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# Development of a High-Efficiency Methane Fermentation Process for Hardly Degradable Rice Straw

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### Abstract

In order to develop the new and high-efficiency methane fermentation process for hardly degradable rice straw, the co-digestion performance of different amount of rice straw and sewage sludge was investigated. The digestion of rice straw and sewage sludge under the investigated condition resulted in VFA (volatile fatty acid) accumulated in the digester. The influences of temperature, moisture content, the ratio of carbon to nitrogen (C:N ratio) and initial pH value on VFA accumulation in the hydrolysis step of methane fermentation were tested. The results showed that the total VFA concentration was increased with initial pH value and moisture content, and was decreased with temperature and the ratio of carbon to nitrogen. Then the feasibility of rice straw pretreatment with the accumulated VFA from the fermentation process was studied, and 10.7% of weight loss was obtained by pretreatment with drip washing VFA solution. The production of methane gas from rice straw was significantly enhanced as nearly as two times by drip washing VFA solution pretreatment than that of untreated rice straw. This suggested that the accumulated VFA from the rice straw fermentation process can be used as a kind of pretreatment reagent for improving biodegradability and methane production. So a low-cost and high-efficiency methane fermentation process for hardly degradable lignocellulosic materials such as rice straw could be established.

#### Introduction

Anaerobic digestion has become one of the major treatment techniques for municipal sewage sludge and manure. In many cases of the world, methane recovered from digestion supplied sufficient energy to

support wastewater treatment plants and farms. And in fact, methane generated from organic wastes has already provided all daily energy requirements for residences in some villages in China (Cui and Xie, 1985). Despite all of advantages of anaerobic digestion process, however, major obstacles still remain to be resolved for the practical application of methane fermentation for an important group of waste --- the lignocellulosic materials, which include forestry products, agricultural crop residues, and urban refuse. Anaerobic digestion of these materials is often a slow and frequently incomplete process, which in most cases makes methane production from these materials uneconomical at current energy prices, because these materials are predominantly composed of various polymers which include cellulose, hemicellulose and lignin in chemical which intimately interconnected and form the complex lignocellulosic structure. Besides this, other factors which are also important in methane production include acidification (Hill et al., 1987), ammonia concentration (Sung and Liu, 2003; Hansen, K.H., 1998), digestion conditions (Zennaki et al., 1996; Sharma et al., 1988; Bardiya and Gaur, 1997; Sundrarajan et al., 1997; Singh et al, 1995), and the nutritional requirements of microbes (Kayhanian and Rich, 1995). However, how to enhance the gas production rate from lignocellulosic material and reduce the digestion failure resulting from the acid accumulation or ammonia inhibition are poorly understood.

Since lignocellulosic materials include literally thousands of different plant species and crop residues, it is by no means that this research would be able to deal with every single material. Several representative lignocellulosic materials were therefore selected for detailed study (Forster-Carneiro, 2007; Vedrenne, 2008; Ting, 2007; Sosnowski, 2003). The criteria of selection were twofold: first, materials representing the broad range of characteristics of lignocellulosic materials were desired so that the influence of material property on biodegradation could be investigated; second, materials having good potential as feed stocks for methane production at commercial scale were sought.

On the other hand, biological wastewater treatment has been used widely in the world, but large amounts of sewage sludge are produced in this process. Rapid urbanization in many areas of the world has resulted in a drastic increase of sewage sludge with a typical person generating over 50 g of dry solids every day (Hudson, 1995). In China alone, about 0.88-1.55 million tons of dry sludge was produced in 2003 (Liu, 2008). The treatment and disposal of sludge have become one of the most important and complex problems. In fact, the main part of sewage sludge is organics (biomass) and it can become a source of energy (e.g., methane). The main component of sewage sludge is also microbial cells, so that it can be used as the inoculum of the fermentation. So in the present study, rice straw and sewage sludge was selected as the substrate of the fermentation.

