



# Anaerobic Co-Digestion of Agricultural Waste with Cow Dung for Biogas Production

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## Abstract

The aim of this study is investigating the methane production through anaerobic co-digestion of rice straw (RS) as one of the famous agricultural residues and cow dung with different total solids. This study is carried out in lab-scale by using batch operation. To evaluate the performance of anaerobic co-digestion process of agricultural residue with cow dung, the experiments of the study were conducted in duplicate or triplicate. The anaerobic co-digestion process was operated in 500 mL batch reactors maintained under Mesophilic conditions (35°C). Rice Straw (RS) produced the maximum value of methane production; methane yield; chemical oxygen demand (COD) removal and VS reduction at 4% total solids (TS).

**Keywords:** Anaerobic co-digestion; Agricultural residue; Cow dung, Methane production.

## 1. Introduction

In many countries, more than 80% of the residents lives in rural areas where more than 90% of the energy being used come from non-commercial sources; the most common is the fuel wood. The increasing cost of conventional fuel in city areas necessitates the searching of other energy sources [1]. Biofuels produced from biomass such as agricultural residue help to reduce both the world's dependence on petrol and CO<sub>2</sub> production. Biofuels can mitigate global warming because the biomass absorbs CO<sub>2</sub> during growth and emits it during combustion. Therefore, biomass helps the atmospheric CO<sub>2</sub> recycling. Therefore, the utilization of biomass resources will be one of the most important factors for environmental protection in the 21st century. Simultaneously, biofuels manufacture beside bio-products can provide new profits and employment opportunity in countries. For the eco-friendly growth of human society and for the abatement of greenhouse gases production, more efficient methods based on renewable technologies has to be used [2].

Rice is internationally the most important food which is consumed every day by at least half of the world's peoples. It presents the most plentiful source of agricultural residues in the world that can be used for the creation of renewable energy.

A common solution for dealing with rice residue is open field burning alongside rice mills, contributing to increased green house gas emissions, including CO<sub>2</sub> [3], which accelerates the increase in atmospheric temperature and can be basis of the global warming phenomenon. Moreover, this air pollutant can negatively affect human being, and cause cancer. Due to the healthy and environmental aspects, many countries have compulsory new regulations preventing field burning activities [4].

Consumption of rice residues for electricity generation has been well developed in many countries due to its wide accessibility at rice grinders. However, this solution can be used only in more developed areas with large rice mills that produce enough rice residues for direct combustion. The chemical composition of a biomass feedstock can also affect its combustion efficiency. High content of (Na and K) and the existence of phosphorus in rice husk and rice straw can decrease the melting temperature of ash. Besides, the low melting temperature of rice residues may lead to fouling and corrosion of the heat transfer surfaces, and the opportunity of agglomeration in a fluidized bed reactor [5].

Another potential utilize of rice biomass for energy creation can be used worldwide, even in the poor countries.

It is the change of rice wastes into the clean-burning fuel through anaerobic digestion. During the anaerobic digestion fermentation, anaerobic and facultative microorganisms convert biomass into biogas, mainly methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) with trace portion of other gases like carbon monoxide (CO) and hydrogen (H<sub>2</sub>), in the absence of oxygen (O<sub>2</sub>). The produced methane can be used as fuel to generate heat and electricity and the digested sludge produced can be used as an organic fertilizer [2, 6, 7].

The main purpose of this research was to examine the appropriateness of rice straw biomass for anaerobic fermentation and to investigate the energy of the digestion of rice straw with cow dung.

## 2. Materials and Methods

### 2.1. Substrate Preparation

Rice straw used for this research, were collected; dried and milled using a laboratory grinder to an average particle size between 1 and 1.5 mm. Cow dung utilized was taken from a livestock farm in Sharkia, Egypt. The foreign materials like stone, wood, metals, straw, and other inorganic materials were manually removed from it.

## 2.2. Equipment

This research was conducted in double or triplicate where three reactors with working volume of 500 mL were loaded with rice straw and cow dung. Other three reactors of 500 mL were control reactors or without rice straw addition. All digesters were put at constant temperature water bath; the temperature for this batch experiments were controlled under 35°C (mesophilic condition).

