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Improved of Biogas Production by Anaerobic Co-digestion of Ziziphus Leaves and Cow Manure Wastes

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ABSTRACT

This study aimed to use low cost materials and environmentally friendly approach to product biogas by anaerobic co-digestion from agriculture waste and animal waste and evaluate the cumulative biogas and methane yield at optimal operating condition at different substrates mono and co-digestion. For this purpose, biogas production from anaerobic co-digestion of locally organic wastes such as Ziziphus leaves waste (ZLW) and Cow manure waste (CMW) in laboratory scale batch reactor, the organic wastes (ZL), and (CM) were characterized by Kjeldahl analysis system. The effects of Mass ratio, Dilution water and pH solution treatment value for difference type of agriculture waste on the biogas production were taken into full consideration. The results showed the ultimate accumulative of biogas yield from co-digesting at optimum condition was estimated to be 4090 and 2380 mL/g VS, for Co-digestion (ZL:CM) and Mono-digestion (CM), respectively. A higher rate of methane concentration was observed at mesophilic condition, which was estimated to be 67.64 and 52.60%, for Co-digestion (ZL:CM) and mono-digestion (CM), respectively. It can be concluded that the addition of ZL:CM in Co-digester is more significant in increasing the methane concentration and biogas production compared to a mono-digester (CM). This study was analyzed by using a kinetic ***modified Gompertz model*** for entire digestion process to get the best fits the experimental data.

1. INTRODUCTION

In recently years there has been increasing interest in the problems of the world environments. It was necessary to change some of development approaches that may pose vast, harm to health, and damages to surrounding environment such as the global warming sources, and the use of renewable energy source for power generation has been seriously considered [1]. Energy sources which has grown up with industrialization and overall development of technology [2]. The continuous use this process application leads to rise in CO2 content in the atmosphere that causes global warming. It is necessary to replace fossil fuel with the renewable and clean energy sources such as biomass energy, solar energy geothermal energy, wind energy ocean energy hydroelectric energy etc. [3].

Modern technologies for renewable and clean energy sources were evolution to provide green healthy, and environmentally friendly source of energy based on consistent and replenished source of feed stock [4]. Anaerobic digestion, which is sustainable approach, has gained a lot of attention as a means of biogas production pathogens desolation, and reduction of organic waste disposal [5]. Today, huge attentions have been prevailed about connecting agriculture with bioenergy so as to obtain many important necessities. There are many plants for biogas operate on a confederation of organic industrial waste, manure, and a number of plants where organic household waste is fermented to produce biogas [6].

Ziziphus leaves waste is agriculture lignocellulosic substrate that is widely spread availability in Iraq. Hence, biogas production from Zizphus leaves waste is very attracting because (i) ZLW is agriculture waste, (ii) ZLW is a low-cost raw material, (iii) ZLW is abundantly available every were. Generally, lignocellulosic substrates contain cellulose, hemicellulose and lignin, and Recently in Iraq, the generation of this type undesirable agro-cellulosic biomass wastes is so abundant and so localized that there is insufficient capacity for its natural degradation. However, currently many types of these materials are of no real or tangible economic value although they can be used as a potential source for biogas recovery as a new approach of renewable clean energy source. This waste has a large potential for the production of biogas through anaerobic digestion [7].

Biogas is a renewable gaseous fuel occur from the anaerobic digestion of different variety of feed stocks that originate from agriculture, municipal and industry [8]. It obtained from a monocular feed stock mainly rely upon the feed stock characteristics. Co-digestion involves the mixing of two or more different feed stocks in a suitable proportions to achieve a complementary characteristics of the feed stocks. Many research papers have been published performance of anaerobic digesters using different organic wastes [9]. Raw biogas production of agriculture leaf waste consists primarily of methane (CH_4), carbon dioxide (CO_2), hydrogen sulphide (H_2S), ammonia (NH_3), nitrogen (N_2) and other trace components [10].

2. MATERIALS AND METHOD

Fresh Ziziphus leaves (ZL) were collected from the local garden, and fresh were collected from Middle Technical University. The cow manure was obtained freshly from a local farm in Baghdad, and the cow manure is recognized to be rich in the methanogenic anaerobic bacteria. Therefore, they were selected to inoculate the anaerobic digester alternatively.

2.1 Characterization

2.1.1 Measurements of total solids (TS) and volatile solids (VS)

These tests were implemented in triplicate corresponding to the standard methods as follows:

Sample of substrate and inoculum were 30-50 g dried at 100℃ to 105℃ to drive off the water in the sample. The samples were cooled, weighed, and burned at 550- 600℃ for 1 h to drive off volatile solids in the sample are shown in Table 1.

