

Lab-Scale Anaerobic Co-Digestion of Manure with Wastewater from Toddy Palm Process for Increased Biogas Production

J. Intanin, K. Hussaro^{*} and S. Teekasap

Abstract— Anaerobic digestion processes have been applied for the management of organic wastes, agricultural residues, and animal manure. In this study, a pilot-scale system, consisting of an anaerobic digester and biogas collection. The experimental codigestion of biogas production was fed with manure and wastewater of toddy palm process. The processes in this research were design and set up biogas production system, the fermentation experiment to find the optimum condition. The monitoring included the determination of quality and quantity of input feedstock, biogas volume, biogas composition (CH₄, CO₂, H₂S), biogas yield, and energy production. Bio-methane potential (BMP) test, which biogas production that operated in 4 liters of digester for 40 days with pH and mesophilic temperature control. The materials mixed in 5 different ratios of manure: wastewater of toddy palm process as follows; 1:0 (D1, Digester 1), 0:1 (D2, Digester 2), 1:1 (D3, Digester 3), 2:1 (D4, Digester 4) and 3:1 (D5, Digester 5). The result was found that the ratio of manure: waste water of toddy palm process as 3:1 (D5, Digester 5) generate the highest volume of biogas with the methane concentration 65% by volume. This co-digestion system provides a good alternative for rural wastewater treatment, which has the potential to become a sustainable and green process.

Keywords-Manure, waste water, toddy palm process, renewable energy, biogas production.

1. INTRODUCTION

Wastewater of toddy palm is a byproduct during toddy palm process, which there is collectively called WWTP and must be treated prior to disposal for environmental protection. Large amounts of organics in wastewater from toddy palm process, which are obtained high carbon content. One potential method, the wastewater be can converted to fuel for renewable energy. In addition, growing concerns about energy security, environmental impacts and increasing energy cost for wastewater treatment have reinstated the anaerobic digestion process as a major renewable energy production technology to the center of the scientific spotlight [1]. However, using anaerobic co-digestion to feeding wastewater from toddy palm process with other organic waste materials is to enhance both biogas production and wastewater treatment process.

Anaerobic digestion (AD) is a complex bioconversion process that can produce abundant benefits for treating organic wastes, such as recovering energy in the form of biogas, producing organic fertilizer, and controlling greenhouse gas emission, which reported by other research [2]. The AD, for the biogas production, is considered as a key technology for the sustainable use of agricultural biomass or residues as a renewable energy source and able to meet the growing energy needs [3]. Biogas mainly contains of generally 60-70 %, CH₄ 30-40 % CO₂, and low amounts of other trace gases. The mechanism of anaerobic digestion within four steps including hydrolysis, acidogenesis, acetogenesis, and methanogenesis, as shown in Fig. 1. For the hydrolysis, organics substrate is consisting of complex materials, which were lipids, polysaccharides, proteins, and nucleic acids are converted to simple organics (soluble compounds) including fatty acids, monosaccharides, amino acids, purines, and pyrimidines. Then, in the fermentation, acetate, hydrogen (H₂), carbon dioxide (CO₂), formate, methanol, methylamines, propionate, and butyrate are produced by acidogenesis. In the next mechanism, the acetogenesis are obtained to acetate, hydrogen (H_2) , and carbon dioxide (CO_2) . At the last stage, methane (CH₄) is produced by two groups of methanogens; (i) acetoclastic (acetate consumer) methanogens split acetate into methane and carbon dioxide; while, (ii) hydrogen-utilizing methanogens are responsible for methane production using CO₂ and hydrogen as electron acceptor and donor, respectively, as shown in reactions (1) - (6) [4].

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \tag{1}$$

 $4\text{HCOO}^{-} + 4\text{H}^{+} \rightarrow \text{CH}_{4} + 3\text{CO}_{2} + 2\text{H}_{2}\text{O}$ (2)

$$4\text{CO} + 2\text{H}_2\text{O} \rightarrow \text{CH}_4 + 3\text{CO}_2 \tag{3}$$

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \tag{4}$$

 $4(CH_3)_3N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3$ (5)

$$CH_3COOH \rightarrow CH_4 + CO_2$$
 (6)

J. Intanin is with the Rattanakosin College for Sustainable Energy and Environment, Rajamangala University Technology Rattanakosin, Salaya, Phutthamonthon, Nakhon Pathom 73170, Thailand.

K. Hussaro is with Rajamangala University Technology Rattanakosin, Salaya, Phutthamonthon, Nakhon Pathom 73170, Thailand.

S. Teekasap is with the Thonburi University, Thayaburi, 248 Phet Kas, Khwaeng Nong Khang Phlu, Khet Nong Khaem, Bangkok, 10160, Thailand.

^{*}Corresponding author: K. Hussaro; Phone: +66-84-757-7653; Fax: +66-2-441-6065; E-mail: <u>hussarokanokorn@gmail.com</u> or kanokorn.hus@rmutr.ac.th.

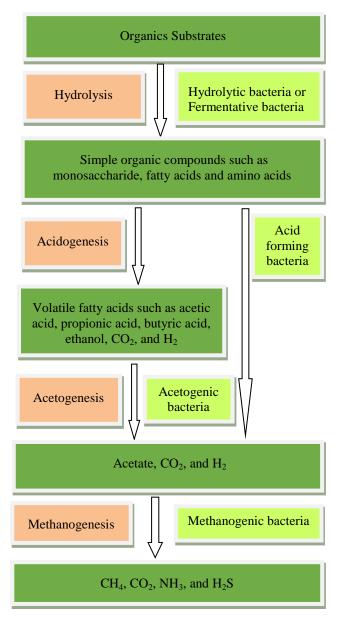


Fig.1. Mechanism of Anaerobic Digestion.

However, the several of bacteria participating in the AD have different optimum pH ranges to collectively ensure efficient digestion and gas production. The process of acidogenesis and methanogenesis require different pH for optimal process control. Acidogenic bacteria are less sensitive and only require pH above 5, while methanogenic bacteria are extremely sensitive and only make well in a pH range of 6.5-7.2. Thus, the optimal pH rage is 6.8-7.4 where both the bacteria can coexist [5]. In normally, the hydrolysis and acidogenesis are facultative and obligate anaerobic digestion bacteria, which isolation of several bacterial kinds such as Clostridium, Peptococcus, Actinomyces, Lactobacillus, Bifidobacterium, Corynebacterium, Desulphovibrio, Staphylococcus, and Escherichia, as reported in the other hand. While, methane production is conducted by two groups of methanogens; the community composition of methanogens is like microbial consortium of ruminant animal's stomach. The main microorganisms which

serve for methane production in the AD are including Mthanobacterium, Methanobacillus, Methanococcus, Methanothrix, and Methanosarcina [6].

