Anaerobic co-digestion of rice straw and digested swine manure with different total solid concentration for methane production

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Abstract: This study aimed to investigate potential methane production through anaerobic co-digestion of rice straw and digested swine manure with different total solids. The research was carried out in bench scale with utilizing batch system. To evaluate the stability of anaerobic co-digestion process, the experiment was run in triplicate. The anaerobic co-digestion process was operated in 500 mL batch digesters under constant agitation speed and temperature. The agitation speed was maintained at 270 r/min. Temperature of the batch system was set and maintained at 35°C. Digested swine manure utilized in this experiment was obtained from semi-continuous digesters run at steady state condition, with 25 days of hydraulic retention time under mesophilic condition. Rice straw (RS) generated the highest methane production at 3% total solids (TS) which was around (1814±47.43) mL, where in this concentration, it had C:N ratio at 10.6:1. Rice straw obtained the highest methane yield at 3% TS, which was around (141.4±3.70) mL CH_4/g volatile solids (VS) added. Rice straw also had the highest chemical oxygen demand (COD) removal and VS reduction at 3% TS which were around (52.97%±1.46%) and (61.81%±1.04%), respectively.

Keywords: anaerobic co-digestion, rice straw, digested swine manure, methane production

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1 Introduction

Anaerobic digestion is a series of processes that convert waste materials into useful products by micro-organisms in the absence of oxygen. Some waste materials include animal wastes, forest residues, agricultural residues and grasses. In the past, anaerobic digestion was related to the treatment process of animal manure and sewage sludge. Since there is an increasing awareness of environmental issues and energy crisis throughout the world, the advancement of technology regarding anaerobic digestion is inevitable[1]. Anaerobic digestion applied may help cut the use of fossil fuels and reduce greenhouse gas emissions that may contribute to climate change[2]. The anaerobic digestion technology is widely known as the technology for treating and managing wastes in order to generate renewable energy.

Currently, fossil fuel resources are not considered as sustainable due to the worsening environment and massive energy usage[3]. About 90% of the energy usage is derived from fossil fuel; it is known that burning fossil fuel to generate energy is closely related to the emission of greenhouse gases to the atmosphere. Some researches revealed that the earth atmosphere receives more than 15 million tons of carbon dioxide (CO_2) annually. Accumulation of carbon dioxide in the

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atmosphere is resulted from burning fossil fuel for energy production. This condition can increase its concentration in the atmosphere that may enhance the trend of global warming[6,7]. Some other greenhouse gases including nitrous oxide and methane are typical gases that may contribute to global warming[8, 9]. Some wastes that end up decaying in landfill may generate methane. It is revealed that the comparative impact of CH_4 on climate change is more than 20 times of that of CO_2 over a period of a hundred years. It occurs as methane can trap more radiation than CO_2[9, 10].

Anaerobic digestion is considered as a good method for treating and handling organic wastes including food waste, animal manure and agricultural residues. It occurs since the anaerobic digestion process can remove the high concentration of organic waste, and then convert it into biogas[11]. Compared with conventional aerobic digestion, running anaerobic digestion process is more beneficial due to low initial investment, low energy requirement, net energy recovery, and lower release of volatile compounds into the atmosphere. The anaerobic digestion process operated in the digester not only can be used for preventing greenhouse gases emissions released to the atmosphere but it may also generate useful byproducts including biogas, and digestate that can be used as soil conditioner or fertilizer. The anaerobic digestion process operated in anaerobic reactor produces biogas containing methane, which can be utilized as a source of renewable energy[12-15]. Biogas generated from the anaerobic digester may help cut the use of fossil fuels for reducing carbon emissions. Thus, this work will meet the Kyoto Treaty that encourages each country throughout the world to cut carbon emissions[16]. Currently, some research revealed that more than 60% of methane derived from human activities including industry and agriculture[10]. Organic decomposition which is not managed properly may contribute to methane accumulation in the atmosphere. As a greenhouse gas, methane has greater capacity to trap heat in the atmosphere compared with carbon dioxide. By trapping much heat in the atmosphere, it can substantially contribute to the global warming trend occurred on the earth[9].

Biogas generated from anaerobic digestion typically consists of methane (60%), carbon dioxide (40%), hydrogen sulfide, and trace elements. During anaerobic digestion process, micro-organisms convert complex organic matter into simpler chemical components. Around 90% of biodegradable organic compounds can be converted into biogas through the anaerobic digestion process[3]. Several major benefits derived from running the anaerobic digestion process for livestock farming as well as animal grazing include waste stabilization, pathogen reduction, odor control, energy production, nutrient recovery and mineralization, reduction of groundwater and surface water contamination potential, stable liquid fertilizer, and high-quality solids for soil conditioner[4, 6]. Some parameters including dry matter or total solids (TS) and volatile solids are typically used for determining the characteristics of organic material loaded into the anaerobic digester, and also to assess methane yields generated from the organic material[5].