Anaerobic digestion of lignocellulosic materials is a very complex process, not only because of bacteria diversity and process complexity, but also because of the nature of the complex polymeric structure of the materials themselves. Previous studies have focused on various aspects of the digestion process. The aim of this research is to optimize the fermentation conditions of acid accumulation and to discuss the fermentation effect of materials pretreated by the drip washing solution which obtained from acid fermentation process. Then, the results obtained from this and other research are applied to the appropriate design of a new high-efficiency methane fermentation process for methane production from lignocellulosic materials.

## *Materials and methods* Fermentation of rice straw

Rice straw used in this study was collected from field near Tsukuba (Ibaraki, Japan) in 2008. The airdried rice straw was cut into small pieces and further milled to powder. The particles having a size between 40-mesh (0.45mm) and 50-mesh (0.36mm) were stored in plastic bag at room temperature until further processed. The sewage sludge (TS=1.77%, VS=79.5%TS) from Kasumigaura sewer office (Ibaraki, Japan) was used as original seed methanogens sludge. It was kept in the refrigerator at 4°C before the fermentation experiment.

The batch fermentation experiment was conducted with rice straw and sewage sludge. Fermentor bottles (500 mL, SIBATA) were used as batch fermentation reactors. 148 g sewage sludge was added into the bottle along with 14 g, 26 g and 40 g of rice straw, respectively. Fermentation of sewage sludge only was used as the control. These two materials were mixed and the pH value of mixture was adjusted to 7 by sodium hydroxide. The bottles were stopped with silica gel stoppers, which were air impermeable even after repeated sampling with syringes. All of bottles were initially flushed with nitrogen gas prior to fermentation test, and then they were incubated at 35°C for 30 days. Gas production and composition from each bottle were monitored periodically every 3 days. Sampling was conducted in a glove-box (COY Laboratory Products, USA).

#### Acidogenic fermentation

The composition of rice straw was as follows: total solids (%, w/w) 92.2, volatile solids (%, TS) 81.9, carbon (%, dwb) 35.11, hydrogen (%, dwb) 5.47, nitrogen (%, dwb) 0.87, where dwb means dry weight basis. The sewage sludge was obtained from Kasumigaura sewer office (Ibaraki, Japan) and used as inoculum. Its composition was as follows: total solids (%, w/w) 1.77, volatile solids (%, TS) 79.5, carbon (%, dwb) 36.36, hydrogen (%, dwb) 4.10, nitrogen (%, dwb) 5.76.

Four batch fermentation experiments were conducted to assess the fermentability of the rice straw and sewage sludge to determine the amount of organic matters that could be converted into VFA. Fermentor bottles (500 mL, SIBATA) were used as batch fermentation reactors. Here, sewage sludge was mixed with rice straw by the ratio of 1:3.8 and added into the bottle. Then the pH value of mixture was adjusted to 6, 7, and 8 by sodium hydroxide, respectively. All of bottles were initially flushed with nitrogen gas prior to fermentation test, and then they were incubated at  $35^{\circ}$ C or  $55^{\circ}$ C for 30 days.

## Preparation of drip washing solution

In order to obtain drip washing solution, batch acidogenic fermentation experiment with rice straw (<60-mesh) and sewage sludge (w/w=1:3.8) was conducted at 35°C. After the pH value of sludge was adjusted to 7.0 by sodium hydroxide, it was mixed with rice straw and then fed into the fermentor bottles (500 mL, SIBATA). Then the fermentors were flushed with nitrogen gas for 2 min to exclude oxygen. The fermentation was operated at 3 days HRT because the results of preliminary experiment showed that the acid concentration reached the maximum value at the third day (data was not shown). For the production of the drip washing solution, distilled water was added into fermentors and kept for 1 h at room temperature. Then the liquid fraction (drip washing solution) was subsequently separated from solid material by screening filter (60-mesh). The drip washing solution was kept at 4°C in the refrigerator for the anaerobic digestion of rice straw.