It was well known; the anaerobic co-digestion of two or more types of substrates could improve buffer capacity and reduce ammonia inhibition of mixture liquid due to better carbon and nutrient balance. In the other hand, anaerobic co-digestion of many biomass with manure (cow manure, pig manure, and chicken manure) had been widely studied and received better results [7, 8]. Cow manure is characterized by a high buffer capacity and contains a wide variety of micro and macro organisms, and low organic matter content [9]. Several positive experiments have been presented about codigestion of manure with complementary substrates as food wastes, vegetable, and other biomass [8]. Moreover, the compositions of the substrates are important for achieving a stable degradation process. A carbon to nitrogen ratio (C/N Ratios) that is too low can lead to high ammonia levels that inhibit the production of bio methane, especially at high process temperatures. As, an improvement aspect of this research, the results of binary mixtures anaerobic co-digestion using cow manure (CM), and wastewater from toddy palm process (WWTP) with different ratios are shown. The binary mixtures analyzed have been designed based on the C/N ratio.

The purpose of this research was to evaluate the effect of adding wastewater from toddy palm process (WWTP) and cow manure (CM) with different ratio of feeding (CM : WWTP = 1:0, 0:1, 1:1, 2:1, and 3:1) for anaerobic co-digestion (at mesophilic condition) on the biogas production. The experiment includes the analysis of the effect of mono and binary mixtures on biogas production in terms of biogas volume and methane content, which including the pH and temperature of digestion.

2. MATERIALS AND METHODS

Experimental design and set-up

The substrates consisted of cow manure (CM) and wastewater from toddy palm process (WWTP). Wet cow manure was derived from a dairy farm near the Wang Toddy Palm Community Enterprise Group, Phetchaburi Province, Thailand during February 2017, that used as its primary source of raw material for feeding. While, wastewater from toddy palm process (WWTP) was obtained in the fresh produce sugar toddy palm from the Wang Toddy Palm Community Enterprise Group, Phetchaburi Province, Thailand during February 2017, as added to CM for binary mixture anaerobic co-digestion. All substrates were shown in Fig. 2.



Fig.2. Photo of Cow Manure and Wastewater from Toddy Palm Process.

A lab-scale experiment was bio-methane potential (BMP) tests, which was fabricated using digesters. there was operated into 4 liters batch digester at mesophilic condition for 40 days with pH control. CM with WWTP was fed into 4 liters batch digester, which were loaded in 5 different ratios of CM: WWTP as follows; 1:0 (D1, Digester 1), 0:1 (D2, Digester 2), 1:1 (D3, Digester 3), 2:1 (D4, Digester 4) and 3:1 (D5, Digester 5). The volume of the biogas produced was measured by water displacement method which considers that the volume of generated gas equals of the expelled water in the water collector. The schematic of lab-scale for anaerobic co-digestion was presented in Fig. 3. Digesters were maintained by feeding 3 liters of mono and binary substrates, which depended on substrates ratio.



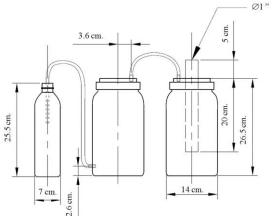


Fig.3. The Schematic of Lab-scale (BMP tests) for Anaerobic Co-digestion.

Analytical methods

The CM and WWTP substrates were initially characterized by determining carbon to nitrogen ration (C/N ratio) and pH value which C/N ratio measured accorded with the Standard Methods (APHA, 2005) and pH value was measured every day by pH meter, respectively, these results for the BMP samples are shown in Table 1. The amount of gas produced was recorded daily to understand the impact of WWTP on anaerobic co-digestion, which was recorded by water displacement method. The methane (CH₄) content was analyzed using a gas bags which was analyzed by gas chromatography (Gas Chromatograph, GC-TCD14B model, Shimadzu, Japan) provided with a thermoconductivity detector (TCD). Helium was used as carrier

gas at a flow rate of 40 mL/min. The temperature of the TCD detector, the injector port, and the oven were 120 °C, 100 °C, and 70 °C, respectively. The pH and temperature of the substrated mixture in the digesters was measured every day by pH meter and data logger (Amron, ZR-RX25), respectively [8].

Table 1. Characteristics of Cow Manure and Wastewater
from Toddy Palm Process (WWTP) for the BMP
Experiment

	-	
Substrates	Cow Manure (CM)	Wastewater from Toddy Palm Process (WWTP)
pH	7.19	5.3
Total Solid (TS) %	14.59	-
Volatile Solid (VS) %	14.25	-
VS:TS Ratios	0.97	-
C/N Ratios	20.99	47.91

The fermentation was operated for approximately 2 months and the temperature of the experimental was carried out mesophilic condition around 30 - 36 °C. Characteristics of carbon to nitrogen ratios (C/N ratios) of mono and binary substrates used in the experiments can be seen in Table 2.

Table 2. Characteristics of Carbon to Nitrogen Ratio (C/N
ratio) in Cow Manure and Wastewater from Toddy Palm
Process (WWTP) for the BMP Experiment

Substrates	C/N Ratios
D1 (CM : WWTP = 1:0)	10.51
D2 (CM : WWTP = 0:1)	20.59
D3 (CM : WWTP = 1:1)	34.95
D4 (CM : WWTP = 2:1)	32.97
D5 (CM : WWTP = 3:1)	25.85

3. RESULT AND DISCUSSION

Biogas Production under Mesophilic Condittions of Anaerobic Digestion for Lab-scale

The biogas production was presented as the volume of biogas for mono and binary with anaerobic digestion process. Fig. 4 and Table 3 were reported daily biogas production for D1-D5. As it can be seen, the biogas production between mono digestion (D1-D2) and binary digestion (D3-D5) did differ significantly, which the binary digestion had the volume of biogas higher than mono digestion (the volume of biogas of D3-D5 >. D1-D2). From day 1-23, the volume of biogas was increased to represent 3,072 ml, 2,832 ml, 2,700 ml, 2,148 ml, and 1,428 ml of D5, D4, D3, D1, and D2, respectively. After that, the volume of biogas had decreaded to minumun on days 24-40, which there were 96.6 ml, 38.4 ml, 31.2 ml, 15.6, and 10.8 of D5, D4, D3, D1, and D2, respectively.