Table 1. Characteristic of feed stock

Characteristic	Cow manure (CM)	Ziziphus leaves (ZL)
TS%	74.18	93.23
VS%	70.04	85.01
VS/TS	0.94	0.91

2.1.2 Measurement of Carbon/Nitrogen (C/N) ratio by Kjeldahl analysis

Kjeldahl analysis system was performed in three main steps including digestion, distillation, and titration to Measurement of (C/N) ratio as shown in Table 2.

Table 2. The result of C/N ratio for ZLW and CLW

Type of waste	ZLW	CMW
O.M%	68.8	43
O.C%	40	25
N%	2.72	1.12
C/N ratio	14.70	22.32

2.2 Experimental work

The experiment of biogas production from mesophilic Co-digestion of different substrates (Ziziphus leaves, Conocarpus leaves) and cow manure were carried out in batch scale digesters.

The leaves of ZLW was washed repeatedly several times with distilled water to remove dirt, dust and other impurities. Then clean leaves were chopped into small pieces (3.5 cm) and exposed to the sunlight for specific time. These leaves were then dried by the oven at 80℃ (353K) for 1h. The dried leaves were crushed ground and sieved to obtain the desired particles of each substrate (0.3-0.5 mm). After sieving, the resulted particles were kept in a desiccators for further use in biogas production.

The waste of cow manure collected freshly from local farm. The filtration was used by cleaned the sample by gloves hand to remove grass and impurities. The manures were kept in a desiccators for further use in biogas production. Effects of different operating parameters were studied to enhance the biogas production by anaerobic co-digestion from different substrates (ZL) and (CL) as shown in below Table 3.

Table 3. Summary of experimental parameters in co-digestion process

Parameter	Ziziphus Leaves	Digestion time-days
Mass ratio CW: ZLW	50:50 To 0:100	30
Water dilution: mixture	1:1 To 4:1	56
pH chemical treatment	6 -7 -8	56

The experiments were achieved to obtain the optimum mass ratios of substrate **(**ZL**)** to cow manure for biogas production. Different masses ratio as shown in Table 4 of substrate to cow manure were mixed with know value of water in each digester. After adding the mixture liquor, the digester was flushed with N_2 for 15 minutes to remove O_2.

Then each digesters were tightly plugged with valve control. All digesters were placed in water bath to maintain at 37℃ (mesophilic temperature).

Table 4. Different masses ratio of substrate to cow manure

Anaerobic digester Number	Ratios of CM:ZL
AD1	50:50
AD2	60:40
AD3	70:30
AD4	80:20
AD5	*100*:0

During the digestion period, the digesters were daily shaken by hand to obtain the homogeneity of the mixed liquor. The accumulative biogas and methane content were measured using displacement water method and gas analyzer device respectively.

2.3 Biogas measurements

In this study, the produced biogas was measured by three different methods as follows:

- ***Water displacement method,*** was used to determine the volume of biogas from anaerobic digestion process in show fig [3]. The biogas was first passed into tube associated with valve digester in which the gas passes into the cylindrical volume to measure the waterfall yield to the downstream by the gas pressure from the batch digestion.
- ***Gas analyzer,*** is a device that measures the concentration of gases (CH_4, CO_2, O_2) produced by anaerobic digestion. A device consists of display show the results with option selection. condensate separator and key pad control user. Operates based on electrochemical measured principle the oxygen content of the sample gas is a measured with two electrode electrochemical sensor. The electrochemical sensors are based on gas diffusion technology the advantage this technology is that the signal generated is direct proportional and linear to the volume concentration (% or ppm) of the analysis gas components.
- ***Gas chromatography (GC),*** was used to evaluate the major components of the bio gas produced as the byproduct of the anaerobic digestion process.