## 2.3. Experimental Procedure

Batch experiments were run to evaluate and investigate the bio-methane potential from rice straw co-digested with cow dung. Several experiments were carried out to assess potential methane production. In the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> experiment, anaerobic co-digestion processes were run at 2%, 4% and 6% total solids (TS) concentration, respectively. Total solids of cow dung were measured in order to measure the amount of biomass that should be added to each reactor. The mixture of rice straw and cow dung loaded into each digester as an influent was prepared homogeneously. During recording of the biogas production examination, first there was no addition of any other nutrient including enzyme and chemicals in order to assess how much bio-methane generated by substrate added. 500 mL of 0.5 Normal sodium hydroxide (NaOH) solutions were freshly prepared and used as a filter flask, in order to entrap CO<sub>2</sub> and H<sub>2</sub>S. Each filter flask containing NaOH was connected to the system of water displacement for measuring the bio-methane production. NaOH can be used to filter biogas generated from anaerobic fermentation process as it may react with both CO<sub>2</sub> and H<sub>2</sub>S; however, it cannot react with bio-methane. Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) will be generated once the CO<sub>2</sub> reacts with the NaOH (Zhao Q et al., 2010). Before starting anaerobic fermentation process, each fermenter was purged with nitrogen (N<sub>2</sub>) gas for around 5 minutes to remove oxygen (O<sub>2</sub>) traces, and ensure anaerobic condition in the digester. To prevent any gas leakage due to high pressure in the digester and to ensure strict anaerobic condition, each reactor and filter flask used were sealed correctly using parafilm.

## 2.4. Analytical Methods

The end of the experiment was taken by the point at which bio-methane production stopped completely. Characterization of materials used including organic matter (OM), moisture content (MC), carbon (C) and nitrogen (N<sub>2</sub>) content of each substrate, pH, total kjeldahl nitrogen (TKN), total organic carbon, chemical oxygen demand (COD), total solids (TS), and volatile solids (VS). All analysis was run based on the "Standard Methods" [8]. Biogas production rates were measured and recorded as bio-methane produced (mL) per day, and the methane yield was calculated based on the cumulative bio-methane produced per gram VS loaded [9, 10]. The organic load of the waste usually can be known by adding the amount of organic biomass (solids) mixed in the culture [11]. To analyze the effectiveness of the treatment, some parameters measured include the percent of COD reduction and the percent of VS removal [12].

## 3. Results and Discussion

### 3.1. Performance Evaluation at Different Solid Concentrations

The study intended to evaluate methane production potential of rice straw co-digested with cow dung through several different total solids concentrations including 2%, 4% and 6% TS. The anaerobic digestion process was run at the steady state condition where the temperature of the process was conducted under mesophilic condition (35°C). The physical-chemical characteristics of substrates loaded are shown in Table 1. Analysis of rice straw contain 73.82% organic matter, 37.42% carbon, 83.4% volatile solids, and 1 965.4 mg/L COD.

Table-1. Analysis of Rice straw

Parameters	Unit	Rice straw
Total solids	%	92.4
Volatile solids	%	83.4
Moisture content	%	7.6
Organic matter	%	73.82
Carbon content	%	37.42
Nitrogen content	%	0.75
Chemical oxygen demand (COD)	mg/L	1 965.4
C:N ratio	-	50.00

The rice straw analysis shown in Table 1 point to the great quantity of organic matter in rice straw, which enable it for the anaerobic co-digestion with cow dung to generate higher bio-methane generation. It has been known that central parameters that affect on the anaerobic fermentation process to produce bio-methane generation include (VS) and (TS). In addition, TS was used to determine whether the digester volume large enough for digestion of substrates, and VS possibly measured as an indicator of organic load transfer into biogas including methane [13]. Moreover, the bio-methane yield perhaps appreciably improved by increasing volatile solids of substrates feeded [14]. It is discovered that by applying agricultural wastes as co-biomass in biogas plants, it will extensively increase the bio-methane generation of a cow dung utility. Though the VS of substrates added is considered as a pointer of bio-methane production, the methane yield based on the VS is not specific. This occurs as there is any disturbance in the VS composition which contain both degradable organic (carbohydrates, lipids, and proteins) and non-

biodegradable organics such as lignocellulosic materials. Consequently, it possibly known that all VS of organic matter are not all the time the same; this condition may produce biodegradation of different rates and extents during anaerobic process Randa, *et al.* [12] and Wilkie [15].

In addition, cow dung as inoculums was taken from the livestock farm. The cow dung utilized had a considerable amount of nutrient materials, which was accessible for co-digestion with rice straw to enhance methane production. Analysis of cow dung before dilution with tap water can be seen in Table 2, which had 16.98% TS, contained 136.62 g VS and 153.05 g COD per kg of diluted cow dung. The ratio of COD to VS was 1.12. The ratio of VS to TS (80.45%) denotes that a large portion of the cow dung was degradable and might act as an important feed for biogas generation. The C/N ratio of the cow dung was found sufficient (27) because it is frequently recommended that the C: N ratio in the biomass or inoculums should be in between 20:1 to 30:1. During all experiments cow dung was diluted to decrease the solids content and prevent shocks due to high concentrations of it, dilution was performed using tap water. Table 3 represents analysis of diluted Cow dung used in all experiments.