Moreover, when a substrate at 1:1:0.33 (D5) of CM:WWTP was fed, the cumulate biogas production (volume of biogas) for 40 days showed higher than fed with substrate mixture of 1:1:0.5 (D4), 1:1:1 (D3), 1:0 (D2), and 0:1 (D1), which were 48,364.8 ml, 43,834.8 ml, 39,006 ml, 29,139.6 ml, and 18,445.2 ml, respectively. The comparison of highest volume of biogas, it found that D5 had higher than other digesters with occured at 23^{th} days. The averaged biogas production were 1,209.1 ml, 1,095.9 ml, 975.2 ml, 728.5 ml and 461.1 ml of D5, D4, D3, D1, and D2, respectively.

These results suggest that the addition of WWTP to a system anaerobic co-digestion effect on the biogas production. Due to, binary co-digestion (D5-D3) is stable at the optimum value of C/N ratio in the range 25-32 [4]. Especially, D5 has been reported in 25.85 of the C/N ratio, which was close to the optimum C/N ratio for codigestion, as shown in Table 2. While, the low C/N ratio of mono digesters was 10.51 and 20.59 of D1 and D2, respectively, which have high buffer capacity and during the gigestion process, the concentration of ammonia increased and inhibit the microbial growth of the anaerobic digestion. To allay this issue, they added 33-50 % wastewater from toddy palm process (WWTP) to cow manure (CM); upon its additition, the C/N ratio of the mixture substrates increased to 25.85-32.97. In these results, it was found that D5 was provided the volume of biogas higher than D4 and D3, which were 9.37 % and 19.35 %, respectively.

However, Analysis of variance for the volume of biogas from anaerobic digestion of mono and binary digesters (D1-D5) were presented in Fig. 5, it was found that the highest mean data from all digesters was obtained in D5 including to normal probability plot had linear, which were the clearly results in these experiments.

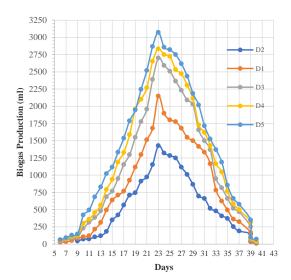


Fig.4. Biogas Production (Volume of Biogas) of D1 (CM:WWTP = 1:0), D2 (CM:WWTP = 0:1), D3 (CM:WWTP = 1:1), D4 (CM:WWTP = 2:1), and D5(CM:WWTP = 3:1).

Table 3. Biogas Production (Volume of Biogas) of D1 (CM : WWTP = 1:0), D2(CM : WWTP = 0:1), D3 (CM : WWTP = 1:1), D4 (CM : WWTP = 2:1), and D5 (CM : WWTP = 3:1)

	Biogas Production (ml)				
Days	D2	Diogas D1	D3	D4	D5
1-5	0	0	0	0	0
6	0	22.8	43.2	52.8	62.4
7	0	33.6	63.6	78.0	91.2
8	0	50.4	81.6	106.8	127.2
9	43.2	82.8	102.0	121.2	144.0
10	70.8	104.4	228.0	300.0	420.0
10	73.2	118.8	313.2	358.8	492.0
12	103.2	213.6	384.0	456.0	684.0
13	118.8	312.0	481.2	562.8	828.0
14	182.4	492.0	684.0	790.8	1020.0
15	348.0	636.0	768.0	936.0	1116.0
16	420.0	708.0	948.0	1188.0	1332.0
17	564.0	768.0	1152.0	1327.2	1536.0
18	708.0	924.0	1296.0	1586.4	1788.0
19	744.0	1116.0	1548.0	1956.0	1944.0
20	912.0	1296.0	1776.0	2100.0	2244.0
21	972.0	1512.0	1956.0	2268.0	2520.0
22	1152.0	1680.0	2388.0	2652.0	2868.0
23	1428.0	2148.0	2700.0	2832.0	3072.0
24	1320.0	1896.0	2592.0	2748.0	2856.0
25	1284.0	1800.0	2508.0	2724.0	2820.0
26	1248.0	1776.0	2364.0	2532.0	2748.0
27	1116.0	1680.0	2232.0	2472.0	2616.0
28	1008.0	1548.0	2088.0	2304.0	2436.0
29	864.0	1500.0	2028.0	2112.0	2184.0
30	696.0	1416.0	1656.0	1728.0	2016.0
31	660.0	1332.0	1500.0	1620.0	1716.0
32	516.0	1164.0	1368.0	1428.0	1524.0
33	480.0	780.0	948.0	1164.0	1368.0
34	408.0	624.0	816.0	1045.2	1188.0
35	370.8	492.0	660.0	768.0	852.0
36	252.0	360.0	516.0	583.2	660.0
37	189.6	324.0	468.0	496.8	576.0
39	147.6	168.0	249.6	315.6	348.0
39	34.8	45.6	68.4	82.8	98.4
40	10.8	15.6	31.2	38.4	69.6
Total	18445.2	29139.6	39006	43834.8	48364.8
Average	461.1	728.5	975.2	1095.9	1209.1

Methane Content under Mesophilic Condittions of Anarobic Digestion for Lab-scale

Methane content of mono and binary digestion from cow manure (CM) and wastewater from toddy palm process (WWTP) was measured from the BMP tests by portable BIOGAS 5000 (Geotech). It was found that the binary substrates (D3-D5) had the highest methane content (CH_4) more than that the mono substrate (D1-D2) at 23th days, which were 65 %, 64 %, 63 %, 61 %, and 53 % of D5, D4, D3, D1, and D2, respectively, as shown in Fig. 6 and Table 4. At the experiments, the CH₄ content of binary substrates was relatively high and the volume of biogas high for binary substrates more than mono substrate, which increased the biogas product for anaerobic co-digestion due to added the rich carbon substrate to digestion. Additionally, the average of methane content were represented 56.2 %, 53.93 %, 51.73 %, 47.6 %, and 43.69 % of D5, D4, D3, D1, and D2, respectively. Moreover, it was found that the range of metane content (for anaerobic digestion with mesophilic temperature in 40 days) were 38-65 %, 36-64 %, 35-63 %, 30-61 %, and 34-53 % of D5, D4, D3, D1, and D2, respectively.