Agricultural residues such as wheat straws, rice straws, and corn stalks are produced annually in large quantities throughout the world. Since agricultural wastes are a plentiful source of organic matter, these can be used as a valuable alternative feedstock for biogas production[17]. Furthermore, these wastes also have a considerable amount of carbon that may be beneficial for anaerobic co-digestion with animal manure. Agricultural residues include the crop residues and processing residues[18]. A million tons of agricultural residues are generated every year. Because of their abundance, they have a great potential in many areas especially in the anaerobic co-digestion process. In the developing world, there is a tendency that rice straw is either disposed or utilized to provide energy for household cooking and heating. Usually it is burned in the field after harvesting, and this will result in severe environmental pollution such as greenhouse gases and nitrogen oxide emission[19,20]. Moreover, in rice producing countries, an important source of agricultural waste is rice crop residues. Although there are methods available to use rice straw as a feedstock for generating useful products, significant amounts remain unused and some of them are burned in the open field. This practice may lead to serious environmental damage due to the air pollution[21].

Therefore, advanced anaerobic digestion may be a promising alternative approach to deal with rice straw disposal problems in concentrated rice production regions[22]. Rice straw is also considered as one of the most abundant lignocellulosic waste materials, and it has great potential in terms of biofuel production. Culms, leaf sheaths, panicle remains are the main parts of rice straw[23]. Like corn stover and wheat straw, rice straw also contains three components: cellulose, hemicelluloses, and lignin[24]. Rice straw is lignocellulosic biomass, which consists of cellulose (36.2% w/w), hemi-cellulose (19% w/w), and lignin (9.9% w/w)[25]. Furthermore, it is known that rice straw has a C/N ratio at around 75, and contains about 0.4% nitrogen[26]. Therefore, to enhance the anaerobic digestion of rice straw, it is necessary to add animal manure which contains a high amount of nitrogen in order to meet an optimum C/N ratio between 25 and 35[26].

An anaerobic digestion process using only one substrate will generate some issues in terms of biogas and methane production. This occurs since there are no sufficient nutrients available in the digester. Therefore, by adding other substrates, it will enable microbes to grow faster to produce biogas and methane. The stability of the process and biogas productivity in the anaerobic digestion system can be enhanced by treating anaerobic digestion with different feedstock[28]. Manure co-digested with other substrates including energy crops, forest wastes, botanical or agricultural wastes, food wastes, and industrial wastes, may significantly enhance biogas production[27].

Conducting anaerobic co-digestion will also be more effective in terms of cost spent and equipment use[29]. Running anaerobic co-digestion also can benefit the anaerobic process since it can improve the digestion process of some wastes containing protein and fat that may not be degraded easily[30]. Some research conducted regarding anaerobic digestion of dairy manure with various co-substrates (corn silage, whey, switchgrass, and waste grease), revealed that the co-digestion had successfully enhanced biogas and methane production[27].

Several studies focusing on the co-digestion of animal manure with straw found that the addition of rice and wheat straw into the cattle manure digester can enhance biogas production. It was also found that by adding rice straw and wheat straw into the cattle manure digester, higher organic biodegradability was achieved[31]. In addition, by combining several kinds of wastes that contain a high buffer capacity, it will overcome negative effects appeared in the anaerobic digestion process[29]. Compared to manure digestion only, animal manure co-digestion that has a low C/N ratio along with feedstock containing low levels of nitrogen (high C/N ratio) may generate operational performance which is more stable and it also can enhance methane production[32]. Literature review revealed that when swine manure was co-digested with fruit wastes, the biogas production rate was increased. Moreover, co-digestion also enables stabilization of the system during the anaerobic process[33].

Study on anaerobic co-digestion of wasted tomatoes and cattle dung for biogas production revealed that volatile fatty acids (VFAs) in the digestion was low; this means that the co-digestion method is effective to generate a stable anaerobic process. In addition, it is extremely risky if solely manure is utilized in anaerobic digestion as it contains a high nitrogen content. Thus, it will inhibit the growth of bacteria during anaerobic digestion, which leads to the reduction of methane production[34]. The purpose of this current study was to investigate and evaluate potential methane production through anaerobic co-digestion of rice straw and digested swine manure with different total solids concentrations run in the mesophilic condition.

2 Materials and methods

2.1 Substrate preparation

Rice straw used as a substrate for this experiment was derived from Japanese short grain heirloom variety called Koshihikari. Rice straw collected were dried and milled using a laboratory grinder (Thomas Wiley Laboratory Mill) to an average particle size between 1 and 1.5 mm. Digested swine manure utilized was taken from an effluent of the semi-continuous reactor operated in mesophilic temperature at the steady state.

