of the importance of renewable energy and energy efficiency to control environmental pollution related to global warming and to reduce dependency on fossil fuels has grown considerably (Hagos *et al.* 2017). Wise use of waste, can, of course, produce a valuable resource. Turning waste into energy sources for the future can also reduce environmental management costs (Panichnumsin *et al.* 2010) while cutting down greenhouse gas emissions and slowing down climate change. However, waste is low in concentrations of nutrients and has a low buffering capacity, which is a known limitation for the formation of biogases, especially methane (Panichnumsin *et al.* 2010). Effluent left over from the anaerobic process can also be used as fertilizer (Okudoh *et al.* 2014). The purpose of this work was to address the feasibility of using Euphorbiaceae waste for the production of biogas by using anaerobic digestion with composting as the seeding.

2. Materials and methods

2.1 Preparation of feedstock for biogas reactor

The raw materials for biogas production included Euphorbiaceae waste was collected from a chip processing factory agricultural wastes: Euphorbiaceae stems and peel. About 100 kg of the Euphorbiaceae waste was isolated from the waste bin and transported to a laboratory for pre-treatment. The collected sample was then washed using tap water, before being dried overnight at 105 ℃ to remove moisture, using a drying oven (IF110BW, Memmert, Germany). The sample was then ground using a heavy duty blender to obtain finer particle sizes (CB-15, Waring Commercial, USA). Then, the ground sample was strained and refrigerated at a temperature above 4℃ and stored until it was used as feedstock for digestion.

2.2 Preparation of feedstock

The feedstocks were prepared in bulk, in a 2L solution. Solutions were prepared using the ratio of 1L:0.25 kg; that is, 1L of distilled water in 250 grams of samples. From this homogenized slurry, samples were taken and fed into each of the digesters at a constant flow rate, Q. The feedstock solutions were then kept at 4℃ prior to their use, as shown in Figure 1.

2.3 Physical and chemical characterization

In this study, determination of the lignocellulosic composition, moisture, total suspended solid (TSS), volatile suspended solid (VSS), temperature (T), pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), volatile fatty acid (VFA), and alkalinity were carried out according to various standards and recommended guidelines established in the literature. The lignocellulosic composition (TAPPI standard- T6), moisture content, TSS (APHA-2540, 2005), VSS (APHA-2540, 2005), VFA (APHA-5560, 2005), BOD5 (APHA-5210B, 2005), COD

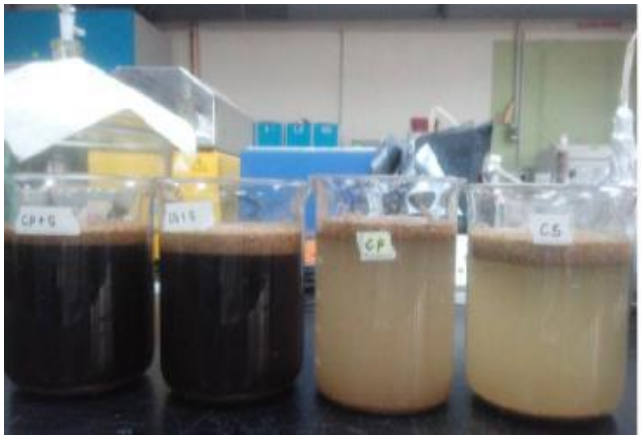


Fig. 1 Feedstocks solutions

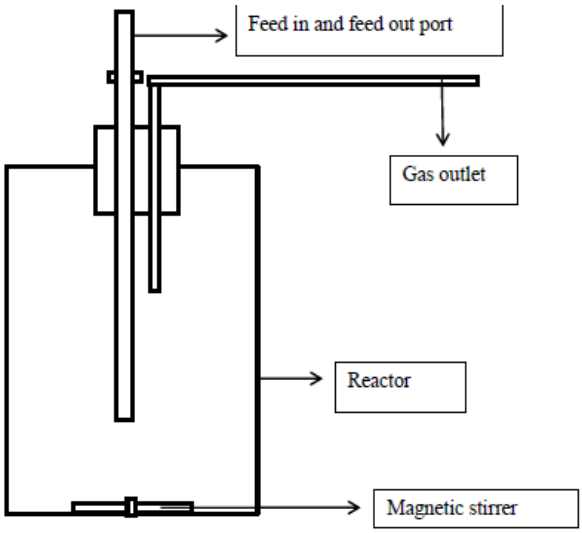


Fig. 2 CSTR schematic layout

(APHA-5220D 2005) and alkalinity (APHA-2320, 2005) were determined using the standard method of the American Public Health Association. A measurement of the pH was taken daily using a laboratory HACH pH meter. The temperature was measured daily using a thermometer. The biogas composition produced was measured using Portable Landfill Gas (GA 2000, Australia).

2.4 Single state continuous stirred tank reactor

The experiment was conducted in a 5.0 L anaerobic digester with a 2L working volume, as shown in Fig. 2. The digester was a white glass rectangle tank (16.5 cm length and 28.5 cm height). The biogas fermentation equipment was composed of two parts: a digester vessel and a gas collector. Gas produced during the digestion flowed through a silicone rubber tube into a 3 L graduated gas bag collector. At the bottom of the reactor, a magnetic stirrer was provided to mix to

the solution. The mixing process was set to 1 minute for every 45 minutes. A peristaltic pump was used to withdraw and feed in the feedstocks. For feeding, port and gas outlets were both located on the top of the reactor (Demirel and Yenigun 2004). Two reactors were used for co-digestion and the seeding used was derived from natural seed cultures produced in laboratories, seeding which was supernatant for composting. The seeding was acclimatized for 30 days before the experiment started. This was to make sure the bacteria could adapt to the new conditions. The volume of biogas produced in the digester was measured using volumetric displacement, via a 1L gas-tight syringe. However, biogas composition was measured using Portable Landfill Gas. The composition and the gas volume were monitored everyday using a gas analyser (GA2000, Australia). A buffer solution; sodium bicarbonate ($NaHCO_3$), was also prepared for pH correction.