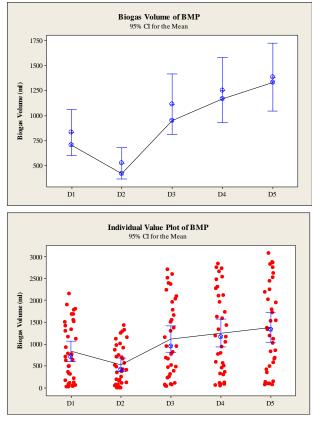


Fig.5. Analysis of Variance for Volume of Biogas from Anaerobic Digestion of All Digesters (D1-D5).

From the analysis of variance for methane content results of D1-D5 were reported in Fig. 7. It was found that the highest mean data from all digesters was obtained in D5 including to normal probability plot had linear, which were the clearly results in these experiments and these results were relevant to the volume of biogas results (as shown in Fig. 5).

	Methane Content (%)				
Days	D5	D4	D3	D2	D1
1-5	0	0	0	0	0
6	38	36	35	30	0
7	39	38	36	33	0
8	44	40	38	36	0
9	45	42	39	37	34
10	46	43	40.5	37	35
11	48	46	43	38	35.5
12	49.5	46.5	44.5	40	36
13	51	48	47	43	39
14	52	49.5	48	44	40.5
15	54	50	48.5	46	43
16	56	52	50	46.5	43.5
17	56.5	53.5	52	48	45
18	58	55.5	54	49.5	46
19	59	57	55.5	51	48
20	58.5	57	56	52	49.5
21	61	59.5	57	52.5	50
22	63	61.5	59	54	51.5
23	65	64	63	61	53
24	64	63.5	62	60	51.5
25	64	63	62	59	51
26	64.5	63	62	59	50
27	64	62	61	58	50
28	63	63	60.5	57	49
29	63	61	59	57	49
30	63	61	58	55	48
31	64	60	56	53.8	48
32	63	60.5	56.5	51	47.5
33	61	59	55	50.5	45
34	61	58.5	55	48	42
35	60	57	53	47	41
36	59	56	52.5	46.5	38
37	56	54	50	44	37.5
38	54	51	49	42.5	36
39	51	49	47	41	33
40	49	47	46	38.5	32
Average	56.2	53.93	51.73	47.61	43.69
Range of CH4 Content	38-65	36-64	35-63	30-61	34-53

Table 4. Methane Content of D1 (CM : WWTP = 1:0), D2 (CM : WWTP = 0:1), D3 (CM : WWTP = 1:1), D4 (CM : WWTP = 2:1), and D5 (CM : WWTP = 3:1)

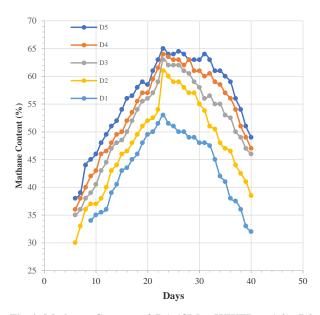


Fig.6. Methane Content of D1 (CM : WWTP = 1:0), D2 (CM : WWTP = 0:1), D3 (CM : WWTP = 1:1), D4 (CM : WWTP = 2:1), and D5 (CM : WWTP = 3:1).

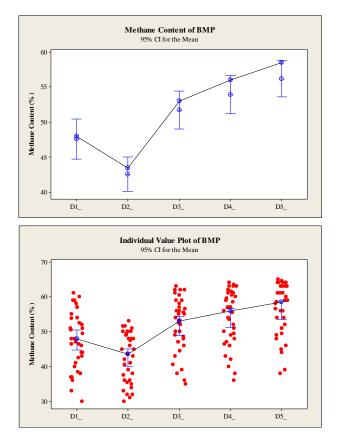


Fig.7. Analysis of Variance for Methane Content from Anaerobic Digestion of All Digesters (D1-D5).

Temperature for Anaerobic Digestion of D1-D5

Temperature of digester is one of the important for anaerobic digestion, which were three range can be consists of psychrophilic (10-29 °C), mesophilic (30-40 °C), and thermophilic (41-55 °C). Microorganisms grow best at temperature ranges of mesophilic and thermophilic. Normally, anaerobic digestion was high biogas production with higher temperature operational, which an increased temperature has a positive effect on the metabolic rate of microorganisms and accelerates the digestion process. But the thermophilic condition is harder to control and needs more energy to maintain the constant temperature of the digester [10]. Therefore, in these studied has been operated at mesophilic temperature. It was found that the average temperature of all digesters was obtained in rage of mesophilic condition, which were 34.4 °C, 33.4 °C, 32.9 °C, 32.7 °C, and 32.6 °C of D5, D4, D3, D1, and D2, respectively. Moreover, the rages of mesophilic for all digesters were obtained between 30.1-36 °C, as shown in Fig. 8 and Table 5. In these results was supported the biogas production and methane content for all experiments.

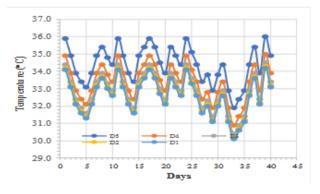


Fig.8. Temperature for Anaerobic Digestion of D1 (CM : WWTP = 1:0), D2 (CM : WWTP = 0:1), D3(CM : WWTP = 1:1), D4 (CM : WWTP = 2:1), and D5(CM : WWTP = 3:1)

The analysis revealed that cow manure and wastewater from toddy palm process is significantly positively correlated with temperature in digester, p-value = 0.002. Since, p-value is lesser than 0.05, which it is concluded that the correlation coefficients are statistically significant at 99% confidence level, which was presented in Fig.9.

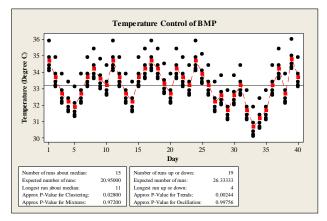


Fig.9. Analysis of Variance for Temperature Control from Anaerobic Digestion of All Digesters (D1-D5).