3. RESUlTS AND DISCUSSIONS

3.1 Effect of mass ratio

The effect of co-digestion (ZL/CM) mass ratio on the daily and accumulative biogas production with the CH_4 concentration amount. The digestion time of the anaerobic process was 30 days. The highest biogas generation for co-digestion of ZL/CM was obtained at reactor AD_1 that was 50:50 ratio higher than other mass ratios, Whereas, the lowest biogas generation was obtained in digester AD_4 where co-digestion ZL/CM contained substrate 80:20, After 30 days of the anaerobic digestion process was observed to be approximately, 4240, 3695, 2375, and 2115 mL/g VS, respectively of the total biogas generation for digesters AD_1,AD_2,AD_3,AD_4 respectively. The highest CH_4 generation for co-digestion of ZL/CM was achieved at reactor AD4 that was 80:20 by ratio concentration 58.03% higher than other mass ratios, Whereas, the lowest CH_4 generation was made in digester AD_2, where co-digestion ZL/CM contained substrate 60:40 by ratio concentration of 45.82% of methane content. Based on the results obtained of methane content and accumulative biogas yield for the co-digestion, it can conclude that the best mass ratio Z:M (80:20) in AD4 appeared a faster generation CH_4 than other digesters was set for the following experiments.

The highest biogas generation for mono-digestion of CM was obtained at reactor AD5 that was 980 mL/g VS. The highest CH_4 generation for mono-digestion of CM was obtained at reactor AD5 that was 100:0 by ratio concentration of 51.08%. However, the results of the biogas indicate that the use of mass ratio enhanced the co-digestion process and anaerobic biodegradation of agriculture feedstock wastes in the order of ZL > CM.

3.2 The effect of water dilution

The effect of co-digestion (ZL/CM) mass ratio on the daily and accumulative biogas production with CH_4 concentration content. The digestion time of the anaerobic process was 56 days. The highest biogas generation for co-digestion of ZL/CM was obtained at 1:2 reactor AD2 that was 4440 mL/g VS higher than other dilution water. Whereas, the lowest biogas generation was obtained in digester AD4 at 1:4 where co-digestion ZL/CM contained substrate approximately 1615 mL/g VS. The highest CH_4 generation for co-digestion of ZL/CM was obtained at 1:2 reactor AD7 by ratio concentration 65.59% higher than other water dilution, Whereas, the lowest CH_4 generation was obtained in digester AD8 at 1:4 where co-digestion ZL/CM contained substrate by ratio concentration 51.20% percentage of methane% of biogas production by gas analyzer concentration device. Based on the results obtained of methane content and accumulative biogas yield for the co-digestion, it can conclude that the best dilution water at ratio Z:M (1:2) in AD_2 appeared a faster generation CH_4 than other digesters was set for the following experiments.

The biogas production of (CM) was observed during 56 days, which present at 1:2 ratio of dilution water in AD5 digester. The highest biogas generation for mono-digestion of CM was obtained at 1:2 reactor AD5 that was 1445 mL/g VS on 56 days at effect of dilution water. The highest CH_4 generation for mono-digestion of CM 100% was obtained at reactor AD_5 by ratio concentration 50.09%.

3.3 The effect of pH solution treatment at optimum

After knowing the best result of mass ratio and dilution water obtained of methane content and accumulative biogas yield for the co-digestion, we will study the optimum condition at different pH solution treatment.

3.3.1 The effect of co-digestion pH solution (ZL/CM) on the biogas yield

Figures 1-2 show the effect of co-digestion (ZL/CM) pH solution at optimum condition on the daily and accumulative biogas production with the CH_4 concentration amount. The digestion time of the anaerobic process was 56 days.

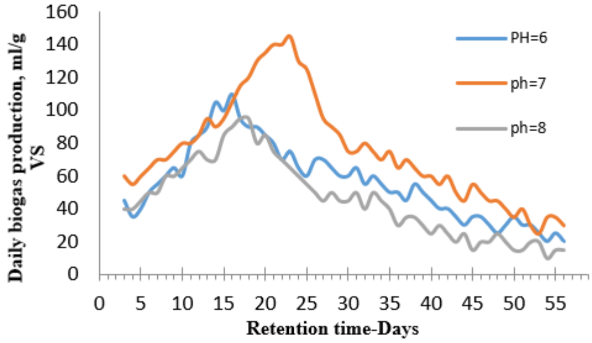

Figure 1. Daily Biogas production Z:M during co-digestion for pH solution at optimum condition

The result can be showed that the daily biogas production increased slightly during the first stages of fermentation-digestion (5 days) for all reactors. Then, with the increase of retention time beyond 5 days up to 20 days, the biogas production daily increased significantly from 7 days to 21 days for all digesters AD_1, AD_2, AD_3 respectively. After 5 days, it rise gradually up to 20 days of retention time, and then it stops and becomes steady. The biogas production sudden change in peak observed at 16-21 days of retention time, due to the formation of excessive quantity of nitrogen during those days, which in turn reduce the C/N ratio at digester.