3. Results and discussions

3.1 Characterization of Euphorbiaceae

Euphorbiaceae waste was collected from a chip processing factory. For biogas production, Euphorbiaceae stems and Euphorbiaceae peel were chosen because they are the most abundant stocks available. For this study, the physical and chemical composition of Euphorbiaceae waste were determined, as shown in Table 1.

Feedstock characteristics are important because of their possible influence on biogas production. Many types of feedstock have been used in previous studies (Anunputtikul and Rodtong 2004). All types of biomass can be used as substrates for biogas production as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components or are biodegradable. Hemicellulose is a physical barrier which surrounds the cellulose fibre and can protect the cellulose from enzymatic attack (Weiland 2010).

Table 1 Physical and chemical characterization of Euphorbiaceae waste

Chemical composition	Euphorbiaceae peel	Euphorbiaceae stem	Seeding
Moisture content	39.50%	26.89%	ND
Volatile suspended solid, VSS	412 mg/g	425 mg/g	0.36 g/L
Total suspended solid, TSS	404 mg/g	400 mg/g	0.735 g/L
Biochemical oxygen demand, BOD	0.28 mg/L	0.14 mg/L	0.07 mg/L
Chemical oxygen demand, COD	378 mg/L	94 mg/L	50 mg/L
Dissolved oxygen, DO	6.70 mg/L	6.73 mg/L	6.68 mg/L
Lignin	6.30%	24.40%	ND
Hemicellulose	63.80%	32.40%	ND
Cellulose	23.60%	19.90%	ND

*ND= Not determined

Table 3 Correlations of parameters and biogas in CP+S reactor

		pH	COD	TSS	VSS	Alkalinity	Biogas	VFA
pH	Pearson Correlation	1	.515**	.440*	.429*	.448	.687**	.087
	Sig. (2-tailed)		.008	.028	.032	.266	0.000151	.838
	N	25	25	25	25	8	25	8
COD	Pearson Correlation	.515**	1	.343	.348	-.280	.288	.122
	Sig. (2-tailed)	.008		.094	.088	.502	.163	.773
	N	25	25	25	25	8	25	8
TSS	Pearson Correlation	.440*	.343	1	.994**	.026	.284	-.367
	Sig. (2-tailed)	.028	.094		.000	.951	.169	.370
	N	25	25	25	25	8	25	8
VSS	Pearson Correlation	.429*	.348	.994**	1	-.140	.266	-.301
	Sig. (2-tailed)	.032	.088	.000		.741	.200	.469
	N	25	25	25	25	8	25	8
Alkalinity	Pearson Correlation	.448	-.280	.026	-.140	1	.801*	.429
	Sig. (2-tailed)	.266	.502	.951	.741		.017	.289
	N	8	8	8	8	8	8	8
Biogas	Pearson Correlation	.687**	.288	.284	.266	.801*	1	.312
	Sig. (2-tailed)	.000	.163	.169	.200	.017		.451
	N	25	25	25	25	8	25	8
VFA	Pearson Correlation	.087	.122	-.367	-.301	.429	.312	1
	Sig. (2-tailed)	.838	.773	.370	.469	.289	.451	
	N	8	8	8	8	8	8	8

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.01 level (2-tailed)

In this study, the first reactor was filled with Euphorbiaceae peel and seeding was added. The second reactor was filled with Euphorbiaceae stems and seeding was added. The seeding was changed with distilled water. The time to feed in and withdraw the sample was the same for the 25 days retention time and began at 1.00 p.m. The differences between the reactors were examined using an ANOVA in order to determine if the means between the treatments were significantly different. The ANOVA analyses were performed in IBM's SPSS statistical software package.

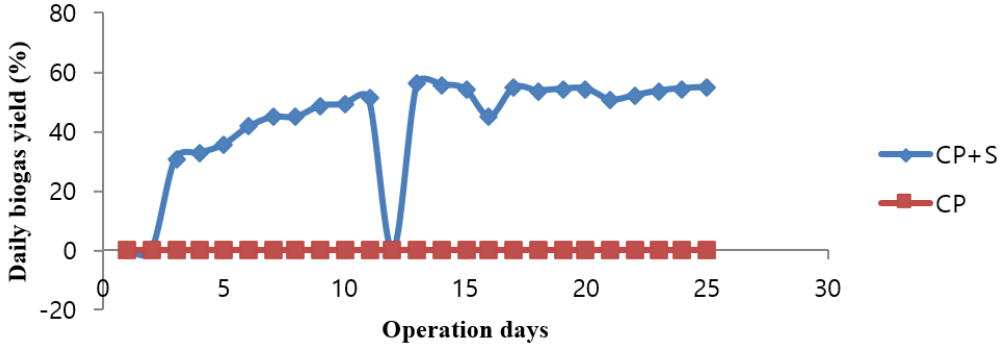
3.2 Biogas yield

In this study, the Euphorbiaceae peel and Euphorbiaceae stem were divided into two parts. The first part was added with seeding (co-digestion) and the other one used as a control (mono-digestion). The control was only mixed with distilled water. The reading was taken from 14 October 2018 until 08 December 2018. Biogas yield was caused by the formation of shorter carbon chains and yeast. Shorter carbon chains are more easily degraded by external enzymes produced by fermentative microorganisms to lower molecular weight molecules so that in the next stage the bacteria would more easily convert these organic materials into biogas.