Table 5. Temperature for Anaerobic Digestion of D1 (CM : WWTP = 1:0), D2(CM : WWTP = 0:1), D3 (CM : WWTP = 1:1), D4 (CM : WWTP = 2:1), and D5 (CM : WWTP = 3:1)

	Temperature of Each Experiment (°C)				(°C)
Days	D5	D4	D3	D1	D2
1	35.9	34.9	34.4	34.2	34.10
2	34.9	33.9	33.4	33.2	33.10
3	33.9	32.9	32.4	32.2	32.10
4	33.4	32.4	31.9	31.7	31.60
5	33.1	32.1	31.6	31.4	31.30
6	33.9	32.9	32.4	32.2	32.10
7	34.9	33.9	33.4	33.2	33.10
8	35.4	34.4	33.9	33.7	33.60
9	34.8	33.8	33.3	33.1	33.00
10	34.4	33.4	32.9	32.7	32.60
11	35.9	34.9	34.4	34.2	34.10
12	34.9	33.9	33.4	33.2	33.10
13	33.9	32.9	32.4	32.2	32.10
14	33.4	32.4	31.9	31.7	31.60
15	34.9	33.9	33.4	33.2	33.10
16	35.4	34.4	33.9	33.7	33.60
17	35.9	34.9	34.4	34.2	34.10
18	35.4	34.4	33.9	33.7	33.60
19	34.5	33.5	33.0	32.8	32.70
20	33.9	32.9	32.4	32.2	32.10
21	35.4	34.4	33.9	33.7	33.60
22	34.9	33.9	33.4	33.2	33.10
23	34.4	33.4	32.9	32.7	32.60
24	35.9	34.9	34.4	34.2	34.10
25	35.1	34.1	33.6	33.4	33.30
26	34.4	33.4	32.9	32.7	32.60
27	33.4	32.4	31.9	31.7	31.60
28	33.8	32.8	32.3	32.1	32.00
29	32.9	31.9	31.4	31.2	31.10
30	33.8	32.8	32.3	32.1	32.00
31	34.4	33.4	32.9	32.7	32.60
32	32.9	31.9	31.4	31.2	31.10
33	31.9	30.9	30.4	30.2	30.10
34	32.4	31.4	30.9	30.7	30.60
35	32.9	31.9	31.4	31.2	31.10
36	34.4	33.4	32.9	32.7	32.60
37	35.4	34.4	33.9	33.7	33.60
38	33.9	32.9	32.4	32.2	32.10
39	36.0	35.0	34.5	34.3	34.20
40	34.9	33.9	33.4	33.2	33.10
Average	34.4	33.4	32.9	32.7	32.6

pH Value from Anaerobic Digestion of All Digesters (*D1-D5*)

Fig. 10 and Table 6 were reported the pH value of mono and binary substrates in anaerobic digestion for all experiments. pH value is one of the key factor in anaerobic digestion process, and the growth of methanogens can be significantly influenced by the pH level. Due to the pH value indicates an activity environment for digester microorganisms. The pH value of mono substrates mixture (D1-D2) was low as 6.1-6.7 and 6.15-6.58 of D1 and D2, respectively, but was raised over 6.2 by mixing cow manure (CM) with the wastewater from toddy palm process (WWTP) at the ratio of 1:1, 2:1, and 3:1. The pH value of binary substrates mixture (D3-D5) was increased as 6.57-7.2, 6.34-7.15, and 6.24-7.1 of D5, D4, and D3, respectively, which was closed to range of optimal pH for anaerobic digestion (about 6.8-7.2) [10]. The observed increase in pH value above indicated that the enhanced biogas production, which seem the positive effect of C/N ratio on biogas production in co-digestion process.

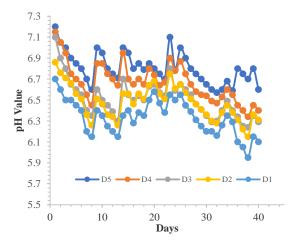


Fig.10. pH Value of D1 (CM : WWTP = 1:0), D2 (CM : WWTP = 0:1), D3 (CM : WWTP = 1:1), D4 (CM : WWTP = 2:1), and D5 (CM : WWTP = 3:1)

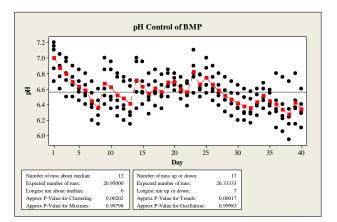


Fig.11. Analysis of Variance for pH Value from Anaerobic Digestion of All Digesters (D1-D5).

Table 6. pH Value of D1 (CM : WWTP = 1:0), D2 (CM : WWTP = 0:1), D3 (CM : WWTP = 1:1), D4 (CM : WWTP = 2:1), and D5 (CM : WWTP = 3:1)