2.2 Equipment

This research was conducted in triplicate where three reactors with working volume of 500 mL were loaded with rice straw and digested swine manure. Other three reactors of 500 mL were control reactors or without rice straw addition. All reactors were placed at thermostatic water bath; the temperature for this batch experiment was maintained and controlled under mesophilic condition at 35°C. For agitation purpose, magnetic bar was put into each reactor and the culture was continuously stirred at 270 r/min.

2.3 Experimental procedure

Batch experiments were conducted to evaluate and investigate the bio-methane potential from rice straw co-digested with digested swine manure. Several experiments were carried out to assess potential methane production. In experiment 1, 2 and 3, anaerobic co-digestion processes were conducted at 2%, 3% and 4% total solids (TS) concentration, respectively. Total solids of digested swine manure were measured in order to measure the amount of biomass that should be added to each reactor. The mixture of rice straw and digested swine manure loaded into each digester as an influent was prepared homogeneously. During measurement of the bio-methane production test, first there was no addition of any other nutrient including enzyme and chemicals in order to assess how much methane generated by substrate loaded. Five hundred mL of 0.4 Normal sodium hydroxide solutions were prepared, and filled into filter flasks. In order to entrap CO_2 and H_2S, each filter flask containing sodium hydroxide was connected to the gas meter and the reactor. Sodium hydroxide can be applied to purify biogas generated from anaerobic digestion process as it may react with both carbon dioxide and hydrogen sulfide; however, it cannot react with methane. Sodium carbonate will be generated once the carbon dioxide reacts with the sodium hydroxide[35]. Before running anaerobic digestion process, each reactor was purged with nitrogen gas for around 5 minutes to remove oxygen traces, and ensure anaerobic condition in the reactor. To prevent any gas loss due to high pressure in the reactor and to ensure completely anaerobic condition, each digester and filter flask utilized were sealed properly using parafilm.

2.4 Analytical methods

The duration of the experiment was determined by the point at which biogas production stopped completely. Some parameters analyzed including organic matter (OM), moisture content of the biomass (MC), carbon and nitrogen content of each substrate, pH, total Kjeldahl nitrogen (TKN), total organic carbon, chemical oxygen demand (COD), total solids (TS), and volatile solids (VS). TS samples were dried in an oven at 105°C, and VS samples were burnt in the furnace at the temperature of 550°C. All analytical assessments were measured based on the "Standard Methods"[36]. Methane production rates were measured as bio-methane generated (mL) per day, and the methane yield was assessed based on the cumulative methane generated per gram VS loaded[37, 38]. The strength of the waste typically can be known by assessing the amount of solids mixed in the culture[39]. To analyze the effectiveness of the digestion process, some parameters measured include the percent of COD removal and the percent of VS reduction.

2.4 Statistical analysis

Experimental data obtained while performing an anaerobic digestion process were statistically analyzed with two factors of analysis of variance (ANOVA) in triplicate at the steady state; the main effects and the interaction among factors (substrates and percent TS) with digestion parameters were analyzed. Descriptive statistics of two-way ANOVA with replication were conducted to specify the influence between potential methane production and investigated parameters. In addition, the data were analyzed by utilizing the ANOVA test at the 5% (α=0.05) level of significance for assessing the influence of percent TS, substrates loaded and digestion parameters of the batch experiment.

3 Results and discussion

3.1 Performance at different solid concentrations

The study intended to evaluate methane production potential of rice straw co-digested with digested swine manure through several different total solids concentrations including 2%, 3% and 4% TS. The anaerobic digestion process was run at the steady state condition where the temperature of the process was maintained at the mesophilic condition (35°C). The physical-chemical characteristics of substrates loaded are mentioned in Table 1. The characteristics of rice straw include organic matter of 72.82%, carbon content of 36.42%, volatile solids of 84.4%, and COD of 1 950 mg/L.

Table 1 Rice straw characteristics (wet basis)

Parameters	Unit	Rice straw
Total solids	%	91.4
Volatile solids	%	84.4
Moisture content	%	8.6
Organic matter	%	72.82
Carbon content	%	36.42
Nitrogen content	%	0.71
Chemical oxygen demand (COD)	mg/L	1 950.48
C:N ratio	-	51.3

The rice straw characteristics presented in Table 1 indicate the abundance of organic matter in rice straw, which enable it for the anaerobic co-digestion with digested swine manure to generate higher methane production. It has been known that vital factors that influence the anaerobic digestion process to generate methane production include volatile solids (VS) and total solids (TS). Moreover, TS was used to determine whether the reactor capacity large enough for fermentation of substrates, and VS may be considered as an indicator of organic matter for conversion into biogases including methane[40]. In addition, the volumetric methane yield may be significantly enhanced by high volatile solids content of substrates loaded[41]. It is revealed that by applying agricultural residues as co-substrates in biogas plants, it will significantly enhance the volumetric methane production of a swine slurry facility. Although the VS content of substrates loaded is regarded as a determinant of potential methane production, the methane yield on the VS basis is not always definite. This occurs as there is any variation in terms of VS composition which consists of both readily degradable organic materials (carbohydrates, proteins, and lipids) and refractory organics such as lignocellulosic materials. Therefore, it may be known that all volatile solids of organic materials are not always the same; this condition may generate biodegradation of different rates and extents during anaerobic digestion[42].