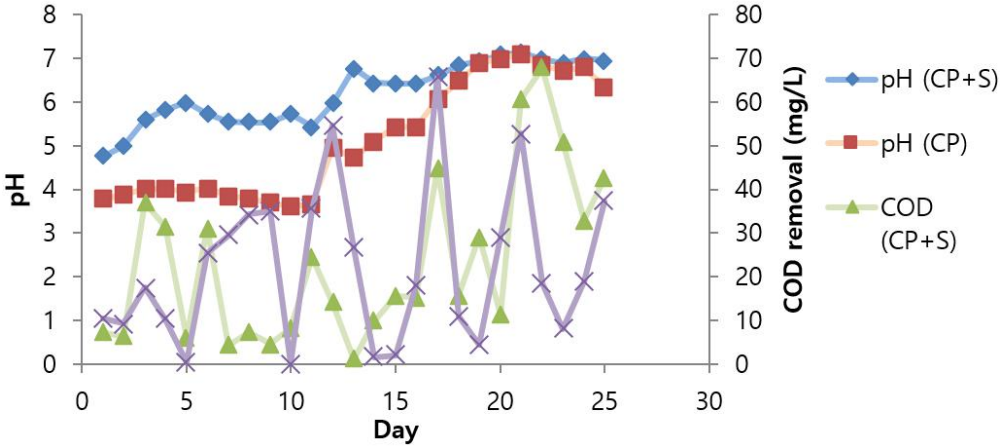
Figs. 3(a), 3(b) and 3(c) show the biogas production from the Euphorbiaceae peels (CP). It is clear from Fig. 3(a) that the biogas yield from reactor CP+S fermentation was high compared to the reactors without added seeding. The biogas was produced as early as day 3 for reactor CP+S. The biogas continued to emit from the third day until day eleven. However, on day twelve, there was no production of biogas from both reactors. For biogas production in reactor CP+S there was a significant drop from 51.2% to 0% production at day twelve. This was probably due to pH value in both reactors not being in the range for biogas production. From Figure 3 (a), it could be observed that the pH value for reactor CP+S was 5.95 and for reactor CP, pH the value was 4.96. Table 3 shows that there was a significant difference ($p < 0.05$) for the pH and biogas yield. The suitable pH range for biogas production is between 6.8 until 7.4, which is not too acidic and not too alkaline (Björnsson *et al.* 2000, Kim *et al.* 2003, Mao *et al.* 2015, Ward *et al.* 2008). To overcome this problem, a buffering solution of sodium bicarbonate ($NaHCO_3$) was employed. The 0.05M $NaHCO_3$ was added until day 15 for both reactors. Just one day after the buffer solution was added, the pH value was increased for reactor CP+S to 6.77. It can be seen in Fig. 4.1(a) that biogas was reproduced at day thirteen. In fact, the biogas produced on day thirteen was the highest within the 25 day retention time, which was 56.1%.

In addition to pH, COD removal was also found to influence the reactor performance. However, from Table 3 it can be seen that the p –value was not significant ($p > 0.05$) and had a low Pearson correlation value of 0.288. At day thirteen, the percentage of COD removal was the lowest: at 1.24 %, showing a high level of pollutant removal. However, by day sixteen, once again the production of biogas decreased significantly: from 54.3 % to 45.1%, with a pH value of 6.43. Acidification of the reactor was connected with alkalinity. Between day 15 and day 18 (Fig. 3(c)), the alkalinity value was high: between 2275 mg $CaCO_3$/L and 3112.5 mg $CaCO_3$/L. Of course, a high alkalinity value may disturb bacteria growth, leading to a decrease in biogas production. Table 3 clearly shows that there was a significant difference between alkalinity and biogas production ($p< 0.05$), with a strong correlation value of 0.801.