1 7.2 7.15 7.1 6.3 2 7.05 7.05 6.9 6.7 3 7 6.95 6.8 6.7 4 6.9 6.75 6.65 6.6 5 6.85 6.7 6.6 6.5 6 6.8 6.65 6.55 6.4 7 6.7 6.55 6.4 6.3 9 7 6.85 6.6 6.5 9 7 6.85 6.5 6.4 10 6.95 6.85 6.5 6.4 11 6.8 6.75 6.45 6.3 12 6.75 6.7 6.39 6.7 13 6.71 6.65 6.5 6.3 14 7 6.95 6.7 6.5 6.5 15 6.95 6.7 6.54 6.5 6.5 16 6.8 6.65 6.55 6.5 6.5						
D5 D4 D3 D 1 7.2 7.15 7.1 6.3 2 7.05 7.05 6.9 6.7 3 7 6.95 6.8 6.7 4 6.9 6.75 6.65 6.6 5 6.85 6.7 6.6 6.6 6 6.8 6.65 6.55 6.4 7 6.7 6.55 6.4 6.5 9 7 6.85 6.5 6.5 10 6.95 6.7 6.35 6.5 11 6.8 6.65 6.5 6.5 12 6.75 6.7 6.55 6.5 13 6.71 6.55 6.5 6.5 14 7 6.95 6.7 6.54 6.5 14 7 6.95 6.5 6.5 6.5 16 6.8			pH Value			
2 7.05 7.05 6.9 6.7 3 7 6.95 6.8 6.7 4 6.9 6.75 6.65 6.0 5 6.85 6.7 6.6 6.1 6 6.8 6.65 6.55 6.1 7 6.7 6.55 6.4 6.1 9 7 6.85 6.5 6.4 9 7 6.85 6.5 6.1 10 6.95 6.85 6.5 6.1 11 6.8 6.75 6.45 6.3 12 6.75 6.7 6.39 6.3 13 6.71 6.64 6.3 6.3 14 7 6.95 6.7 6.54 6.5 15 6.95 6.7 6.54 6.5 6.5 16 6.8 6.65 6.5 6.5 6.5 18 6.79 6.67 6.54 6.5 <td< td=""><td>D1 D2</td><td>D1</td><td>D3</td><td>D4</td><td>D5</td><td>Days</td></td<>	D1 D2	D1	D3	D4	D5	Days
3 7 6.95 6.8 6.7 4 6.9 6.75 6.65 6.6 5 6.85 6.7 6.6 6.5 6 6.8 6.65 6.55 6.4 7 6.7 6.55 6.4 6.3 9 7 6.85 6.5 6.4 10 6.95 6.85 6.5 6.4 11 6.8 6.75 6.45 6.3 12 6.75 6.7 6.39 6.3 13 6.71 6.64 6.3 6.3 14 7 6.95 6.7 6.5 15 6.95 6.7 6.54 6.5 16 6.8 6.65 6.5 6.4 18 6.79 6.65 6.5 6.4 20 6.8 6.74 6.65 6.6 21 6.75 6.64 6.57 6.5 22 6.7 <t< td=""><td>86 6.7</td><td>6.86</td><td>7.1</td><td>7.15</td><td>7.2</td><td>1</td></t<>	86 6.7	6.86	7.1	7.15	7.2	1
3 7 6.95 6.8 6.7 4 6.9 6.75 6.65 6.6 5 6.85 6.7 6.6 6.5 6 6.8 6.65 6.55 6.4 7 6.7 6.55 6.4 6.3 9 7 6.85 6.5 6.4 10 6.95 6.85 6.5 6.4 11 6.8 6.75 6.45 6.3 12 6.75 6.7 6.39 6.3 13 6.71 6.64 6.3 6.3 14 7 6.95 6.7 6.5 15 6.95 6.7 6.54 6.3 14 7 6.95 6.5 6.5 15 6.95 6.7 6.54 6.5 16 6.8 6.65 6.5 6.5 18 6.79 6.65 6.5 6.5 20 6.8 6.		6.76	6.9		7.05	2
5 6.85 6.7 6.6 6.5 7 6.7 6.55 6.4 6.5 7 6.7 6.55 6.4 6.5 9 7 6.85 6.6 6.5 9 7 6.85 6.6 6.5 10 6.95 6.85 6.5 6.4 11 6.8 6.75 6.45 6.5 12 6.75 6.7 6.39 6.5 13 6.71 6.64 6.3 6.5 14 7 6.95 6.7 6.55 6.5 15 6.95 6.7 6.54 6.5 6.6 17 6.85 6.7 6.54 6.5 6.6 18 6.79 6.65 6.5 6.6 20 6.8 6.74 6.65 6.6 21 6.75 6.67 6.5 6.6		6.71	6.8		7	3
6 6.8 6.65 6.55 6.4 7 6.7 6.55 6.4 6.3 8 6.6 6.45 6.35 6.3 9 7 6.85 6.6 6.3 10 6.95 6.85 6.5 6.4 11 6.8 6.75 6.45 6.3 12 6.75 6.7 6.39 6.3 13 6.71 6.64 6.3 6.3 14 7 6.95 6.7 6.55 6.3 15 6.95 6.7 6.54 6.3 6.4 16 6.8 6.65 6.5 6.4 6.5 6.4 16 6.8 6.65 6.5 6.3 6.4 6.3 6.4 17 6.85 6.7 6.54 6.5 6.4 18 6.79 6.74 6.65 6.5 6.5 20 6.8 6.74 6.65 6.5	66 6.5	6.66	6.65	6.75	6.9	4
7 6.7 6.55 6.4 6.3 8 6.6 6.45 6.35 6.3 9 7 6.85 6.6 6.4 10 6.95 6.85 6.5 6.4 11 6.8 6.75 6.45 6.3 12 6.75 6.7 6.39 6.3 13 6.71 6.64 6.3 6.3 14 7 6.95 6.7 6.3 15 6.95 6.7 6.55 6.3 16 6.8 6.65 6.5 6.3 17 6.85 6.7 6.54 6.3 18 6.79 6.65 6.5 6.3 19 6.85 6.8 6.63 6.6 20 6.8 6.74 6.65 6.5 21 6.75 6.64 6.57 6.3 22 6.7 6.65 6.5 6.3 23 7.1	56 6.45	6.56	6.6	6.7	6.85	5
8 6.6 6.45 6.35 6.7 9 7 6.85 6.6 6.3 10 6.95 6.85 6.5 6.4 11 6.8 6.75 6.45 6.3 12 6.75 6.7 6.39 6.3 13 6.71 6.64 6.3 6.3 14 7 6.95 6.7 6.5 15 6.95 6.7 6.55 6.3 16 6.8 6.65 6.5 6.4 18 6.79 6.65 6.5 6.4 19 6.85 6.8 6.63 6.6 20 6.8 6.74 6.65 6.4 21 6.75 6.64 6.57 6.4 22 6.7 6.67 6.54 6.4 23 7.1 6.9 6.8 6.6 24 6.79 6.75 6.6 6.4 25 7	51 6.4	6.51	6.55	6.65	6.8	6
9 7 6.85 6.6 6.1 10 6.95 6.85 6.5 6.4 11 6.8 6.75 6.45 6.7 12 6.75 6.7 6.39 6.7 13 6.71 6.64 6.3 6.7 14 7 6.95 6.7 6.5 15 6.95 6.7 6.55 6.7 16 6.8 6.65 6.5 6.6 17 6.85 6.7 6.54 6.6 18 6.79 6.65 6.5 6.6 20 6.8 6.74 6.65 6.6 21 6.75 6.64 6.57 6.6 22 6.7 6.67 6.54 6.6 23 7.1 6.9 6.75 6.6 24 6.79 6.75 6.6 6.6 25 7	36 6.2	6.36	6.4	6.55	6.7	7
10 6.95 6.85 6.5 6.4 11 6.8 6.75 6.45 6.5 12 6.75 6.7 6.39 6.5 13 6.71 6.64 6.3 6.5 14 7 6.95 6.7 6.55 6.5 15 6.95 6.7 6.55 6.5 6.5 16 6.8 6.65 6.5 6.5 6.5 18 6.79 6.65 6.5 6.5 19 6.85 6.8 6.63 6.62 20 6.8 6.74 6.65 6.5 21 6.75 6.64 6.57 6.5 22 6.7 6.65 6.62 6.62 23 7.1 6.9 6.75 6.64 24 6.79 6.75 6.64 6.29 25 7 6.87 6.55 6.4	26 6.15	6.26	6.35	6.45	6.6	8
11 6.8 6.75 6.45 6.7 12 6.75 6.7 6.39 6.7 13 6.71 6.64 6.3 6.7 14 7 6.95 6.7 6.55 6.7 15 6.95 6.7 6.55 6.7 6.54 6.7 16 6.8 6.65 6.5 6.7 6.54 6.7 17 6.85 6.7 6.54 6.7 6.54 6.7 18 6.79 6.65 6.5 6.7 6.54 6.65 20 6.8 6.74 6.65 6.6 6.67 21 6.75 6.64 6.57 6.65 6.65 23 7.1 6.9 6.75 6.6 6.65 24 6.79 6.75 6.6 6.65 6.55 28 6.75 6.41 6.4 6.29 6.7 <	51 6.4	6.51	6.6	6.85	7	9