Moreover, digested swine manure as inoculums was taken from the semi-continuous reactor. The digested swine manure utilized still had a considerable amount of nutrients, which was available for co-digestion with rice straw to enhance methane production. The characteristics of digested swine manure can be seen in Table 2 which also showed the total Kjeldahl nitrogen (TKN) and total organic carbon. The C:N ratio utilized for the experiment was 1.52:1. The digested swine manure utilized as inoculums also contained high COD, TOC and VS, which were (13 853 ± 2 962) mg/L, (860 ± 121.2) mg/L and (78.19% ± 1.64%), respectively. It also had a neutral pH, which was suitable for the anaerobic digestion process.

Table 2 Digested swine manure characteristics

Parameter	Unit	Digested swine manure
pH	-	7.29 ± 0.28
Total Kjeldahl nitrogen (TKN)	mg/L	566.75 ± 92.49
Total organic carbon (TOC)	mg/L	860 ± 121.29
Total solids (TS)	%	1.02 ± 0.08
Volatile solids (VS)	%	78.19 ± 1.64
Chemical oxygen demand (COD)	mg/L	13853.33 ± 2962.1

A study revealed that inoculums applied to the anaerobic digestion process may significantly enhance the performance of the process. It is also mentioned that the better performance of the inoculated digesters may be associated with accelerated reproduction of microorganisms that contribute to the fermentation of organic material in digesters[43]. Moreover, another study mentioned that inoculum has a substantial role for starting up anaerobic digestion process since it is able to balance the populations of some bacteria that include *syntrophobacter* which is responsible for degrading propionate as well as butyrate, and *methanogens*[44].

As shown in Table 1, rice straw has a high percentage of both VS and TS. The carbon content of rice straw (RS) is also adequately high, indicating that the substrate utilized should be appropriate for co-digestion with digested swine manure. Carbon to nitrogen ratio of rice straw is pretty high, which is about 51.3. However, this C:N ratio is still not feasible to increase methane production through the anaerobic digestion process as the optimum C:N ratio for running anaerobic digestion is from 20:1 to 30:1[45]. Therefore, by co-digesting rice straw with digested swine manure, it may enhance performance of the anaerobic digestion process to generate methane production.

To optimize the co-digestion performance and to compare anaerobic co-digestion effects of rice straw at different TS concentrations, and to achieve the maximum productivity of methane, three experiments were carried out. The first experiment was run at 2% TS where 500 mL of digested swine manure and 5.58 g of rice straw were loaded into the reactor. In this experiment, methane production stopped completely at day 25 of digestion. Influent data of the process were summarized in Table 3. As mentioned in Table 3, it is known that all digesters operated in the optimum pH range between 6.5 and 8.0, which enabled them to run in proper anaerobic digestion environment for methane generation[46]. This result is in agreement with a previous finding that the anaerobic digestion process run at pH between 7 and 8 was effective for degrading total suspended solids as well as volatile suspended solids during the anaerobic digestion process[47].

Table 3 Influent data of anaerobic digestion at 2% TS concentration

Analysis	Unit	Control (digested swine manure)	Rice straw
COD	mg/L	13 500	31 700
TOC	mg/L	994	801.01
TS	%	0.95	2.06
VS	%	76.8	79.56
pH	-	7.16	7.19
TKN	mg/L	574.72	821.81

As mentioned in Table 3, it can be known that the COD of rice straw (RS) co-digested with digested swine manure was substantially higher than that of control (digested swine manure alone). As illustrated in Figure 1, control reactors run at 2% TS started to produce methane at the first day of digestion (29 ± 10) mL. The shape of the curve looks like a sigmoid curve, representing cumulative methane production within 25 days of the digestion process. Maximum production was reached at day 22 at around (379.6 ± 16) mL.