## Analyses

Both rice straw and sewage sludge contain varying amounts of water. The oven-drying method was used to determine their moisture content (or total solid). A certain amount of sample were weighed and placed in a porcelain crucible, and then the sample (with stopper removed) was placed in a 105°C oven (NRG-1400, Nihon Freezer Co., Ltd.) and dried to constant weight overnight. The sample was then placed in a desiccator for cooling and then weighed again. The moisture content, represented by the loss in weight at 105°C, is reported as percentage of the original sample weight. Analyses were performed in triplicate. The air-dried sample of rice straw and sewage sludge was grounded into the powder and their carbon and nitrogen content was analyzed by element analyzer (Perkin Elmer JST 2400).

The ash content is a measure of the inorganics present in the materials. The analysis was carried out using porcelain crucibles. The sample was first dried and weighed, and then fired in a 600°C-muffle furnace (Fuji Electronics Industry Co., Ltd.) overnight. The final residual remaining in the crucible is reported as the ash content (dry weight basis). The lost weight is reported as the volatile solid.

Biogas analyses of fermentor headspace were performed using a chromatography (GC-8A, SHIMAZU, Japan) equipped with a thermal conductivity detector (80°C) and a Porapak Q column (60°C). Nitrogen gas was used as carrier gas at a flow rate of 1 mL/min. Certified gas standards were employed for the standardization of hydrogen, methane and carbon dioxide. Standards were run on each day that samples were measured. Each sample was analyzed at least twice, and a 1 mL volume of gas was injected for analysis.

About 1 g of substrate was taken out from the fermentor and was added into 10 mL of distilled water. The mixture was then centrifuged in a centrifugal machine. The supernatant was quantified by HPLC (Jasco International Co., Japan), equipped with UV/ VIS detectors after filtrated by 0.45  $\mu$ m membrane. Formic acid, acetic acid and propionic acid in the samples were determined by a Inertsil ODS-4 column at 40°C. 0.05 mol/L of ammonium dihydrogen phosphate solution (pH=2.4) was used as eluent at a flow rate of 1.0 mL/min.

## **Results and discussion**

# Performance of rice straw and sewage sludge anaerobic digestion

The chemical composition of the mixture of rice straw and sewage sludge is given by Table 1. The amount of sewage sludge in four fermentors is same, while the addition amount of rice straw is 14, 26, and 40 g, respectively. According to the calculation, the

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	Fermentor NO.1	Fermentor NO.2	Fermentor NO.3	Fermentor NO.4
Rice straw (g)	0	14	26	40
Sewage sludge (g)	148	148	148	148
The initial pH value	7.0	7.0	7.0	7.0
Moisture content (%)	98.2	90.0	85.0	80.0
C:N ratio	6.3	21	26	30

Table 1. Chemical composition of mixture of rice straw and sewage sludge.

moisture content and ratio of C: N are in the range of 98.2% to 80.0% and 6.3 to 30, respectively. Because the sewage sludge was acidic, and its pH value is 6.4, the initial value of pH of the substrate was adjusted to 7 by sodium hydroxide.

Fig. 1 showed the methane production of different fermentors. In 30 days, sewage sludge (fermentor NO.1) produced methane gas continuously and the total volume of methane was 270 mL. But with the addition of rice straw (fermentor NO.2, NO.3 and NO.4), the cumulative methane production of substrate in these four fermentors was decreased gradually. At the end of the fermentation process, they were achieved 64, 27, and 12 mL for the addition of 14, 26,

and 40 g rice straw, respectively. It is obviously that no methane gas was released from the degradation of the rice straw, and it can be presumed that the addition of rice straw has negative effect on the production of the methane gas.

Fig. 2 showed the biogas production of different fermentors. Similar to methane production, sewage sludge produced biogas continuously in the whole fermentation process, and its total volume achieved 487 mL. In the contrary case, gas production with rice straw addition is mainly concentrated in the first 10 days. The cumulative biogas production was 397, 755, and 880 mL, respectively. Comparison with biogas production of sewage sludge, the production of

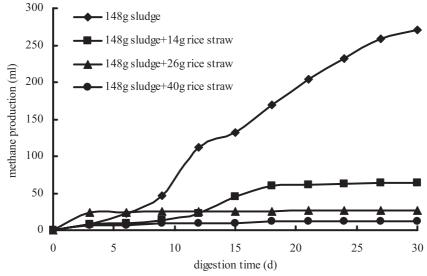


Fig. 1. Cumulative methane production with different rice straw addition.