12 6.75 6.7 6.39 6.7 13 6.71 6.64 6.3 6.7 14 7 6.95 6.7 6.55 6.7 15 6.95 6.7 6.55 6.7 6.55 6.7 16 6.8 6.65 6.5 6.7 6.54 6.7 18 6.79 6.65 6.5 6.7 6.54 6.7 19 6.85 6.8 6.63 6.64 6.57 6.54 20 6.8 6.74 6.65 6.64 6.57 6.64 21 6.75 6.64 6.57 6.64 6.59 6.67 22 6.7 6.78 6.59 6.67 6.44 6.64 23 7.1 6.9 6.75 6.66 6.64 6.29 6.64 6.29 6.64 6.29 6.64 6.29 6.64 6.29	46 6.35	6.46	6.5	6.85	6.95	10
13 6.71 6.64 6.3 6.7 14 7 6.95 6.7 6.5 6.7 15 6.95 6.7 6.55 6.7 16 6.8 6.65 6.5 6.4 17 6.85 6.7 6.54 6.4 18 6.79 6.65 6.5 6.4 19 6.85 6.8 6.63 6.64 20 6.8 6.74 6.65 6.64 21 6.75 6.64 6.57 6.4 22 6.7 6.67 6.54 6.63 23 7.1 6.9 6.75 6.6 24 6.79 6.78 6.59 6.6 25 7 6.87 6.55 6.4 26 6.9 6.75 6.6 6.4 29 6.7 6.55 6.41 6.4 30	36 6.25	6.36	6.45	6.75	6.8	11
14 7 6.95 6.7 6.5 15 6.95 6.7 6.55 6.4 16 6.8 6.65 6.5 6.4 17 6.85 6.7 6.54 6.5 18 6.79 6.65 6.5 6.4 19 6.85 6.8 6.63 6.64 20 6.8 6.74 6.65 6.64 21 6.75 6.64 6.57 6.64 22 6.7 6.67 6.54 6.67 23 7.1 6.9 6.75 6.6 24 6.79 6.78 6.59 6.6 25 7 6.87 6.65 6.5 26 6.9 6.75 6.6 6.4 29 6.7 6.55 6.4 6.2 30 6.65 6.53 6.4 6.2 31 6.6	34 6.2	6.34	6.39	6.7	6.75	12
15 6.95 6.7 6.55 6.7 16 6.8 6.65 6.5 6.4 17 6.85 6.7 6.54 6.4 18 6.79 6.65 6.5 6.4 19 6.85 6.8 6.63 6.6 20 6.8 6.74 6.65 6.6 21 6.75 6.64 6.57 6.4 23 7.1 6.9 6.8 6.7 24 6.79 6.78 6.59 6.6 25 7 6.87 6.65 6.6 26 6.9 6.75 6.6 6.4 28 6.75 6.58 6.46 6.4 29 6.7 6.55 6.41 6.4 30 6.65 6.53 6.4 6.2 31 6.6 6.47 6.3 6.4 33 6.6	26 6.15	6.26	6.3	6.64	6.71	13
16 6.8 6.65 6.5 6.4 17 6.85 6.7 6.54 6.3 18 6.79 6.65 6.5 6.4 19 6.85 6.8 6.63 6.0 20 6.8 6.74 6.65 6.0 21 6.75 6.64 6.57 6.4 22 6.7 6.67 6.54 6.4 23 7.1 6.9 6.8 6.7 24 6.79 6.78 6.59 6.0 25 7 6.87 6.65 6.4 20 6.7 6.55 6.4 6.4 24 6.79 6.75 6.6 6.4 26 6.9 6.75 6.4 6.4 29 6.7 6.55 6.41 6.4 30 6.65 6.53 6.4 6.3 31 6.6 6.49 6.29 6. 32 6.57 <td>56 6.35</td> <td>6.56</td> <td>6.7</td> <td>6.95</td> <td>7</td> <td>14</td>	56 6.35	6.56	6.7	6.95	7	14
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	56 6.4	6.56	6.55	6.7	6.95	15
18 6.79 6.65 6.5 6.5 19 6.85 6.8 6.63 6.6 20 6.8 6.74 6.65 6.6 21 6.75 6.64 6.57 6.6 22 6.7 6.67 6.54 6.5 23 7.1 6.9 6.8 6.7 24 6.79 6.78 6.59 6.6 26 6.9 6.75 6.6 6.6 26 6.9 6.75 6.6 6.6 27 6.8 6.65 6.55 6.4 28 6.75 6.58 6.46 6.4 29 6.7 6.55 6.41 6.4 30 6.65 6.53 6.4 6.3 31 6.6 6.49 6.29 6.7 33 6.6 6.47 6.3 6.3 34 6.68 <td>46 6.28</td> <td>6.46</td> <td>6.5</td> <td>6.65</td> <td>6.8</td> <td>16</td>	46 6.28	6.46	6.5	6.65	6.8	16
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	56 6.38	6.56	6.54	6.7	6.85	17
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	51 6.35	6.51	6.5	6.65	6.79	18
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	64 6.5	6.64	6.63	6.8	6.85	19
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	68 6.58	6.68	6.65	6.74	6.8	20
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	56 6.47	6.56	6.57	6.64	6.75	21
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	51 6.38	6.51	6.54	6.67	6.7	22
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	75 6.55	6.75	6.8	6.9	7.1	23
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	61 6.5	6.61	6.59	6.78	6.79	24
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	65 6.55	6.65	6.65	6.87	7	25
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	57 6.45	6.57	6.6	6.75	6.9	26
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	51 6.39	6.51	6.55	6.65	6.8	27
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	46 6.31	6.46	6.46	6.58	6.75	28
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	41 6.25	6.41	6.41	6.55	6.7	29
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	36 6.2	6.36	6.35	6.54	6.65	30
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$.3 6.2	6.3	6.29	6.49	6.6	31
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	28 6.16	6.28	6.3	6.47	6.57	32
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	38 6.26	6.38	6.4	6.53	6.6	33
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	45 6.35	6.45	6.49	6.6	6.68	34
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	38 6.29	6.38	6.4	6.55	6.59	35
38 6.7 6.34 6.24 6.7 39 6.8 6.45 6.35 6.7 40 6.6 6.4 6.29 6.7 Average 6.81 6.67 6.51 6.4 Range 6.57- 6.34- 6.24- 6.1	31 6.1	6.31	6.34	6.45	6.8	36
39 6.8 6.45 6.35 6.3 40 6.6 6.4 6.29 6.3 Average 6.81 6.67 6.51 6.4 Range of pH 6.57- 6.34- 6.24- 6.1	22 6.05	6.22	6.25	6.4	6.75	37
40 6.6 6.4 6.29 6.3 Average 6.81 6.67 6.51 6.4 Range of pH 6.57- 6.34- 6.24- 6.1	15 5.95	6.15	6.24	6.34	6.7	38
Average 6.81 6.67 6.51 6.4 Range of pH 6.57- 6.34- 6.24- 6.1	36 6.15	6.36	6.35	6.45	6.8	39
Range of pH 6.57- 6.34- 6.24- 6.1	31 6.1	6.31	6.29	6.4	6.6	40
of nH 0.5/- 0.54- 0.24- 0.1	48 6.33	6.48	6.51	6.67	6.81	Average
vale 7.2 7.15 7.1 6.3		6.15- 6.58	6.24- 7.1	6.34- 7.15	6.57- 7.2	of pH