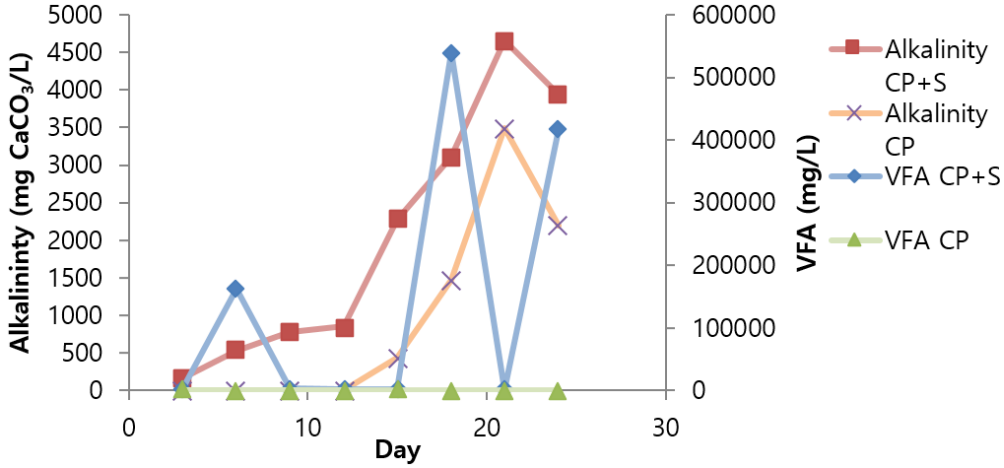
However, for reactors CP the pH value was still within the acidic range, at 4.73, and there was still no biogas produced after the 0.05M $NaHCO_3$ was added. As shown in Fig. 3(a), from day one until day 25, no biogas was produced from the CP reactor. From Fig. 3(b) it is clear that the pH range in reactor CP was present in the biogas production from day 19 until day 22: from 6.89 to 6.83. This was still within the ideal pH range, in spite of fluctuations, but unfortunately, no biogas was observed. Normally, in such a situation, biogas production does not only depend on the pH value, but also on TSS, VSS, COD, alkalinity, and other parameters. However, the first step of AD was assumed not to be fully completed. This step is called the hydrolysis step and is the most important step in AD, because it can be the rate-limiting step. At this stage, the complex compound would be converted to soluble and smaller organic molecules. Also, a simpler bacteria substrate that would be used in the next step of AD is also produced (Deublein and Steinhauser 2011, Maharaj and Elefsiniotis 2001). If the first step failed, the biogas cannot be produced. Since the pH value was still not in the ideal range, 0.1M of $NaHCO_3$ was added at day 16 to buffer the solution. The buffer solution was then added until day 21. After the addition of the $NaHCO_3$ solution, the pH value was slightly increased to 6.6 at day 17, and continued to increase until day 25 for reactor CP+S, even though the buffer solution was only added until day 21. The pH value increased above the normal range starting from day 23 until day 25. In terms of biogas production, the biogas produced slightly increased from day to day from day 17 until day 25, except for day 21. On day 21, the biogas had decreased by 3.7% compared to day 20. From Fig. 3(b), it was observed that COD removal was high for CP+S at day 21 which is 60.66 mg/L. This shows a low



(a) Daily biogas yield for CP+S and CP



(b) Factor affecting biogas yield



(c) Factor affecting biogas yield

Fig. 3 Graph of daily biogas yield for CP+S and CP (a), factors affecting biogas yield (b) and (c)

level of pollutant removal on that day. In addition, the highest level of alkalinity was recorded at day 21, with a value of 4662.5 mg $CaCO_3$/L.

Table 4 Descriptive statistics of parameters and biogas in the CS+S reactor

	Mean	Std. Deviation	N
pH	7.6632	.42697	25
COD	26.5120	23.43364	25
TSS	13.9940	14.67306	25
VSS	13.1070	13.94246	25
Alkalinity	3570.6875	2036.66846	8
VFA	499.6696	743.60989	25
CS_S	29.7160	22.15774	25

Table 5 Correlations of parameters and biogas in the CS+S reactor

		pH	COD	TSS	VSS	Alkalinity	VFA	CS_S
pH	Pearson Correlation	1	.430*	.363	.344	-.269	-.019	.329
	Sig. (2-tailed)		.032	.074	.093	.519	.929	.108
	N	25	25	25	25	8	25	25
COD	Pearson Correlation	.430*	1	-.035	-.044	.047	.230	-.151
	Sig. (2-tailed)	.032		.869	.834	.912	.268	.473
	N	25	25	25	25	8	25	25
TSS	Pearson Correlation	.363	-.035	1	.995**	-.004	-.205	.498*
	Sig. (2-tailed)	.074	.869		.000	.993	.325	.011
	N	25	25	25	25	8	25	25
VSS	Pearson Correlation	.344	-.044	.995**	1	.004	-.223	.480*
	Sig. (2-tailed)	.093	.834	.000		.992	.285	.015
	N	25	25	25	25	8	25	25
Alkalinity	Pearson Correlation	-.269	.047	-.004	.004	1	-.004	.175
	Sig. (2-tailed)	.519	.912	.993	.992		.993	.679
	N	8	8	8	8	8	8	8
VFA	Pearson Correlation	-.019	.230	-.205	-.223	-.004	1	-.153
	Sig. (2-tailed)	.929	.268	.325	.285	.993		.466
	N	25	25	25	25	8	25	25
CS_S	Pearson Correlation	.329	-.151	.498*	.480*	.175	-.153	1
	Sig. (2-tailed)	.108	.473	.011	.015	.679	.466	
	N	25	25	25	25	8	25	25

*. Correlation is significant at the 0.05 level (2-tailed)
**. Correlation is significant at the 0.01 level (2-tailed)

As shown in Fig. 3(c), the VFA produced was directly proportional to the alkalinity value. However, as shown in Table 3, there was no significant difference for alkalinity and VFA value ($p>0.05$) and a low correlation, with the value at 0.429. Also, the correlation for the biogas yield and the VFA value also showed no significant different ($p>0.05$).