As depicted in Figure 1, RS digesters run significantly better at 2% TS than control reactors. There was a short lag phase that occurred at the beginning of the anaerobic digestion process. It can be seen that RS reactors operated at 2% TS produced only (60 ± 30) mL CH_4 on the first day of the digestion process. As shown in Figure 6, there was a considerable increase in methane production between day 2 and day 5 of the digestion process. It continuously produced methane with a slow increase until reaching a peak at day 21 of the digestion process (965.7±40.5) mL. Table 4 summarizes the effluent data performed at 2% TS. As can be observed, it is known that each reactor still ran in the optimum pH value range for anaerobic digestion. This may indicate that the low gas production generated by some reactors (control reactors) may not be caused by acid accumulation in digesters.

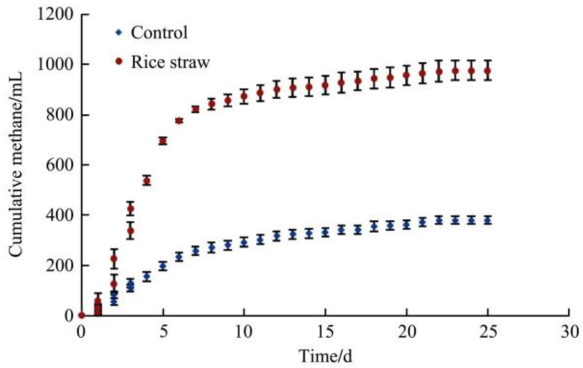

Figure 1 Cumulative methane production of rice straw and control reactors at 2 % TS

Table 4 Effluent data of anaerobic digestion at 2% total solids concentration

Analysis	Means and standard deviations		
	Unit	Control (digested swine manure)	Rice straw
TS	%	0.87 ±0.01	1.44 ±0.01
VS	%	73.84 ±0.23	71.45 ±0.77
COD	mg/L	10 766.67 ±677.15	15 826.67 ±336.06
TOC	mg/L	481.68 ±47.62	542.84 ±24.07
TKN	mg/L	621.22 ±6.48	757.37 ±10.29
pH	-	6.80 ±0.20	6.73 ±0.03
Total methane production	mL	379.67 ±15.95	977 ±37.58

Table 4 showed that RS reactors operated at 2% TS produced methane around three times that of digested swine manure (control) reactors. Total methane production generated by RS reactors within 25 days of digestion was (977 ± 37.58) mL. As depicted in Figure 1, a low degree of homogeneity of mixture in RS reactors may lead to more variations in the daily methane production. This phenomenon also occurred to the previous study conducted on co-digested rice straw with cattle manure, where during the co-digestion periods the daily biogas production was less stable due to solid accumulation[48]. Based on the TS data presented in Tables 3 and 4, the performance of each digester during the anaerobic digestion process can also be known where TS reduction in RS digesters run at 2% TS was 30.1% ± 0.3%.

In the second experiment performed at 3% TS, rice straw added to each reactor containing 500 mL of inoculums was 10.67 g. Under the steady state, the duration of the digestion process was 31 days when methane production stopped completely. Table 5 shows initial conditions of co-digestion process run at 3% TS. It can be noticed that the pH value of RS 7.57, was still in the optimum pH value range between 6.5 and 8.0 for anaerobic digestion[46, 49].

Table 5 Influent data of anaerobic digestion at 3% TS concentration

Analysis	Unit	Control (digested swine manure)	Rice straw
COD	mg/L	15 850	48 300
TOC	mg/L	757	1157
TS	%	0.99	2.98
VS	%	77.78	86.14
TKN	mg/L	655.00	622.50
pH	-	7.62	7.57

Figure 2 depicts the performance of control reactors run at 3% TS. As can be observed, there is not a significant difference among control reactors run at 2%, 3% and 4% TS (Figures 1, 2 and 3). This condition happened since each of control reactors was loaded with the same culture derived from semi-continuous reactors operated in the steady state. There was a small lag phase that occurred a few hours after running experiment. Each of the control reactors run at 3% TS started to produce methane from the second day of digestion process with methane production at around (32±6) mL. The maximum methane production of control reactors performed at 3% TS was reached on day 28 of digestion at (384.3±8) mL.

As shown in Figure 2, RS digesters operated at 3% TS still performed better compared with digested swine manure alone or control reactors. Furthermore, compared with RS performed at 4% TS, cumulative methane generation from RS at 3% TS doubled (Figure 3). Methane production had begun from the first day of digestion process (68±2) mL after having a lag phase during the first few hours of the experiment. Even though at the beginning of digestion process it produced methane the same as RS reactors operated at 4% TS, it produced significantly more methane on day 5 of digestion (901±22) mL. The result showed that on day 5 of the digestion process, RS reactors run at 3% TS produced methane around three times that from RS performed at 4% TS (366±19) mL, and approximately 30% higher than from RS performed at 2% TS (695.7±14.36) mL. In addition, RS reactors performed at 3% TS reached a maximum methane production on day 29 of the digestion process (1814±47.4) mL, which was around two times that from RS operated at 4% TS (954.7±45.4) mL on day 29 of the digestion process (Figure 3).