The analysis revealed that the cow manure and wastewater from toddy palm process is significantly positively correlated with pH value in digester, p-value = 0.00017. Since, *p*-value is lesser than 0.05, which it is concluded that the correlation coefficients are statistically significant at 99% confidence level, which was presented in Fig.11.

4. CONCLUSION

Anaerobic co-digestion of wastewater (WWTP) from toddy palm process can be considered promising microorganisms for improving the performance of anaerobic co-digesters and biogas efficiency of cow manure (CM). In this work, the optimal biogas production was obtained 3,072 ml and 65 % of the highest biogas volume and methane content, respectively, which was operated by BMP test on 3:1 of CM : WWTP ratio with mesophilic temperature (about 31.9-36 °C) at 40 days. Anaerobic co-digestion of feedstock between cow manure with wastewater from toddy palm process can be enhance biogas generation, which was added WWTP about 30 % with CM (D5) increased the volume of biogas by 9.37 % and 19.35 % compared to the anaerobic digestion when was added 50 % (D4) and 100 % (D3) of WWTP to CM. The highest and average methane content were increased ranged from 63 - 65 % and 51.73 % - 56.2 % of D3-D5. Therefore, the anaerobic co-digestion of CM with WWTP was successful and prove to be promising alternatives. The advantage of a scale-up system of the next research would be beneficial, better understand the mechanisms of anaerobic co-digestion and to know real environmental at a larger scale of biogas production. A more valuable environment benefit can be operated by more other biomass source in the large scale. Therefore, the different substrates mixture will be further evaluated on scale-up with circulate system.

ACKNOWLEDGMENT

The authors would like to thank the Wang Toddy Palm Community Enterprise Group, Phetchaburi Province for providing animals manure and waste water from toddy palm for collecting some experimental data and for produced biogas. This research has been financially supported by Rajamangala University Technology Rattanakosin, Salaya, Phutthamonthon, Nakhon Pathom 73170, Thailand.

REFERENCES

•

- [1] Maragkaki, A.E., Vasileiadis, I.,Fountoulakis, M., Kyriakou, K. and Manios, T. 2017. Improving biogas production from anaerobic co-digestion of sewage sludge with a thermal dried mixture of food waste, cheese whey and olive mill wastewater. *Waste Management*, accetpted 8 August 2017.
- [2] Li, Y., Zhang, R., He, Y., Zhang, C., Liu, X., Chen, C. and Liu, G. 2014. Anaerobic co-digestion of chicken manure and corn stover in batch and continuously stirred tank reactor (CSTR). *Bioresource Technology* 156: 342-347.

- [3] Kalamaras, S.D. and Kotsopoulos, T.A. 2014. Anaerobic co-digestion of cattle manure and alternative crops for the substitution of maize in South Europe. *Bioresource Technology* 172: 68-75.
- [4] Neshat, S.A., Mohammadi, M., Najafpour, G.D. and Lahijani, p. 2017. Anaerobic co-digestion of animal manure and lignocellulosic residues as a potent approach for sustainable biogas production. *Renewable and Sustainable Energy Reviews* 79: 308-322.
- [5] Kumaran, P., Hephzibah, D., Sivasankari, R., Saifuddin, N. and Shamsuddin, A.H. 2016. A review on industrial scale anaerobic digestion systems deployment in Malaysia: Opportunities and challenges. *Renewable and Sustainable Energy Reviews* 56: 929-940.
- [6] Metcalf, E. 2003. Wastewater engineering, treatment and reuse. New York: McGraw-Hill.

- [7] Wang, M., Sun, X., Li, P., Yin, L., Liu, D., Zhang, Y., Li, W. And Zheng, G. 2014. A novel alternative feeding mode for semi-continuous anaerobic codigestion of food waste with chicken manure. *Bioresource Technology* 164: 309-314.
- [8] Hussaro, K., Intanin, J. and Teekasap, S. 2017. Biogas production from food waste and vegetable waste for the Salaew Temple Community Angthong Provice Thailand. *GMSARN International Journal* 11: 82-89.
- [9] Abalde, A.R., Flotats, X. And Fernández, B. 2017. Optimization of the anarobic co-digestion of pasteurized slaughterhouse waste, pig slurry and glycerine. *Waste Management* 61: 521-528.

[10] Hagos, K., Zong, J., Li, D., Liu, C. and Lu, X. 2017. Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. *Renewable and Sustainable Energy Reviews* 76: 1485-1496.