Table 3 showed the total Kjeldahl nitrogen and total organic carbon. The C/N ratio used for the experiment was 1.52:1. The diluted cow dung used as inoculums contained high COD, total organic carbon and volatile solids, which were (13 854) mg/L, (861) mg/L and (79.19%), respectively. It had a neutral pH, which was appropriate for the anaerobic process.

Table-2. Analysis of Cow dung

Analysis	Cow dung
pH	7.48
Total solid (g/kg)	169.80
Volatile solids (% of TS)	80.45
Chemical oxygen demand (g/L)	153.05
Soluble COD (g/L)	66.15
Total organic carbon (g/L)	41.58
Total phosphorus (g/L)	2.53
Total kjeldahl nitrogen (g/L)	3.97
Ammonia nitrogen (g/L)	2.34
Free ammonia (g/L)	0.09

Table-3. Analysis of diluted Cow dung used in all experiments

Parameter	Unit	Diluted cow dung
pH	-	7.3 ± 0.29
Total Kjeldahl nitrogen	mg/L	567.75 ± 93.49
Total organic carbon	mg/L	861 ± 122.29
Total solids	%	1.03 ± 0.09
Volatile solids	%	79.19 ± 1.65
Chemical oxygen demand	mg/L	13854.33 ± 2963.1

A research discovered that inoculums applied to the anaerobic fermentation process, may considerably improve the performance of the treatment. It is also reported that the optimum performance of the inoculated reactors possibly coupled with accelerated growth of microorganisms that contribute to the digestion of organic matter in reactors Randa, *et al.* [12] and Lopes, *et al.* [16]. In addition, another research revolved that inoculums has a significant job for starting up fermentation process since it is capable of balance the populations of microorganisms such that *syntrophobacter* which is accountable for biodegrading propionate as well as butyrate, and *methanogens* [17].

As mentioned in Table 1, rice straw has a high portion of both TS and VS. The carbon content of rice straw is also effectively high, representing that the substrate used should be suitable for co-digestion with cow dung. C /N ratio of rice straw is seemed high, which is about 50. However, this C/N ratio is still not reasonable to increase bio-methane production through the anaerobic process as the optimum C/N ratio for conducting anaerobic fermentation is from 20:1 to 30:1 [18]. As a result, by co-digesting rice straw with cow dung, it may improve performance of the anaerobic fermentation process to produce methane generation.

To optimize the co-digestion process performance and to evaluate anaerobic co-digestion effects of rice straw at special TS concentrations, and to attain the maximum production of methane, three experiments were approved. The first experiment was run at 2% TS where 500 mL of diluted cow dung and 5.5 g of rice straw were added into the reactor. In this experiment, biogas generation stopped at day 30 of fermentation. Feed data of the digestion process were shown in Table 4. As summarized in Table 4, it is well known that all reactors utilized in the optimum pH in the range of 6.5 - 8.0, which enabled them to conduct in appropriate anaerobic fermentation environment for biogas production [19]. This result is agreed with a previous study that the anaerobic process conduct at pH in the range of 7 - 8 was efficient for biodegrading suspended solids and volatile suspended solids during the anaerobic process [20].