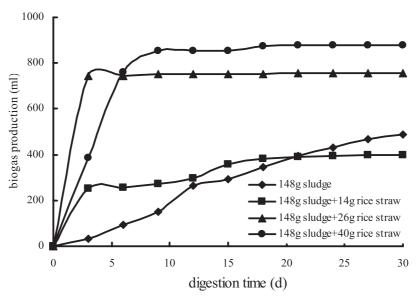


Fig. 2. Cumulative biogas production with different rice straw addition.

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rice straw addition increased. This indicated that rice straw was also degraded in the fermentation process, and released a certain volume of biogas. The more addition amount of rice straw was added, the more biogas was produced. But combined with the result of methane production, the majority of the biogas produced from rice straw should be hydrogen and carbon dioxide. It is well-known that the first step of the degradation of rice straw is hydrolysis, and the main product is hydrogen. Therefore, large quantity of the hydrogen was released in the earlier stage, and the carbon dioxide was released in the later stage.

Figs. 3, 4, 5 and 6 showed the component of the biogas in each experiment. For the fermentation of sewage sludge, no hydrogen gas was produced in the whole fermentation process, and the methane gas accounted for 70% of the biogas. For the 16 g

addition of rice straw, the hydrogen gas could be detected just in the first 3 days, and the methane gas could also be detected in the whole process while its percentage just achieved 57%. For the 24 g addition of rice straw, hydrogen, methane and carbon dioxide was exist from the beginning to the end. The highest value of hydrogen was 46% on the third day and the methane achieved 39% at the end of the fermentation. For the 40 g addition of rice straw, hydrogen gas accounted for 27% at the end of the fermentation and the methane gas was not higher than 20% in the whole process.

Fig. 7 showed the change of pH value of four fermentors in the fermentation process. The pH value of sludge fermentation was maintained at 7.4 while three others were maintained at 4.7, 5.2, and 5.5 at the end of fermentation process, respectively. This

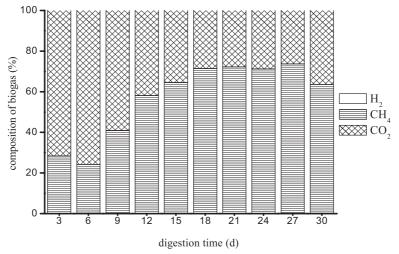


Fig. 3. Composition of biogas produced in fermentor NO.1.

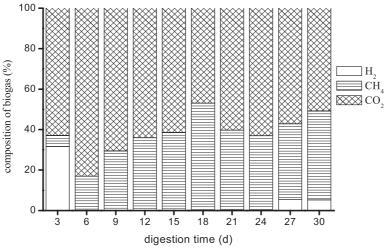


Fig. 4. Composition of biogas produced in fermentor NO.2.

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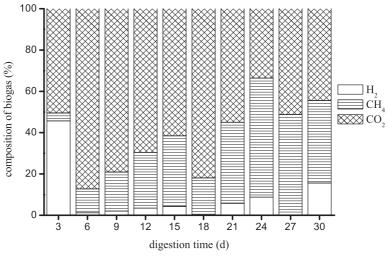
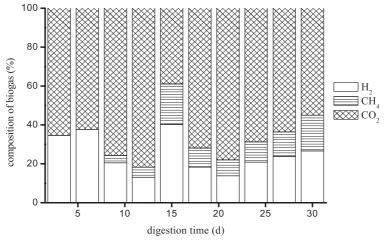
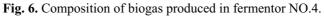


Fig. 5. Composition of biogas produced in fermentor NO.3.