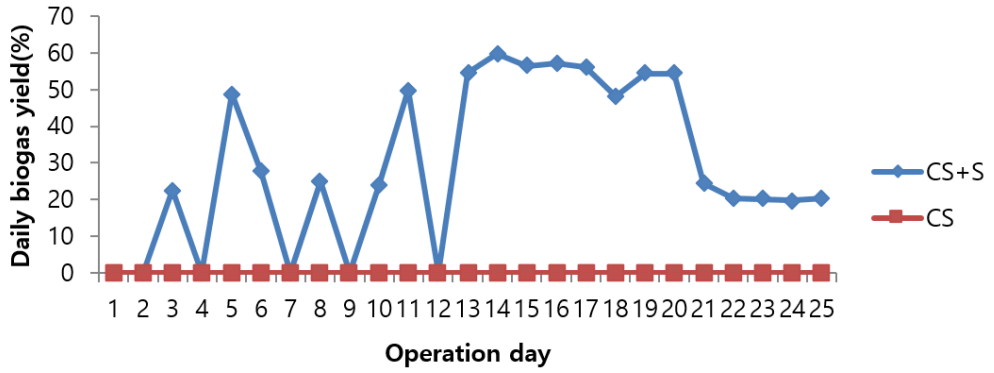
3.3 Biogas yield from Euphorbiaceae stems

Figs. 4(a), 4(b) and 4(c) show the biogas production from the Euphorbiaceae stems. In Fig. 4 (a), the graph shows that the biogas yield from reactor CS+S fermentation was high compared to the CS reactor with no seeding. Biogas was produced in reactor CS+S from day three. In fact, biogas was produced alternately from day six until day ten, drastically falling and increasing during that period. This was due to a few factors which influenced the production of the biogas. Figure 4 (c) shows that the alkalinity value kept increasing, starting from day six until day twelve; from 1925 mg $CaCO_3$/L to 2187.7 mg $CaCO_3$/L. Even though the alkalinity was high, there were a few days in which the CS+S reactor produced biogas: days 6, 8, and 10 day retention time. The pH value on those days was within the ideal range: from 7.06 to 7.37. However, as shown in Table 6, there was no significant difference ($p>0.05$) for both of the parameters, alkalinity and pH. The alkalinity level was not significant, with a weak correlation value of 0.175.

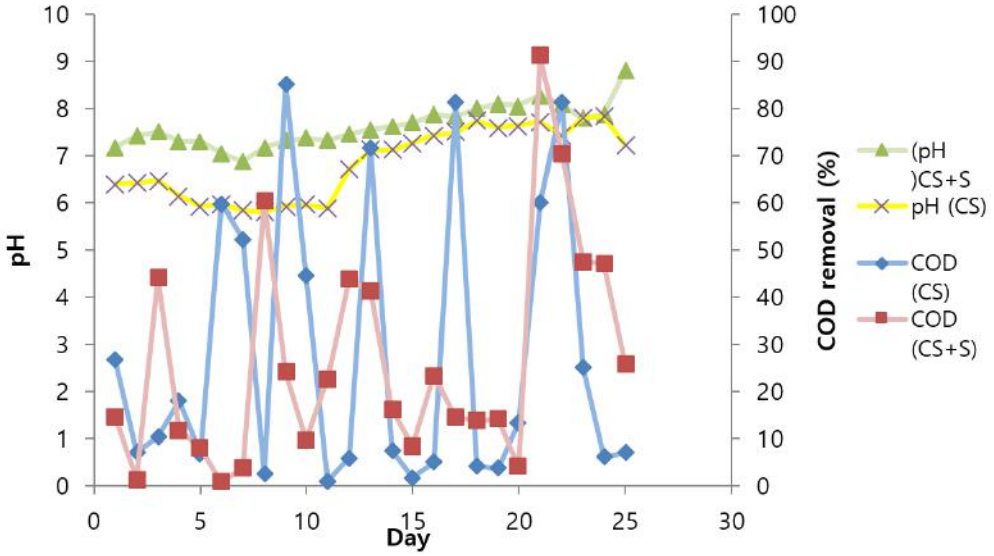
Unfortunately, on day twelve there was no biogas yield in both reactors. As for both reactors, the pH values were within the normal range for biogas production. However, no biogas was detected. Fig. 4(b) shows that the pH value was within the range and the COD removal was high for the CS+S reactor, being 43.85%, which means a low level of pollutant removal. Table 5 illustrates that COD removal and biogas yield was negatively correlated. However, there was no significant difference between the COD removal and the biogas yield. In addition, the alkalinity reading was also high at day twelve, with a value of 2187.5 mg $CaCO_3$/L, showing that the reactor was in a souring condition. The highest amount of biogas was produced was at day fourteen, being 59.8%, while the lowest was at day 24, at 19.8%. Unfortunately, as seen from Table 5, there was no significant difference ($p > 0.05$) between COD and biogas yield.

From day fourteen the biogas produced slightly decreased until day eighteen, and then slightly increased on day nineteen until day twenty before drastically falling at day twenty one, from 54.2% to 24.5% effectively. This decrease in production lasted until day 24. Fig. 4 (b) illustrates that the alkalinity and pH value did not represent ideal conditions and were not suitable for biogas production. The alkalinity reading was in range 3375.0 mg $CaCO_3$/L at day fifteen and 5065.5 mg $CaCO_3$/L at day 21. This showed that the reactor was in a souring condition caused by the decrease in the pH value and that the reactor had become acidic. Reactor acidification through reactor overload is one of the most common reasons for process failure in anaerobic digesters. This occurs because of a build-up of VFAs which are produced by acidogenic and acetogenic bacteria and reflects a kinetic uncoupling between the acid producers and consumers. This condition shows that the reactor was, effectively, filled with fatty acids, with the methane-forming bacteria necessary to convert the VFA to biogas being absent (Franke-Whittle *et al.* 2014). However, the biogas had slightly increased by day 25: from 19.8% to 20.4%. Table 4 shows that the alkalinity was not statistically significant ($p> 0.05$) and negatively correlated with the biogas yield, with a value of -0.314.