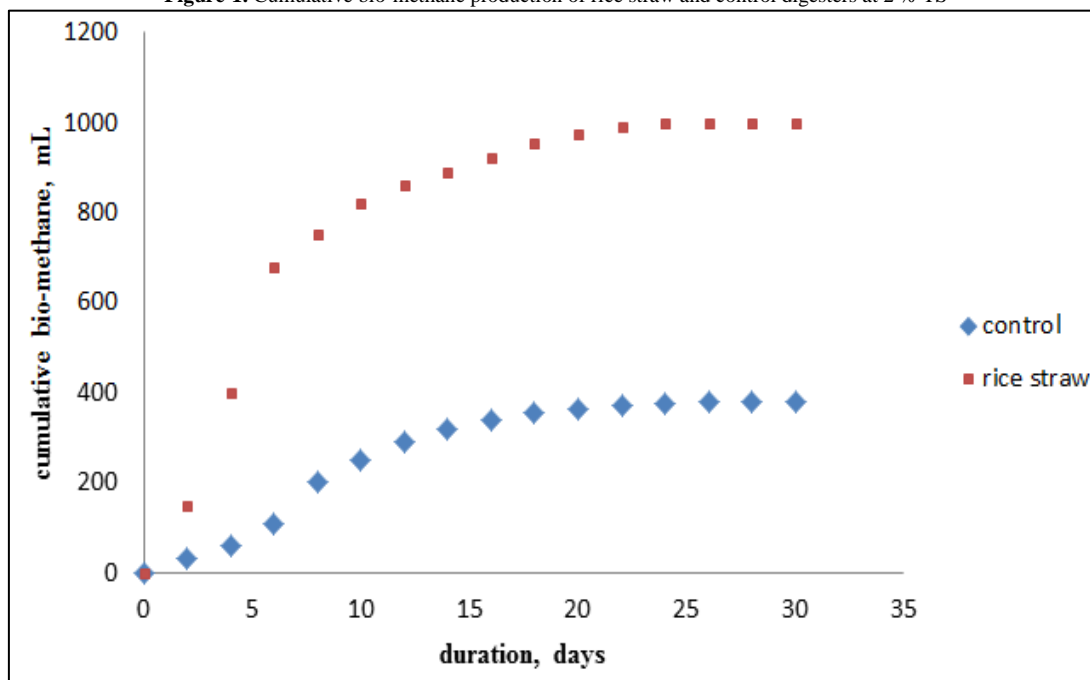
**Table-4.** Feed data of anaerobic process at 2% TS concentration

Analysis	Unit	Control (diluted cow dung)	Rice straw
COD	mg/L	13 400	31 600
TOC	mg/L	995	802.01
TS	%	0.96	2.07
VS	%	77.8	80.56
pH	-	7.17	7.29
TKN	mg/L	575.72	822.81

As shown in Table 4, it can be identified that the COD of rice straw co-digested with diluted cow dung was significantly higher than that of control (diluted cow dung). As illustrated in Figure 1, control digesters conduct at 2% TS began to generate bio-methane at 2<sup>nd</sup> day of digestion process (30) mL. The shape of the drawn curve looks similar to a sigmoid curve, corresponding to cumulative methane generation within 30 days of the anaerobic fermentation process. Maximum production was achieved at day 26 at around (380.6) mL.

As illustrated in Figure 1, rice straw reactors operated appreciably better at 2% TS than control digesters. There was a short lag phase that obtained during start-up of the digestion process. It can be noticed that rice straw digesters run at 2% TS generated only (150) mL CH<sub>4</sub> on the 2<sup>nd</sup> day of the anaerobic digestion. As given in Figure 1, there was a significant increase in bio-methane production between day 2 and day 10 of the anaerobic digestion process. It continuously generated bio-methane with a slow increase until getting a peak at day 24 of the digestion process (1000) mL. Table 5 depicts the effluent data obtained at 2% TS. As can be concluded, it is well known that each digester still conduct in the optimum pH value in the range of anaerobic digestion. This can point to that the low biogas production by some digesters (control digesters) can not be caused by accumulation of acid in reactors.

**Figure-1.** Cumulative bio-methane production of rice straw and control digesters at 2 % TS



**Table-5.** Effluent data of digestion process at 2% TS concentration

Analysis	Unit	Control (cow dung)	Rice straw
TS	%	0.9	1.45
VS	%	74.84	72.45
COD	mg/L	10 770	15 800.6
TOC	mg/L	485.61	545.81
TKN	mg/L	625.24	768.17
pH	-	6.82	6.74
Total methane Production	mL	380.67	1000

Table 5 represented those rice straw digesters utilized at 2% TS produced bio-methane around three fold that of cow dung (control) digesters. Total bio-methane produced by rice straw digesters within 30 days of digestion process was (1000) mL. As shown in Figure 1, a low degree of homogeneity of mixture in rice straw digesters can lead to more variations in the daily methane generation. This phenomenon also obtained to the previous research run on co-digested of rice straw with cattle manure as inoculums, where during the co-digestion process the daily biogas generation was less stable due to accumulation of solid [21]. Based on the TS information obtained in Tables 4 and 5, the performance of each reactor during the digestion process may also be known where TS removal in rice straw reactors operated at 2% TS was 30.01% %.