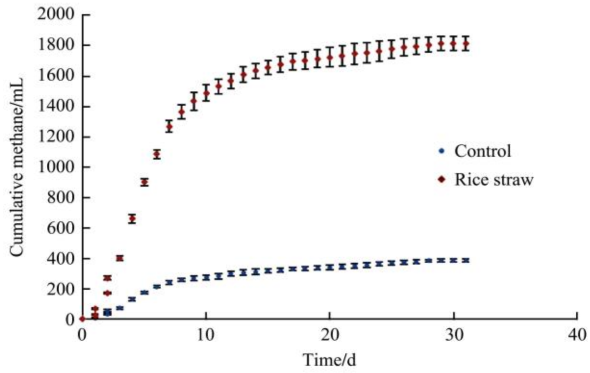

Figure 2 Cumulative methane production of rice straw and control reactors under 3% TS

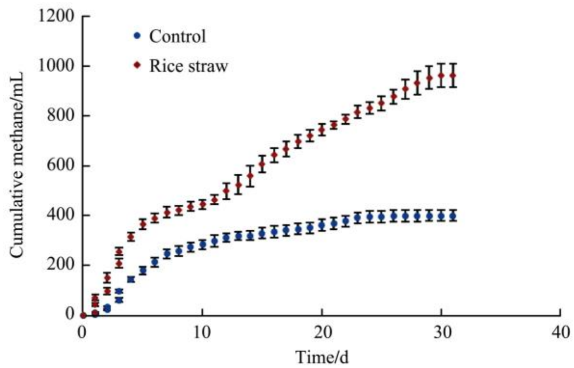

Figure 3 Cumulative methane production of rice straw and control reactors at 4 % TS

Table 6 summarizes the effluent data of anaerobic digestion operated at 3% TS. As can be noticed, pH values of each reactor were still in the optimum range required for anaerobic digestion. This indicated that each reactor was highly stable during anaerobic digestion without any significant inhibition. Table 6 showed that RS produced the most methane (1 814±47.43) mL at 3% TS. This means that RS digesters operated at 3% TS produced methane almost five times that of control reactors (386±10.54) mL with a retention time of 31 days.

Table 6 Effluent data of anaerobic digestion at 3% TS concentration

Analysis	Means and standard deviations		
	Unit	Control (digested swine manure)	Rice straw
TS	%	0.87 ±0.01	1.92 ±0.02
VS	%	73.93 ±0.71	70.35 ±0.56
COD	mg/L	10 326.67 ±336.06	22 716.67 ±709.46
TOC	mg/L	493 ±25.62	901 ±21.67
TKN	mg/L	604.86 ±16.30	734.06 ±41.08
pH	-	6.9 ±0.04	6.92 ±0.04
Total methane production	mL	386 ±10.54	1 814.33 ±47.43

The experiment performed at 4% TS, RS loaded into each reactor was 16.96 g. When digested swine manure were co-digested with rice straw, the C:N ratio of the culture was 18.5. The C:N ratio value was in agreement with the optimum C:N ratio ranges mentioned by previous study, where the C:N ratio ranges from 15.5 to 19 was discovered to be the optimum range in terms of maximum methane production[50].

Based on Table 7, it is also revealed that RS had higher total organic carbon and volatile solids compared with digested swine manure alone. The high organic content is commonly related to the high biodegradability that enables the substrate to be highly preferred for anaerobic digestion[51]. In the case of RS reactors showed in Figure 3, the shape of curve generated is quite different from control reactors operated at 4% TS. It started to generate methane at the first day of digestion which was about (72±12) mL. This condition represents a high degradation rate from rice straw, where the material is highly biodegradable that lead to easy access by microbes. After 29 days of digestion, RS reactors operated at 4% TS produced methane twice that of control reactors where it produced methane at around (954.7±45.4) mL.

Table 8 summarizes the values of effluent data and total methane production running with a retention time of 30 days, which was operated at 4% TS. As can be observed, there was a slight decrease of pH values from each influent to effluent culture. However, pH values of each effluent culture performed at 4% TS were still in the neutral range between 6.6 and 7 required for proper anaerobic digestion[52].