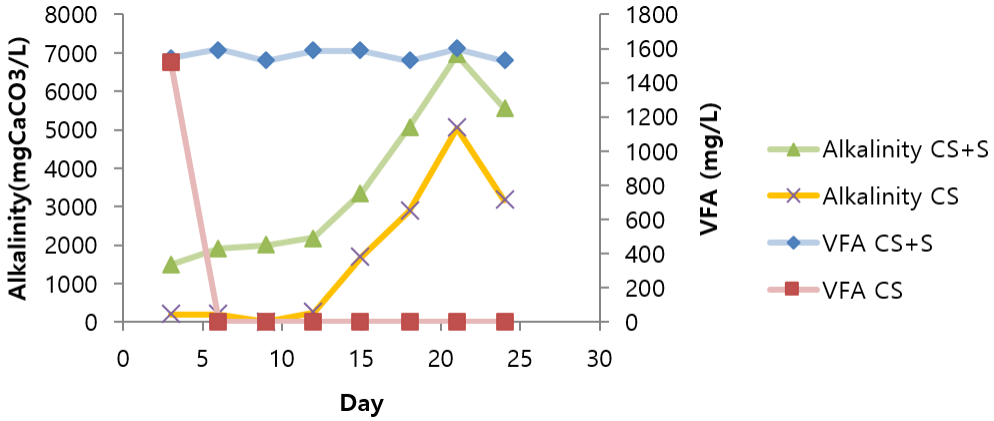
However, for the CS reactor there was no production of biogas from day one of fermentation until day 25 of the retention. Fig. 4(b) shows that the parameters tested exceeded normal readings, especially for the pH reading which was too acidic and too alkaline for biogas production. As stated in the literature, agricultural residue consists of lignocellulose which can slow down the breakdown or degradation of the sample (Amin *et al.* 2017). This can affect the hydrolysis process of breaking down the sample from a complex organic molecule to simpler organic molecules, a step which produces bacteria that will be used in the acidogenesis stage. In addition, agricultural biomass is also low in nutrients which are a source for degrading microorganisms, compared to



(a) Daily biogas yield for CS+S and CS



(b) Factor affecting biogas production



(c) Factor affecting biogas production

Fig. 4 Graph of daily biogas yield for CS+S and CS (a), factors affecting biogas production (b) and (c)

Table 6 Descriptive Statistics of parameters and biogas in CP+S reactor

	Mean	Std. Deviation	N
pH	6.2028	.70757	25
COD	23.2168	18.82212	25
TSS	12.8688	13.12261	25
VSS	11.1104	11.87145	25
Alkalinity	2037.5000	1712.14152	8
VFA	141393.2835	217822.02228	8
Volume	642.8000	302.00055	25
methane	2.6560	2.55181	25

farming biomass. Therefore, biogas cannot be produced for CS fermentation. CS+S fermentation can produce biogas with the help of the seeding. Based on Figure 4 (b), the overall pH in the CS+S reactor was at a higher level compared to the overall pH in the CS reactor. However, to improve the pH value, a buffer solution was added. A buffer solution was also added to the CP+S reactor. This was begun on day twelve and continued until day fifteen using 0.05M of $NaHCO_3$. After adding the buffering solution, the pH continued to increase in both reactors. Although the pH reading was a little acidic, it still produced biogas but this production decreased from day to day: from 59.8% to 56.4%. Because of that, 0.01M of $NaHCO_3$ was added from day 16 until day 21. The biogas still decreased even though the buffering was added. This shows that the Euphorbiaceae stems are less capable of buffering up the pH. From Fig. 4(a), it clear that the alkalinity reading was too high in the CS+S reactor, being at 3375 mg $CaCO_3$/L until 6950 mg $CaCO_3$/L. Alkalinity was a useful tool for an early warning of overloading because it would lead to acidic conditions in a reactor which could harm the acidogenic bacteria.

3.4 Volume of biogas and methane yield

Biogas consists of a mixture of a few gases such as CO_2, O_2, CH_4, H_2, N_2 and H_2S. In this work, the focus was only on CO_2, O_2 and CH_4, using the Gas Analyzer GA2000 model. The remaining gas which was not detected was shown as balance. The 'balance' gases were H_2S, N, CO and NH_3. The quality of the biogas produced could be improved if it contained long-chain hydrocarbon compounds. However, the addition of thelong-chain hydrocarbon compounds cannot be too high in order to avoid acidity (Deublein and Steinhauser 2011).

The methane formation is affected by the acetic acid formation in acidogenesis phase and the volume of biogas produced is affected by the TSS removal. If the acetic acid produced is high, the methane production will increase. However, if the percentage removal of TSS is high, the methane yield will decrease, but the composition of CO_2 will be increased. As discussed above, the biogas production only occurred in CP+S and CS+S reactors. If no biogas was produced in CP and CS reactors, so there will be also no methane produce in those reactors.