In the 2<sup>nd</sup> experiment occurred at 4% TS, rice straw feeded to each digester containing 500 mL of inoculums was 17 g. Under the steady state condition, the duration period of the anaerobic digestion process was 35 days when bio-methane generation completely stopped. Table 6 illustrates initial conditions of anaerobic co-digestion process operated at 4% TS. It can be observed that the pH value of rice straw 7.58, was still in the optimum pH value range between 6.5 and 8.0 for anaerobic digestion process [19, 22].

Table-6. Influent data of digestion process at 4% TS concentration

Analysis	Unit	Control (diluted cow dung)	Rice straw
COD	mg/L	15 855	59801
TOC	mg/L	758	1558
TS	%	1.00	3.99
VS	%	78.78	87.14
TKN	mg/L	656.00	1623.50
pH	-	7.63	7.58

Figure 2 represents the performance of control digesters operated at 4% TS. As can be noticed, there is not a considerable difference among control digesters operated at 2%, 4% and 6% TS (Figures 1, 2 and 3). This operation condition occurred since each of control digesters was feeded with the same culture. There was a small lag phase that obtained a few hours after starting experiment. Each of the control digesters utilize at 4% TS began to produce bio-methane from the 2<sup>nd</sup> day of anaerobic process with methane generation at around (38) mL. The maximum methane production of control reactors performed at 4% TS was reached on day 30 of digestion process at (395.3) mL.

As shown in Figure 2, rice straw reactors run at 4% TS still operated better compared with cow dung alone or control digesters. Additionally, compared with rice straw operated at 6% TS, cumulative methane production from rice straw at 4% TS doubled as shown in Figure 3. Bio-Methane generation had started from the 2<sup>nd</sup> day of anaerobic process (82) mL after having a lag phase during the 1<sup>st</sup> few hours of the experiment. Although at the beginning of anaerobic process it generated bio-methane the same as rice straw digesters run at 6% TS, it generated significantly more bio-methane on 6<sup>th</sup> day of digestion process (1110) mL. The result showed that on 6<sup>th</sup> day of the anaerobic process, rice straw digesters run at 4% TS produced bio-methane around three fold that from rice straw performed at 6% TS (380) mL, and approximately 38% higher than from rice straw operated at 2% TS (680.7) mL. Moreover, rice straw digesters operated at 4% TS reached a maximum bio-methane generation on day 32 of the anaerobic process (2520.13) mL, which was around 1.5 fold that from rice straw run at 6% TS (1584.85) mL on day 30 of the anaerobic process (Figure 3).

Figure-2. Cumulative bio-methane generation of rice straw and control digesters under 4% TS

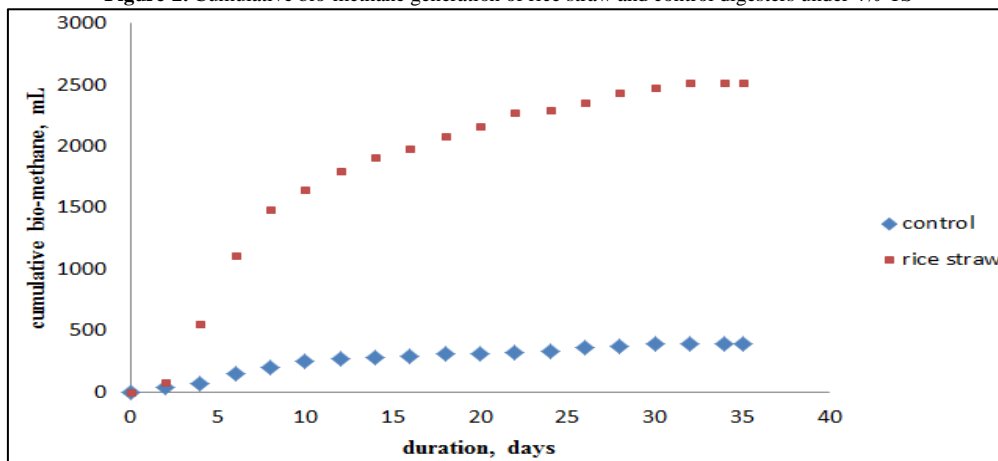


Figure-3. Cumulative bio-methane generation of rice straw and control digesters at 6% TS