Table 7 Influent data of anaerobic digestion at 4% TS concentration

Analysis	Unit	Control (digested swine manure)	Rice straw
TS	%	1.101	3.9465
VS	%	80	88
COD	mg/L	15 260	30 150
TOC	mg/L	830	1312
TKN	mg/L	470.53	768.76
pH value	-	7.1	7.58

Table 8 Effluent data of anaerobic digestion at 4% TS concentration

Analysis	Means and standard deviations		
	Unit	Control (digested swine manure)	Rice straw
TS	%	0.89 ±0.00	3.06 ±0.02
VS	%	75.56 ±0.04	74.71 ±0.09
COD	mg/L	12 700 ±1 040.43	27 936.67 ±1 628.51
TOC	mg/L	753.17 ±223.30	1 971.33 ±193.28
TKN	mg/L	538.61 ±66.80	711.4 ±19.13
pH value	-	6.77 ±0.10	6.83 ±0.08
Total methane production	mL	400.67 ±21.59	962.67 ±46.70

Based on Table 8, it is revealed that RS generated higher methane production compared to digested swine manure alone (control reactors). Figure 3 depicts that RS reactors produced methane more than 100% higher compared with control reactors. According to Table 7 and Table 8, total solid reductions of control and RS reactors obtained were (19%±0.34%), (22.6%±0.6%), respectively. In terms of VS reduction obtained at 4% TS concentration, control, and RS reactors had (22.69%±0.16%), (59.71%±0.19%), respectively. These results showed that RS reactors operated at 4 % TS performed very well compared with control reactors (Figure 3).

According to the ANOVA test conducted, there is a statistically significant difference between percent TS and effluent digestion parameters (pH, TKN, COD, TOC, VS, TS, and methane production) in anaerobic digestion of rice straw (p value = 2.07×10^{-18}; F_{test} = 124.97; F_{crit} = 3.22; df = 2). In addition, the ANOVA analysis also revealed that there is significant difference between percent TS applied and methane gas production where this may also indicate that there is any relationship as well as influence between TS and methane production in anaerobic digestion process of rice straw (p value =

3.77×10^{-14}; F_{test} = 270; F_{crit} = 3.55). Furthermore, statistical analysis by applying the ANOVA test at 5% level of significance showed that there is an interaction between factors (substrates and percent TS) with methane gas production (p value = 5.6×10^{-6}; F_{test} = 282; F_{crit} = 2.928; df = 4). This condition may indicate that there is a relationship as well as influence between percent TS applied and substrates loaded into the digesters with methane production.

3.2 Biodegradation efficiency

Some studies had revealed that methane production is substantially influenced by biodegradation and availability of the primary constituents contained in biomass, such as carbohydrates, protein, and lignin contents[21, 53]. The study about methane fermentation of selected lignocellulosic materials revealed that biodegradability is influenced by lignocellulosic biomass and also restricted by some factors including the lignin content, the availability of surface area and cellulose characteristics inside the biomass[54]. The methane yield presented in terms of mL CH_4/g VS added indicates the biodegradation efficiency[37]. The digestibility and composition of substrates was the major determinant of the maximum methane yield. It is revealed that several factors that influence methane yields include temperature, biodegradability, loading rate, and retention time[55].

In addition, ANOVA analysis revealed that there is an interaction between factors (substrates and percent total solids applied) with methane yields (p value = 2.48×10^{-13}; F_{test} = 141; F_{crit} = 2.9277; df = 4). As presented in Table 9, it is known that RS reactors performed at 2% TS had the highest methane yield (119.3±4.59) mL CH_4/g VS added, which was almost 15% higher than control reactors (104.1±4.37) mL CH_4/g VS added. RS reactors run at 2% TS also had the highest percentage of VS reduction (35.66%±2.41%), which was two times higher than that of control reactors (14.73%±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 147.26% higher than control reactors. These phenomena allowed RS reactors to generate more methane within 25 days of digestion process compared with CH_4 in control reactors. ANOVA test also revealed that there is an interaction between factors (substrates and percent TS) with COD removal (p value = 2.44×10^{-7}; F_{test} = 26.5; F_{crit} = 2.927; df = 4). This condition may indicate that there is a relationship as well as influence between TS applied in the digesters and COD removal.

Table 9 Efficiency of digestion at 2% TS concentration

Analysis	Unit	Control (digested swine manure)	Rice straw
VS reduction	%	14.73 ±1.02	35.67 ±2.41
COD removal	%	20.25 ±5.02	50.07 ±1.06
Methane yield	mL CH_4/g VS added	104.08 ±4.37	119.34 ±4.59
Total methane production	mL	379.67 ±15.95	977 ±37.58

Table 10 summarizes anaerobic digestion efficiency operated at 3% TS. As can be observed, RS reactors had the highest methane yield ((141.4±3.7) mL CH_4/g VS added), which was 41.8% higher than control reactors. Furthermore, good performance of RS reactors ran at 3% TS was also shown in the percentage of COD removal, where they gained (52.97%±1.46%), which was higher than control reactors by 52 %. In addition, RS reactors also had the highest VS reduction (61.81%±1.04%), which was three times of that of control digesters. These phenomena enabled RS reactors to reach the highest cumulative methane production within 31 days of the digestion process, where in terms of total methane production, they gained (1 814±47.43) mL, which was extremely higher compared with control reactors (386± 10.54) mL CH_4, where RS generated methane at around 370% higher than control reactors.