3.5 The volume of biogas and biogas yield from Euphorbiaceae peel

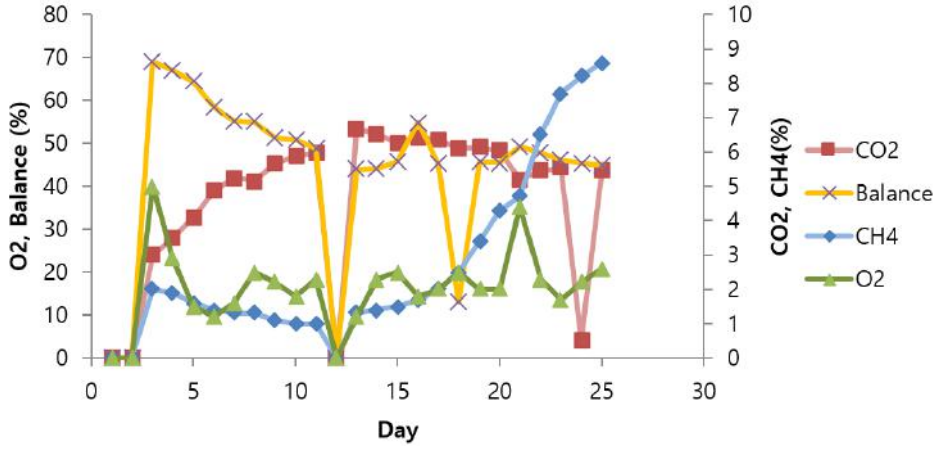
The methane yield and volume of biogas for reactors CP+S and CP was shown in Figs. 5(a)-5(c). It is clear that methane was only detected in the CP+S reactor. This was due to the production of biogas only occurring in the CP+S reactor during the experiment. The trend of methane

Table 7 Correlations of parameters and biogas in CP+S reactor

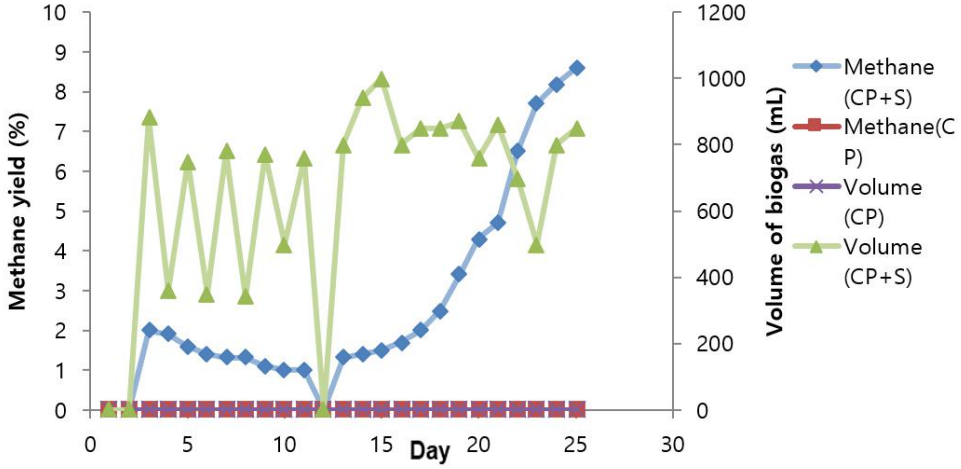
		pH	COD	TSS	VSS	Alkalinityy	VFA	Volume	methane
pH	Pearson Correlation	1	.515**	.440*	.429*	.448	.087	.613**	.725**
	Sig. (2-tailed)		.008	.028	.032	.266	.838	.001	0.000041
	N	25	25	25	25	8	8	25	25
COD	Pearson Correlation	.515**	1	.343	.348	-.280	.122	.218	.689**
	Sig. (2-tailed)	.008		.094	.088	.502	.773	.296	0.000141
	N	25	25	25	25	8	8	25	25
TSS	Pearson Correlation	.440*	.343	1	.994**	.026	-.367	.305	.198
	Sig. (2-tailed)	.028	.094		1.5555E-23	.951	.370	.138	.343
	N	25	25	25	25	8	8	25	25
VSS	Pearson Correlation	.429*	.348	.994**	1	-.140	-.301	.271	.198
	Sig. (2-tailed)	.032	.088	1.5555E-23		.741	.469	.191	.344
	N	25	25	25	25	8	8	25	25
Alkalinity	Pearson Correlation	.448	-.280	.026	-.140	1	.429	.418	.321
	Sig. (2-tailed)	.266	.502	.951	.741		.289	.303	.439
	N	8	8	8	8	8	8	8	8
VFA	Pearson Correlation	.087	.122	-.367	-.301	.429	1	-.292	-.024
	Sig. (2-tailed)	.838	.773	.370	.469	.289		.483	.955
	N	8	8	8	8	8	8	8	8
Volume	Pearson Correlation	.613**	.218	.305	.271	.418	-.292	1	.357
	Sig. (2-tailed)	.001	.296	.138	.191	.303	.483		.080
	N	25	25	25	25	8	8	25	25
methane	Pearson Correlation	.725**	.689**	.198	.198	.321	-.024	.357	1
	Sig. (2-tailed)	0.000041	0.000141	.343	.344	.439	.955	.080	
	N	25	25	25	25	8	8	25	25

*. Correlation is significant at the 0.05 level (2-tailed)