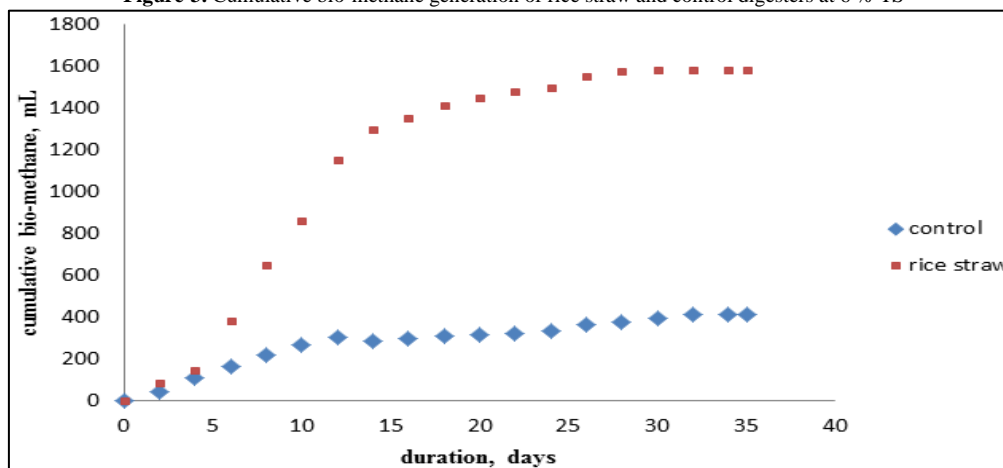




Table 7 summarizes the effluent data of fermentation process utilized at 4% TS. As can be noticed, pH values of each digester were still in the suitable range required for anaerobic process. This indicated that each digester was stable enough during anaerobic process without any considerable inhibition. Table 7 showed that rice straw produced the most bio-methane (2520) mL at 4% TS. This means that rice straw digesters utilized at 4% TS produced bio-methane almost six fold that of control digesters (395) mL with a retention time of 35days.

Table-7. Effluent data of anaerobic process at 4% TS concentration

Analysis	Unit	Control (diluted cow dung)	Rice straw
TS	%	0.9	1.95
VS	%	73.91	71.35
COD	mg/L	10 354.67	27 710.67
TOC	mg/L	492	795
TKN	mg/L	612.86	930.06
pH	-	6.88	6.90
Total methane production	mL	395	2520.33

The experiment performed at 6% TS, rice straw loaded into each digester was 27.96 g. When cow dung was co-digested with rice straw, the C/N ratio of the culture was suitable for anaerobic digestion. The C/N ratio value was in agreement with the optimum C/N ratio ranges reported by previous research, where the C/N ratio ranges from 15.5 to 19 was revealed to be the optimum range in terms of maximum bio-methane generation [23].

Based on Table 8, it is also discovered that rice straw had higher total organic carbon and volatile solids compared with cow dung alone. The high organic content is generally associated to the high degradability that enables the substrate to be highly preferred for anaerobic process [24]. In the case of rice straw digesters depicted in Figure 3, the shape of curve produced is quite different from control digesters utilized at 6% TS. It began to produce bio-methane at the 2<sup>nd</sup> day of digestion process which was about (82) mL. This condition represents a high biodegradation rate from rice straw, where the material is highly degradable that lead to consume by microbes. After 35 days of anaerobic digestion, rice straw digesters utilized at 6% TS generated bio-methane twice that of control digesters where it produced bio-methane at around (1584.85) mL.

Table 9 summarizes the values of effluent data and methane generation running with a retention time of 30 days, which was utilized at 6% TS. As can be noticed, there was an insignificant decrease of pH values from influent to effluent culture. However, pH values of each effluent culture performed at 6% TS were still in the neutral range 6.6 - 7 required for proper anaerobic process [25].

Table-8. Influent data of anaerobic digestion at 6% TS concentration

Analysis	Unit	Control (diluted cow dung)	Rice straw
TS	%	1.101	5.9465
VS	%	80	90
COD	mg/L	15 260	89197.5
TOC	mg/L	830	2375
TKN	mg/L	470.53	2378.76
pH value	-	7.1	7.58

Table-9. Effluent data of anaerobic digestion at 6% TS concentration

Analysis	Unit	Control (diluted cow dung)	Rice straw
TS	%	0.89	4.56
VS	%	75.56	74.71
COD	mg/L	12 700	50160.67
TOC	mg/L	753.17	1 687.25
TKN	mg/L	538.61	2280.41
pH value	-	6.77	6.83
Total methane production	mL	410.67	1584.67

Based on Table 9, it is discovered that rice straw produced higher methane generation compared to cow dung alone (control digesters). Figure 3 depicts that rice straw digesters produced bio-methane more than 300% higher than control digesters. According to Table 8 and Table 9, TS reductions of control and rice straw digesters occurred were (19%), (23.36%), respectively. In terms of VS removal occurred at 6% TS concentration, control, and rice digesters had (23.69%), (36.47%), respectively. These results illustrated that rice straw digesters utilized at 6% TS performed very well compared with control digesters (Figure 3).