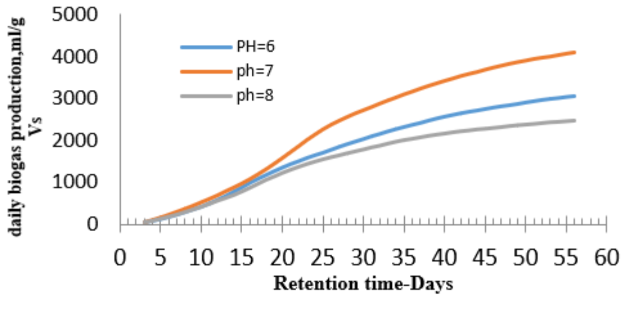

Figure 2. Specific cumulative production Z:M during co-digestion for pH solution ratios at optimum condition

These plots as given higher rate of specific cumulative biogas production at optimum condition founded approximately at natural treatment (pH=7) 4090 ml/g vs. This observation investigated the physical breakdown of substrate structure had a potential effect on the agriculture (lignocelluloses) destruction, and a lower rate of biogas production founded approximately at alkaline solution treatment (pH=8) 2470 ml/g VS.

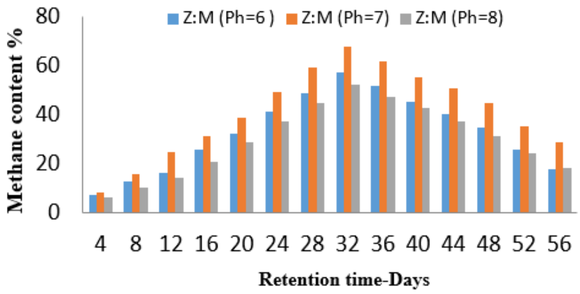

Figure 3. Percentages of CH_4 content during co-digestion for Z:M at a different pH solution ratio at optimum condition

Figure 3 shows the variation of methane content concentration % of biogas generated for co-digestion ZL/CM at different pH ratios in digester AD_1, AD_2, AD_3, respectively.

A maximum methane yield obtain at pH=7 ZL neutral solution in (AD2) of chemical treatment on 56 days by ratio concentration 58.97% at observed on 28 days, after that concentration of methane reduce with digestion time to achieve steady-state with biogas production yield, Also the superiority of neutral solution as a strong chemical reagent compared to alkaline and acid solutions pretreatment of ZL, Due to the dissociation of neutral solution, highly reactive free radicals in particular hydroxyl radicals will form, therefore, the neutral solution has been widely used for removal of organic matter . A lower methane observation at pH=8 (AD3) by ratio concentration of 52.30% of ZL yield.

The results of this section were in good agreement with a previous of reported study by Naseem Khayum et al. (2018) for cumulative biogas generation from anaerobic co-reactor of different masses ratio spent tea waste with animal waste (CM). The cumulative biogas yield production is observed of retention time are found approximately 54.92 to 1669.25 ml/kg and average methane content about 67% [11]. Katukuri et al. (2017) This study transact with the pretreatment of Miscanthus floridulus (a kinds of perennial grass) with natural medium to enhance biogas production. These findings are consistent with the previously reported studies for biogas production from lignocellulosic materials [12]. Ambar Pertiwiningrum et al. (2017), the study to investigate the effect rumen fluid addition on biogas rate and the amount of additional of livestock cow manure and water 1:2 with average volume 450 ml in varied four amounts treatment which is,0,20,30,40% of the total volume slurry the conducted observation for 30 days recorded measurement of biogas volume and methane concentration by GC mass device the result showed the peak of maximum volume of biogas production is carried out on last days (30 day) at a maximum methane concentration (54.88%) for water ratio 1:2 compare with another amount of digesters [13].

3.3.2 The effect of pH solutions to mono-digestion (CM)

The biogas production of (CM) was observed during 56 days as depicted in Figures 4-5, which present by different ratios of pH solutions treatment.

As given in these plots of CM for mono-digestion a higher rate of biogas production founded approximately of acidic solution (pH=6) 2380 ml/g VS, and a lower rate of biogas production established approximately at alkaline solution treatment (pH=8) 1725 ml/g VS, when the neutral solution observed in plots at medium value state between acid and alkaline solutions at value approximately 2115 ml/g VS compared with co- digestion for ZL and CL at a higher rates value of substrate.