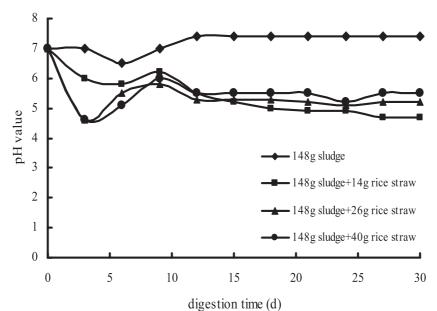


Fig. 7. Change of pH in the fermentation process.

resulted from the production of volatile fatty acid which generated in the hydrolysis of rice straw. It means that acidification was occurred in the fermentor NO.2, NO.3 and NO.4, the degradation of rice straw resulted in the acidification of the fermentation system.

The co-digestion of sewage sludge and different amount of rice straw indicated that digestion of sewage sludge produced methane gas continuously while the addition of rice straw decreased the methane production but increased the hydrogen production. The degradation of rice straw made contributions to the production of VFA and hydrogen.

# Acidogenic fermentation of rice straw and sewage sludge

Based on the presented study, three major factors which affect the methane fermentation with rice straw and sewage were chosen to be studied here: (1) temperature, (2) initial pH value, (3) moisture content, (4) C:N ratio. A better understanding of how these factors control the hydrolysis of rice straw and the VFA production should improve knowledge about the mechanisms of lignocellulosic degradation, the design of methane fermentor, and the understanding of best environmental conditions for reactor operation. In order to find out the optimal conditions for the acid accumulation and to obtain the drip washing solution with concentration as high as possible, acidogenic fermentation of rice straw and sewage sludge was carried out.

The effect of temperature on acidogenesis of rice straw and sewage sludge was showed in Table 2. For the acidogenic fermentation at 35°C, acetic, propionic and butyric acid had been detected in the fermentor. The concentration of acetic acid was increased continuously while that of propionic and butyric acid had no obvious change from the third day. The highest value of these three acids was 18525, 15315 and 10356 ppm, respectively. The TVFA achieved its highest value of 44197 ppm on the sixth day, and the acetic acid accounted for the main content.

For the acidogenic fermentation at 55°C, besides acetic, propionic and butyric acid, formic acid had also been detected in the fermentor. The concentration of formic and butyric acid had no obvious change in the whole fermentation process, while the acetic acid concentration was continuously increased and that of propionic acid was decreased. The TVFA concentration achieved its highest value of 42312 ppm on the seventh day. At that time, acetic acid accounted for the maximum proportion of 41.6%, then was propionic and butyric acid which accounted for 22.8% and 23.7%, respectively, and the least one was formic acid accounted for 12.0%.

Compare this two fermentor's performance, the concentration of acetic acid and butyric acid was nearly same but that of propionic acid at 35°C was much higher than that at 55°C. That is to say, more acid was produced at 35°C. Therefore, 35°C is suitable for the VFA accumulation. This coincides with the VFA distribution observed by Penaud et al. (1997) who reported a change in VFA composition due to temperature and, more specifically, higher percentages of propionate at lower temperature.

Effect of initial pH value on acidogenesis of rice straw and sewage sludge was presented in Table 3. The acid products of acidogenic fermentation at initial pH of 6 were as follows: formic, acetic, propionic and butyric acid. The highest value of acid concentration was obtained on the sixth day, and they were 4312, 13703, 13638, and 11120 ppm, respectively. The TVFA concentration was achieved 42775 ppm on that day.

**Table 2.** Effect of temperature on acidogenesis of rice straw and sewage sludge.

	Temperature (°C)	
	35	55
TVFA (ppm)	44197	42312
Formic acid (ppm)		5084 (12.0%)
Acetic acid (ppm)	18524 (41.9%)	17583 (41.6%)
Propionic acid (ppm)	15315 (34.7%)	9631 (22.8%)
Butyric acid (ppm)	10356 (23.4%)	10013 (23.7%)

The acids produced at the initial pH of 8 were just acetic, propionic, and butyric acid, formic acid had not been detected. At pH of 8, TVFA concentration was 49123 ppm on the fourth day, which was the highest value obtained in the whole fermentation period. The highest value of acetic, propionic and butyric acid was 21519, 15581, 12024 ppm, respectively.