### 3.2. Biodegradation Efficiency

Some researchers had discovered that methane generations significantly influenced by degradation and availability of the primary constituents contained in biomass, such as carbohydrates, protein, and lignin contents [26]. The study about bio-methane digestion of selected lignocellulosic matter revealed that degradability is influenced by lignocellulosic matter and also controlled by some operating factors including the lignin content, the

availability of surface area and cellulose characteristics inside the matter [27]. The methane yield presented in terms of mL CH<sub>4</sub>/g VS added indicates the efficiency of degradation

Lo, *et al.* [9]. The digestibility and composition of substrates was the major determinant of the maximum bio-methane yield. It is revealed that several operating factors that influence bio-methane yields include temperature, degradability, loading rate, and retention time [15].

In addition, results analysis revealed that there is an interaction between factors (substrates and percent total solids applied) with methane yields. As presented in Table 10, it is known that RS reactors performed at 2% TS had the highest methane yield (119.9±4.59) mL CH<sub>4</sub>/g VS added, which was almost 17% higher than control reactors (102.1±4.37) mL CH<sub>4</sub>/g VS added. RS reactors run at 2% TS also had the highest percentage of VS reduction (37.02%±2.41%), which was more than three times higher than that of control reactors (10.73%±1.02%). Moreover, good performance of RS reactors operated at 2% TS was also shown in the percentage of COD removal, where they obtained 50.07%±1.06% reduction, which was 150% higher than control reactors. These phenomena allowed RS reactors to generate more methane within 30 days of digestion process compared with CH<sub>4</sub> in control reactors. It is revealed also that there is an interaction between factors (substrates and percent TS) with COD removal. This condition may indicate that there is a relationship as well as influence between TS applied in the digesters and COD removal.

**Table-10.** Efficiency of anaerobic treatment at 2% TS concentration

Analysis	Unit	Control (diluted cow dung)	Rice straw
VS reduction	%	10.73	37.02
COD removal	%	19.63	50.07
Methane yield	mL CH <sub>4</sub> /g VS added	102.06	119.9
Total methane production	mL	380.67	1000

Table 11 summarizes anaerobic digestion efficiency operated at 4% TS. As can be observed, RS reactors had the highest methane yield (145.4) mL CH<sub>4</sub>/g VS added, which was 45% higher than control reactors. Furthermore, good performance of RS reactors ran at 4% TS was also shown in the percentage of COD removal, where they gained (52.97%), which was higher than control reactors. In addition, RS reactors also had the highest VS reduction (60.81%), which was four times of that of control digesters. These phenomena enabled RS reactors to reach the highest cumulative methane production within 35 days of the digestion process, where in terms of total methane production, they gained (2520) mL, which was extremely higher compared with control reactors (395) mL CH<sub>4</sub>, where RS generated methane at around 500% higher than control reactors.

**Table-11.** Efficiency of anaerobic treatment at 4% TS concentration

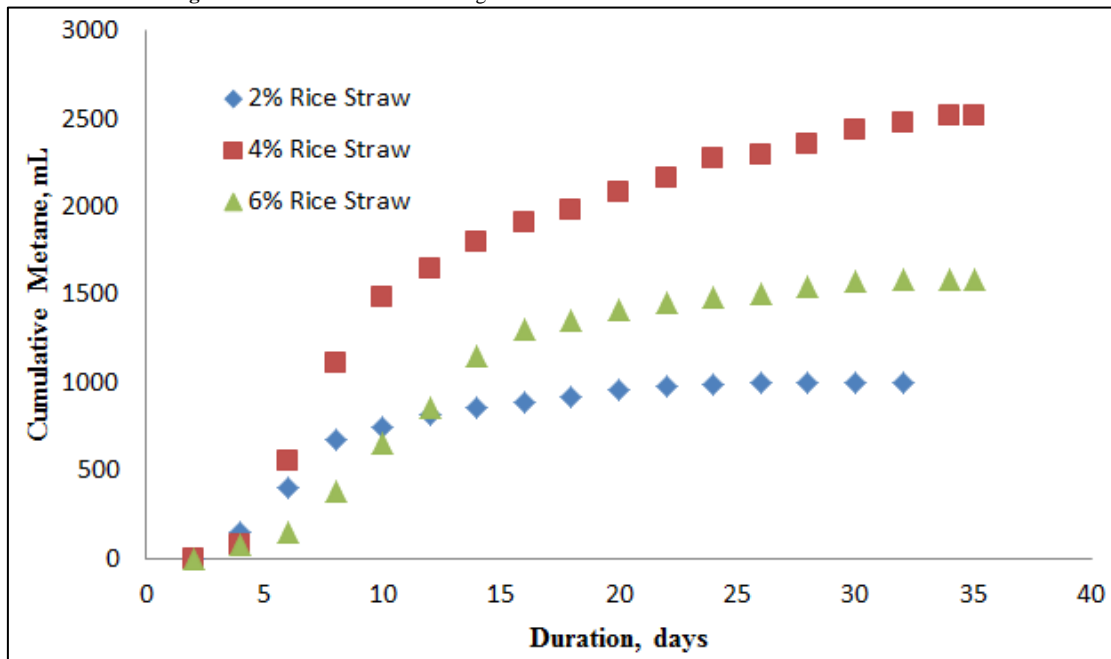
Analysis	Unit	Control (diluted cow dung)	RS
VS reduction	%	15.7	59.95
COD removal	%	34.69	52.9
Methane yield	mL CH <sub>4</sub> /g VS added	100.25	112.44
Total methane Production	mL	395	2520.13