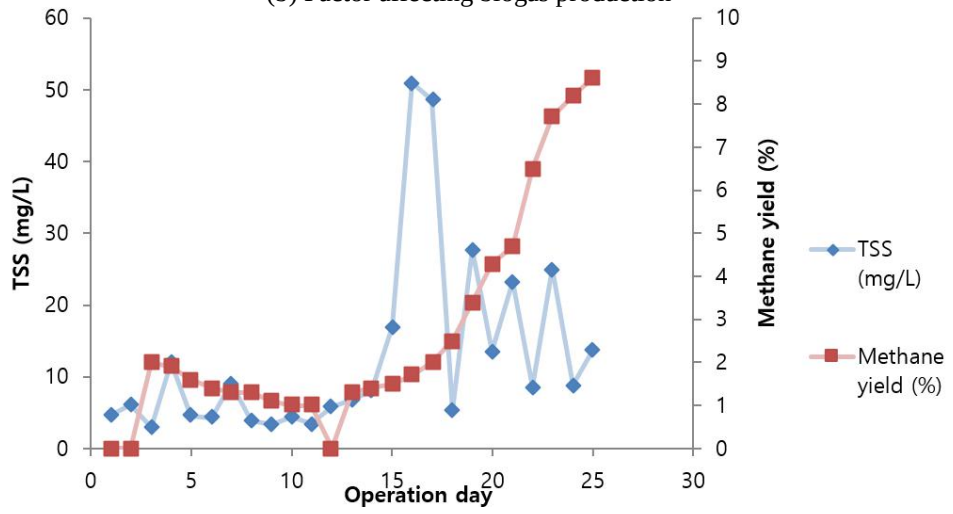
**. Correlation is significant at the 0.01 level (2-tailed)



(a) Daily biogas yield for CS+S and CS



(b) Factor affecting biogas production



(c) Factor affecting biogas production

Fig. 4 Graph of daily biogas yield for CS+S and CS (a), factors affecting biogas production (b), and (c)

produced showed a decrease in the first 11 days of fermentation before a significant reduction on day twelve because there was no biogas produced on that day, followed by an increase on day 13 of fermentation. The highest biogas production was on the final day of fermentation: on day 25 at 8.9 %. However, the lowest amount of methane produced was on days ten and day eleven, at 1.0 %.

From Fig. 5(b) it is clear that the methane yield was inversely proportional to the CO_2 yield. When the methane yield was high, the CO_2 yield was observed to decrease. At day one and day three, there is no methane detected because no biogas was produced. This is because the slurry of the sample was still undergoing the hydrolysis process. The composition of methane yield depends on the type of VFA produced (Wang *et al.* 2009).

In the other hand, the trend of the volume of biogas yield was fluctuating from the first day of the experiment until the final day of the experiment. The highest volume of biogas produced was on day 15 of the experiment, when the biogas volume was at 1000 mL, whereas the lowest volume was recorded on day 8, at 340 mL. From Fig. 5(c) it can be seen that the TSS value was indirectly proportional to the methane yield. On day 15,

The TSS was 16.935 g/L, with a biogas volume of 1000 mL. Due to the lower TSS reading, the CO_2 soluble was higher in the slurry, making the CO_2 composition less in the gas phase. This phenomenon caused better quality methane to be produced because the methane yield concentration was high. The volume of biogas produced in the last three days of the fermentation was from 500 mL to 850 mL. However, the highest methane yield recorded was on day 25 with a value of 8.6 %, while the lowest was on days ten and eleven, at 1.0%.

The methane yield was observed to decrease from day three until day eleven and for CO_2, the gas composition continued to increase. However, no methane gas was detected on day twelve because there was no production of biogas on day twelve. The spike of CO_2 composition gave a signal of a potential souring effect occurring, which might slow down and sometimes even halt the AD process. This can occur when too much waste is added at one time to the digester, or when a highly decomposable waste and the bacterial population became unbalanced. This can cause the acid-forming bacteria to dominate the process, thereby lowering the pH of the reactor, potentially killing off the methanogenic bacteria necessary for methane production. Table 6 illustrates how there was a significant difference ($p < 0.05$) for pH, COD to the methane yield, with high Pearson correlation value.

For the volume of biogas yield, the volume produced was not constant and fluctuated from day to day. The volume of biogas produced depended on the TSS reading. If the TSS was high, so the volume of biogas produced would also be high. This was due to the fact that if the substrate is low, the volume produced would be low because the conversion of the substrate will not occur at a maximum level. However, after day thirteen of fermentation, the methane yield steadily increased day by day until day 25 of the retention day. It was clear that the CO_2 composition was lower than the methane composition. This was due to CO_2 being soluble in the slurry.

4. Conclusions

This study was conducted in a co-digestion environment. The seeding used was derived from the composting process. There were two samples tested, which were Euphorbiaceae peel and Euphorbiaceae stems. Each sample was divided into two parts: co-digestion and mono-digestion. From the results and analysis, biogas was produced was from both sets of co-digestion fermentation: from both the CP+S and the CS+S reactors. In general, agricultural residues consist

of a lignocellulose layer which could slow down the degradation process, due to the lignin, cellulose, and hemicellulose present in the waste. This can contribute the failure of the reactor to produce biogas. The highest volume of biogas was observed when using the seeding volume, at 1000mL. However, the lowest volume of biogas yield was from both co-digestion reactors, with value of 340 mL. For methane yield, the highest methane production rate was 0.16 L CH_4/mg. The COD with yield was at 8.6%, with the lowest at 0.5%. The highest level of methane was from the reactor of Euphorbiaceae peel with added seeding, while the lowest methane yield was from the reactor of Euphorbiaceae stems with added seeding. In this study, sodium bicarbonate ($NaHCO_3$) was used as a buffering solution to correct the pH in the reactor if the reactor condition was in a souring or acidic condition. The laboratory work has shown promising results as potential recovery of Euphorbiaceae waste from being disposed in the landfill, instead it could be diverted and valorised for potential biogas utilisation but further work is needed to optimize the seed and Euphorbiaceae stem as to meet standard biogas minimum requirement for large scale application.

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