Table 10 Efficiency of digestion at 3% TS concentration

Analysis	Unit	Control (digested swine manure)	RS
VS reduction	%	18.90 ±3.00	61.81 ±1.04
COD removal	%	34.85 ±2.12	52.97 ±1.46
Methane yield	mL CH_4/g VS added	99.79 ±2.72	141.44 ±3.70
Total methane production	mL	386 ±10.54	1814.33 ±47.43

Table 11 shows digestion efficiency obtained from the 4% TS process. As can be observed, RS reactors had a lower methane yield (55.44±2.69) mL CH_4/g VS added compared with control reactors ((90.98±4.90) mL CH_4/g VS added). RS reactors had higher VS reduction (59.7%±0.2%) compared with control reactors (22.69%±

0.16%); however, they had lower COD removal (7.34%±5.4%), which was about 128.61% lower than control reactors (16.78%±6.8%). It is known that RS reactors still generated more cumulative methane within 31 days of digestion process compared with control reactors. However, the low methane yield as well as COD removal revealed that they experienced issues in the digestion process. Moreover, it also can be known by comparing total methane production of RS operated at 4% TS with that of 2% and 3% TS concentrations (Tables 9, 10, and 11).

Table 11 Efficiency of digestion at 4% TS concentration

Analysis	Unit	Control (digested swine manure)	RS
VS reduction	%	22.69 ±0.16	59.71 ±0.19
COD removal	%	16.78 ±6.82	7.34 ±5.40
Methane yield	mL CH_4/g VS added	90.98 ±4.90	55.44 ±2.69
Total methane production	mL	400.67 ±21.59	962.67 ±46.70

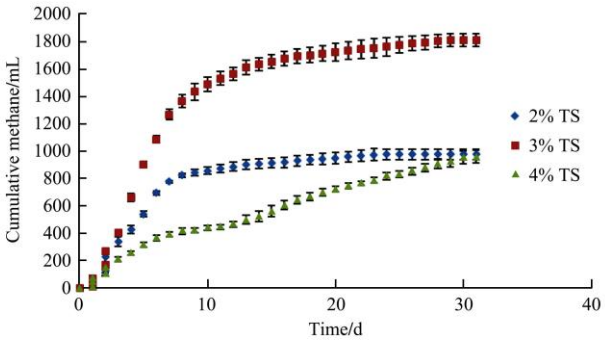

Figure 4 Cumulative methane production of rice straw with different TS concentrations

In terms of total methane production, it was revealed that RS reactors operated at 4% TS, generated methane 1.49% lower than that at 2% TS, and 88.47% lower than RS run at 3% TS. Furthermore, ANOVA analysis also showed that at the 5% level there was a statistically significant difference between percent TS applied and biodegradable parameters (COD removal, methane yield and VS Reduction) in anaerobic digestion of rice straw (p value = 9.94×10^{-20}; F_{test} = 447.96; F_{crit} = 3.403; df = 2). This is very obvious that RS run at 4% TS did not perform very well due to solid accumulation that lead to lower digestion efficiency. This condition occurs since higher TS as well as VS loaded into the digester may generate a lot of VS in the digester that may affect the alkalinity of the digester.

Higher TS concentration applied to the reactor also can influence the volatile loading rate in the available detention or the retention time period. Thus, sufficient time (retention time) should be allowed for the micro-organisms to degrade the organic materials and convert it into biogas[6, 56]. In addition, a previous study also revealed that there is an upper limit for TS content applied in anaerobic digestion, above which the material was not considered slurry capable for processes such as mixing[55].

4 Conclusion

A number of total solids concentrations (2%, 3% and 4% TS) were tested to assess methane production potential of individual substrates. RS showed better performance for all TS concentrations tested. RS generated the most methane at 3% TS which was around (1 814 ±47.43) mL where the C:N ratio of RS was 10.6:1. RS still produced more methane at 2% TS than 4% TS even though at 2% TS, methane production was completed in 25 days of digestion. This occurred since in the mixture with 4% TS, RS had issues of solid accumulation in the digester that led to improper mixing during the digestion process and required a longer retention time to convert biomass into methane. Biodegradation efficiency was evaluated for each substrate. RS had the highest methane yield at 3% TS, which was around (141.4 ± 3.70) mL CH_4/g VS added. RS also had the highest COD removal and VS reduction at 3% TS which were around (52.97% ± 1.46%) and (61.81% ± 1.04%), respectively. These results may indicate that 3% TS is an optimum condition for RS to produce methane with a stable anaerobic digestion process.

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