For the different initial pH value, the acid concentration increased with the pH value increased. In particularly, acetic acid concentration increased sharply. The concentration of propionic acid and butyric acid had a slightly increase with the increase of the pH value. In totally, the total concentration of VFA increased as the pH value increased. This is coincided with the other reseachers such as Lim (2008) and Bengtsson (2008) that high pH value is benefit to the production of VFA.

Effect of moisture content on acidogenesis of rice straw and sewage sludge was presented in Table 4. For these five experiments, acetic, propionic and butyric acid were detected. The highest value of TVFA at 70% moisture content was achieved 22367 ppm at sixth day, and the VFA speciation results indicate that acetic and butyric were the most prevalent VFA generated throughout the experiment.

For other four experiments, the highest value of VFA at 75%, 80%, 85% and 90% moisture content were 20178, 13408, 8508, and 6939 ppm, respectively. Acetic and butyric acid were also the most prevalent VFA generated.

According to above results, it can be concluded that TVFA concentration increased with increasing

	pH		
	6	7	8
TVFA (ppm)	42775	44197	49123
Formic acid (ppm)	4312		
	(10.1%)		
Acetic acid (ppm)	13703	18524	21519
	(32.0%)	(41.9%)	(43.8%)
Propionic acid (ppm)	13638	15315	15581
	(31.9%)	(34.7%)	(31.7%)
Butyric acid (ppm)	11120	10356	12024
	(26.0%)	(23.4%)	(24.5%)

Table 3. Effect of initial pH value on acidogenesis of rice straw and sewage sludge.

Table 4. Effect of moisture content on acidogenesis of rice straw and sewage sludge.

	Moisture content (%)				
	70	75	80	85	90
TVFA (ppm)	22367	20178	13408	8508	6939
Acetic acid (ppm)	6932 (31.0%)	6324 (31.3%)	4221 (31.5%)	2978 (35.0%)	2367 (34.1%)
Propionic acid (ppm)	3682 (16.5%)	2930 (14.5%)	2510 (18.7%)	2059 (24.2%)	1199 (17.3%)
Butyric acid (ppm)	11752 (52.5%)	10925 (54.1%)	6677 (49.8%)	3472 (40.8%)	3374 (48.6%)

moisture content. This suggests that low moisture content is benefit for the acid accumulation. It may be explained by the fact that low moisture content has bad fluidity, and it is easily result in partial acidification in the fermentor. On the contrary, the moisture in substrate will promote the uniform distribution of the acids so that the acid concentration was decreased.

Table 5 showed the effect of C:N ratio on acidogenesis fermentation. For these three experiments, the VFAs identified include acetic, propionic, and butyric acid. The highest value of VFA was achieved 19841 ppm at C:N ratio of 10, while that of other two conditions were 18042 ppm at C:N ratio of 20 and 11999 ppm at C:N ratio of 30. Acetic was the most prevalent VFA generated for all of three experiments. The results showed that the VFA concentration decreased with the increasing C:N ratio. The higher VFA concentration was achieved at low C:N ratio.

# Methane fermentation of rice straw pretreated by drip washing solution

In experiment, 200 ml drip washing solution was

obtained. The HPLC results showed that the acid components in drip washing solution was mainly composed of four kinds of organic weak acids, formic acid, acetic acid, propionic acid and butyric acid. Its concentrations were 0.21, 9.43, 1.27, 0.53 g/L, respectively. It was obvious that the concentration of acetic acid is much higher than other components. Acetic acid was the main acid part of the drip washing solution. The rice straw was then pretreated by actual drip washing solution which obtained from an acidification fermentor for 2 h at 1:20 of solid to liquid ratio. The weight loss was achieved 10.7%.

Methane production from rice straw which is untreated and pretreated by actual drip washing solution was carried out at 35°C for 30 days. The fermentation of sewage sludge was also conducted as control under the same condition. Fig. 8 showed methane productivity of the fermentors with addition of 1.0 g untreated or pretreated dry rice straw. The data were averages of three replicates. The final methane productivity of pretreated rice straw achieved  $1.21l_{ch4}/l_{fermentor} d$ while that of untreated rice straw was just  $1.13l_{ch4}/$ 

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Table 5. Effect of C:N ratio	on actuogenesis	of fice straw an	a sewage sludge.