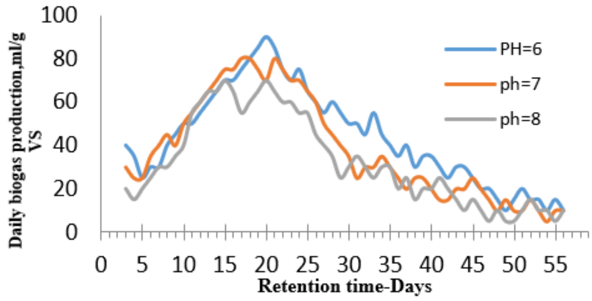

Figure 4. Daily Biogas during mono-digestion for CM 100% at different pH solutions treatment

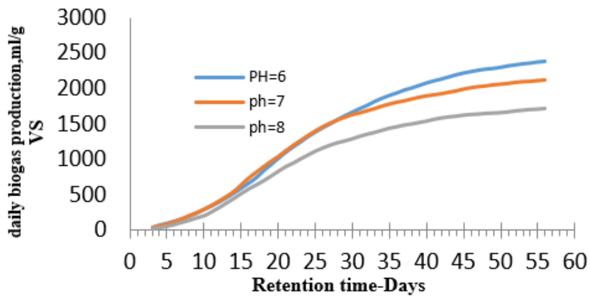

Figure 5. Specific cumulative biogas production mono-digestion for CM 100% at different pH solutions treatment

The variation of methane content concentration % of biogas generated in Figure 6 shows for mono-digestion CM100% at different pH ratios for natural, acid and alkaline solution at condition value state.

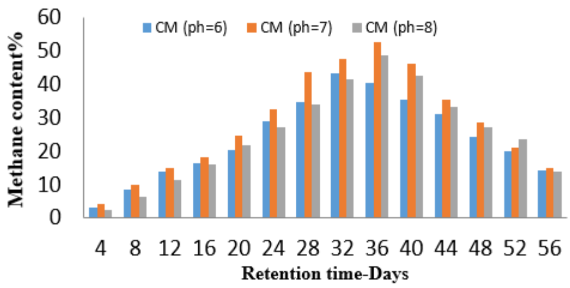

Figure 6. Percentages of CH_4 content during mono-digestion for CM 100% at different pH solution ratio

The highest CH_4 generation for mono-digestion of CM 100% was obtained on 36 days yield by ratio concentration 52.60%, profiles of methane content and biogas production from acid compare with other pH solutions is well observed this could be due to the efficient of separation of animal waste kinds such as fatty acid, amino acids, and sugars ready for conversation of biogas by series of biochemical transformers reaction. Where, the lowest CH_4 generation was obtained in digester AD_8 of pH=7 contained mono-substrate by ratio concentration of 49.26% of CH_4 yield at 56 days.

Table 5. Results of optimum condition at kinetic Gompertz model

Substrate at optimum condition	$G_{(t)}$ exp. ($mL\ CH_4/g$ VS)	Gompertz model parameters				R^2
		λ (day)	$R_{max.}$ ($mL\ CH_4/g$ VS)	G_0 ($mL\ CH_4/g$ VS)	$G_{(t)}$ model ($mL\ CH_4/g$ VS)	
ZL: CM	4090	32.43	126	4090	4025	0.991
CM	2380	27.40	52	2380	2275	0.955

4. KINETIC MODEL

The kinetic model of biogas production rate is identical to the specific growth rate of fermentation methanogenic bacteria in the bio-reactor of batch conditions. Accordingly, the predicted and measured biogas production at optimum condition rate will obey ***Modified Gompertz Model*** as follows:

$G_{(t)}= G_0.\exp\{- \exp [((R_{max}.e)/G_0) (\lambda-t) +1]\}$
where,
$G_{(t)}$ = the cumulative volume of biogas yield at a retention time (mL/g VS)
G_0 = the potential substrate of biogas production (mL/g VS)
R_{max} = maximum average methane production content (mL/g VS-d)
λ = lag phase (day)
t = retention time (day)
e = exp (1) = 2.7183.

A nonlinear regression analysis least-square was carried out using SPSS statistics 25 (2019) to evaluate λ, R_{max}, and the cumulative volume predicted of biogas with methane content as shown in Table 5, at 56 day. The cumulative volume measured and predicted values of biogas production rate are given in Plots in Figures 7, 8. It is well shown that the predicted rates of biogas production using the kinetic modified Gompertz model are good fitted with the measured rates at for agriculture waste and animal waste in co-digestion and mono-digestion at different parameters at optimum condition.