Table 12 shows digestion efficiency obtained from the 6% TS process. As can be observed, RS reactors had a lower methane yield (59.24) mL CH<sub>4</sub>/g VS added compared with control reactors (93.98) mL CH<sub>4</sub>/g VS added). RS reactors had higher VS reduction (36.4%) compared with control reactors (23.6%); in addition, they had higher COD removal (43.76%), which was more higher than control reactors (16.78%). It is known that rice straw digesters still produced more cumulative bio-methane within 35 days of anaerobic process compared with control digesters. However, the low yield of methane as well as COD reduction revealed that they experienced issues in the anaerobic process. In addition, it also can be known by comparing bio-methane generation of rice straw utilized at 6% TS with that of 2% and 4% TS concentrations (Tables 10, 11, and 12).

**Table-12.** Efficiency of anaerobic treatment at 6% TS concentration

Analysis	Unit	Control (diluted cow dung)	RS
VS reduction	%	23.6	36.4
COD removal	%	16.78	43.76
Methane yield	mL CH <sub>4</sub> /g VS added	93.182	59.24
Total methane Production	mL	410.67	1584.85

Figure-4. Cumulative bio-methane generation of rice straw with different TS concentrations



In terms of total methane production, it was revealed that RS reactors operated at 6% TS, generated methane 58% higher than that at 2% TS, and 37% lower than RS run at 4% TS. Furthermore, the results also showed that there was a statistically significant difference between percent TS applied and degradable parameters (COD Reduction, bio-methane yield and VS removal) in anaerobic process of rice straw. This is very obvious that rice straw conducted at 6% TS did not operate very well as in case of 4% TS due to accumulation of solid that lead to lower digestion process efficiency. This condition obtain since higher TS as well as VS loaded into the reactor may produce a lot of VS in the reactor that may affect the alkalinity of the reactor.

Higher TS concentration applied to the digester also can affect on the volatile loading rate in the available retention time period. Therefore, enough retention time should be allowed for the micro-organisms to biodegrade the organic matter and convert it into methane [15, 28]. Moreover, a previous research also discovered that there is a maximum limit for TS content applied in anaerobic process, above which the matter was not considered slurry capable for processes such as mixing [15].

#### 4. Conclusion

A number of experiments at different total solids concentrations (2%, 4% and 6% TS) were examined to assess bio-methane generation potential of individual substrates. Rice straw obtained better performance for all total solids concentrations examined. Rice straw produced the most bio-methane at 4% TS which was around (2520) mL where the C/N ratio of rice straw was more enough. Rice straw still generated more bio-methane at 4% TS than 6% TS even though at 6% TS, bio-methane generation was stopped in 35 days of fermentation. This obtained since in the mixture with 6% TS, rice straw had issues of solid accumulation in the reactor that led to unacceptable mixing during the fermentation process and required a longer retention time to convert biomass into bio-methane. Degradation efficiency was evaluated for each substrate. Rice straw had the highest bio-methane yield at 4% TS, which was around (145) mL CH<sub>4</sub>/g VS added. Rice straw also had the highest COD reduction and VS removal at 4% TS which were around (52.97%) and (60%), respectively. These results may point to that 4% TS is an optimum condition for rice straw to generate bio-methane with a stable anaerobic fermentation process.

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