	C:N ratio		
	10	20	30
TVFA (ppm)	19841	18042	11999
Acetic acid (ppm, %)	13703, 71.4	7976, 44.2	6614, 55.1
Propionic acid (ppm, %)	3913, 19.7	4569, 25.3	2731, 22.8
Butyric acid (ppm, %)	1764, 8.9	5497, 30.5	2654, 22.1

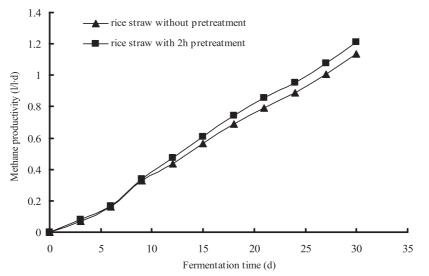


Fig. 8. Methane productivity of rice straw pretreated by drip washing solution.

 $l_{fermentor} \cdot d$ . Before 12 days, the methane production of two fermentors was almost same while after that there is a marked increase in the methane volume of the pretreated substrate than that of untreated rice straw. The methane gas released in former 12 days perhaps mainly come from the degradation of volatile organic compound in the sewage sludge because it is relatively easy degraded in the comparison with rice straw. On the other hand, methanogens could rapidly increase in this period for microbial degradation of the rice straw in the next step. From the 12<sup>th</sup> day, the methane productivity of these two substrates shows different. Table 6 presented total gas volume evolved from sewage sludge and 1 g of rice straw with and without pretreatment. For the untreated rice straw, the total methane production of 1 g of pretreated sample was just 73 mL until the 30th day, while the final the methane production of acid pretreated rice straw was increased up to 154 mL. The total biogas volume was also increased from 106 mL to 227 mL after pretreatment. Compared to untreated rice straw, the methane gas production of pretreated rice straw was increased as nearly as two times. The difference between the methane production and biogas production of untreated and pretreated rice straw was evaluated by ANOVA using Microsoft Excel, and the result showed that both methane gas production and biogas production of pretreated samples was significantly (p  $\leq 0.05$ ) higher than that of untreated one. The present results showed that the drip washing solution pretreatment was a beneficial as a method to improve the anaerobic biodegradability of biomass.

#### Conclusions

The digestion of sewage sludge produced methane gas continuously while the addition of rice straw decreased the methane production but increased the hydrogen production. It is clear that digestion of rice straw and sewage sludge under the investigated condition resulted in the acidification more easily. The degradation of rice straw made contributions to the production of VFA and hydrogen.

For the pretreatment by actual drip washing solution obtained from an acidified methane fermentation process, 10.7% of weight loss was achieved. The production of methane gas from rice straw was enhanced as nearly as two times by pretreatment. This also illustrated that actual dripping solution with low concentration can be used as a kind of pretreatment method for improving biodegradability of rice straw.

In the hydrolysis step of methane fermentation with rice straw and sewage sludge, was increased with initial pH value and moisture content, and was decreased with temperature and the ratio of carbon to nitrogen. The increase of temperature mainly resulted in the decrease of propionic acid, and the increase of pH value mainly due to the increase of acetic acid. Higher moisture content resulted in the higher percentage of butyric, while higher C:N ratio led to higher percentage of acetic acid. The optimal conditions for VFA accumulation were 35°C, initial pH of 8, moisture content of 70% and C:N ratio of 10.

This research has attempted to investigate the acidification performance of methane fermentation, and tried to enhance methane productivity from hardly degradable rice straw by using drip washing VFA solution obtained from an acidified methane fermentation system. It could be possible to develop a lowcost and high-efficiency methane fermentation process for hardly degradable rice straw.

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	CH₄ production (mL)	biogas production (mL)
Sewage sludge	1297±16	1909±12
Sewage sludge + untreated straw	1370±15	2015±18
Sewage sludge + pretreated straw	1451±20	2136±18

Table 6. Total gas volume evolved from sewage sludge and 1 g of rice straw.

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