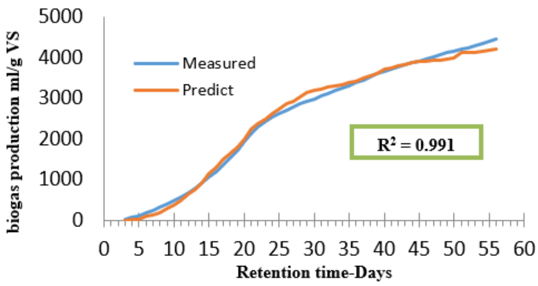

Figure 7. Data as Measured and predicted for Z:M at optimum condition obtained in "Gompertz model"

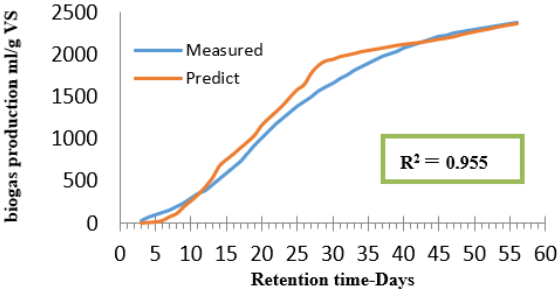

Figure 8. Data as Measured and predicted for CM at optimum condition obtained in "Gompertz model"

Figures 7, 8 describes the experimental data and predicted data obtained at Gompertz model. All constant and correlation coefficients for the Gompertz model are presented in Table 5 the Table and figures show the values of the experimental are good fitted with predicted values by using modified Gompertz model. Hence, the Gompertz model was able to describe the experimental perfectly.

The results of this kinetic are in good agreement with Budiyono et al. [14] reported the measured values of biogas from the reactor of animal waste (cattle manure) in batch mode are good fitted with the predicted values obtained by modified Gompertz model. Kafle et al. [15] proved that the measured values from the bio-reactor of fish waste of biogas produced are good fitted with the predicted data using modified Gompertz model.

5. CONCLUSIONS

This study was evaluated the potential substrate of anaerobic mono-digestion and co-digestion for biogas production using available lignocellulosic agriculture leaves waste feed stock and cow manure waste of no economic value as the substrate. The co-digestion process was investigated using alternatively two types of waste materials which were Zizphus leaves wastes (ZLW), and Cow manure wastes (CMW). The main conclusions that can be drawn from this study are as follows:

- Results revealed that the lignocellulosic agriculture waste materials have the potential for production of biogas in order of ZLW > CMW.
- The experimental set-up (work) explained that the cumulative volume of biogas and methan content are significantly affected by mass ratio, water addition, pH chemical treatment, all of these effecting under temperature (mesophilic) conditions.
- The maximum biogas yield value from co-digester of mass ratio was evaluated to be 2115 and 980 mL/g VS, ZLW and CMW respectively. However, higher rate of methane concentration% production was observed at mesophilic condition which were 58.03 and 51.08 %, for ZLW, and CMW respectively during 30 days. These results indicate the effect of mass ratio on the digestion process.
- The ultimate biogas production from co-digestion of water addition wastes was evaluated to be 4440 and 1445 mL/g VS, whereby, higher rate of methane concentration% production it was 65.59, and 50.93% for ZLW and CMW respectively during 56 days under temperature condition.
- During 56 days observation period, maximum biogas production from chemical PH treatment ZLW and CMW were 4090 and 2380 mL/g VS, respectively at mesophilic temperature condition. However, higher rate of methane

concentration% production was observed at mesophilic condition which were 67.64 and 52.60%, for ZLW and CMW respectively.

- Furthermore, examinations may be performed at various operating conditions (rate of organic load, C/N ratio, etc., and at different digesters (continuous-system and fed-batch digester system), which is not determined in this research),and Investigate the feasibility of biogas production using different type of agriculture lignocellulosic waste materials and different pretreatment method.

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NOMENCLATURE

(ZL)	Ziziphus leaves
(CM)	Cow manure
(C/N)	Carbon C to Nitrogen N_2
(AD)	Anaerobic digester
(TS)	Total solid
(VS)	Volatile solid
(CH_4)	Methane concentration
(CP)	crude protein
(GC)	Gas chromatography
(AD)	Anaerobic digester

APPENDIX

Result of Methane Concentration at optimum condition

Wastes materials	Mass ratios	Water ratio	pH mixture	Methane CH_4 %
(CM: ZL)	80:20	1:2	7	67.64
(CM)	100:0	1:2	6	52.60