



## Regular article

# Effect of pretreatment solutions and conditions on decomposition and anaerobic digestion of lignocellulosic biomass in rice straw

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## HIGHLIGHTS

- Decomposition of rice straw is observed with all pretreatment methods.
- Sulfuric acid pretreatment inhibits subsequent anaerobic biodegradation.
- Hot water and alkali pretreatment are better for enhancing biodegradability.
- Hot water pretreatment substantially enhances methane production potential.

## ARTICLE INFO

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## ABSTRACT

This study assessed the effects of hot water, acid, and alkali pretreatments on the lignocellulosic composition of rice straw and methane production potential of the pretreated solids-reagent mixture. Autoclaving (121 °C, 1.45 atm, 60 min) after addition of 2% H<sub>2</sub>SO<sub>4</sub> showed the highest lignocellulose decomposition efficiency of 65.4%. However, the methane production potential was even smaller than that of the untreated sample, indicating the inhibitory effect of the acid in the biogas production process. On the other hand, hot water- and alkali-pretreated samples showed a factor of 2.1 greater methane production potential than untreated sample despite the relatively lower lignocellulose decomposition efficiency of 17.0–50.4%. By simply keeping the rice straw immersed in water at 100 °C for 30–60 min. or autoclaving prior to anaerobic digestion, the methane production potential of 805.8–824.2 mL of CH<sub>4</sub>/g total carbon and the overall lignocellulose degradability of 73.6–84.4% was achieved. The results of this study show the potential of hot water pretreatment as an efficient, chemical-free method to improve the feasibility of methane production from rice straw.

## 1. Introduction

Rice straw is one of the most abundantly produced agricultural residues considering the worldwide dependence of rice as a food source [7,10,16,17]. Although rice straw is conventionally used as animal feed, heat retaining cover for trees and crops, fiber for producing newsprint [10] and so on, the current utilization practices fail to account for the immensely produced amount, resulting in the disposal of a large proportion as waste [11]. This calls for the development of energy generating processes to utilize rice straw such as methane production via anaerobic digestion [3,5,7,11,14].

As a substrate for anaerobic digestion, rice straw has both opportunities and challenges. Rice straw mainly consists of cellulose and hemicellulose (50–80% of dry mass [4,27]), which are relatively easily biodegradable fraction of plant biomass. The fraction of the biodegradation-resistant lignin in rice straw is 10–15% of the dry mass,

which is a moderate value among the lignin fractions of common agricultural biomass [4,27]. Lignin acts as a protective barrier for the plant biomass to enzymatic attack, which leads to the low hydrolysis efficiency of cellulose and hemicellulose in rice straw, and, in turn, leads to the relatively low feasibility of biological methane production from rice straw by anaerobic digestion [4,24,36]. Therefore, an effective pretreatment method to enhance the hydrolysis efficiency of rice straw needs to be developed.

Chemical and thermal pretreatment of rice straw improves the performance of the subsequent anaerobic biological processes [25,26,30,31,37]. Pretreatment of lignocellulosic components of rice straw has been shown to significantly enhance methane production in the anaerobic digestion process [19,24,30] and conversion efficiency to other valuable organic products as well [2,25,26]. The pretreatment methods demonstrated for effectiveness include acid (e.g. [8,28,33]), base (e.g. [7,27,30]), or hot water (e.g. [18,38,42]), application to the

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raw material in the presence or absence of heating and pressurization.

Despite the relatively large body of literature on the lignocellulosic biomass pretreatment, studies are rare that comprehensively evaluated the effect of the pretreatment methods. Most studies reported in the literature focused on the optimization of one type of the pretreatment approaches (i.e., either acid, base, or hot water) rather than comparing the effectiveness across the different pretreatment types (e.g., [8,23,28,30,38]). These studies mostly employed chemical analyses (e.g., sugar yield) only for the evaluation of the pretreatment efficiency without further investigating the eventual effect on the subsequent biological biomass valorization process (i.e., biological analyses) (e.g. [30,38]). To the best of our knowledge, no studies have comprehensively investigated the effectiveness of acid, base, and hot water pretreatment employing both chemical and biological analyses using rice straw as a sole substrate.

The mechanism and effect of pretreatment to enhance the lignocellulosic biomass biodegradability may be different for each pretreatment approach. Acidic approaches have been proven to be effective for degrading hemicellulose [2,28,33,35]. Concentrated acids were shown to hydrolyze a substantial fraction of cellulose as well [32,33]. Dilute sulfuric acid pretreatment not only solubilizes hemicellulose but also enhances the availability of cellulose for enzymatic hydrolysis by removing the protecting shield of lignin and hemicellulose [28]. The mechanism of alkali pretreatment can be explained by saponification of intermolecular ester bonds with crosslinked structure of xylan hemicelluloses and lignin or other hemicellulose [32]. The porosity of lignocellulosic materials increases with the removal of the crosslinks and the lignin structure may be further decomposed [32]. Alkali treatments are also known for their high efficiency to reduce the crystallinity of cellulose so that the enzyme accessibility to cellulose is increased [12,30,37]. Pretreatment by hot water under high pressure can hydrate cellulose and remove hemicellulose and some fraction of lignin [34,39,40]. The cellulose crystalline structure can be changed by disrupting the hydrogen bonding of cellulose chains by simple hydrothermal pretreatment methods [37]. The difference in the mechanism and effect among the different pretreatment approaches suggests that i) a separate evaluation and optimization of the pretreatment method is needed for each type of lignocellulosic biomass and ii) single chemical analysis (e.g., sugar yield or reduction of measurable lignocellulose content) may not be sufficient for cross-comparison of the various pretreatment approaches.

To address the research needs described above, the present study is aimed to investigate and compare the effect of the three different pretreatment approaches – hot water, acid, and alkali – under various conditions on the anaerobic digestion process targeted on methane production. The changes in lignin, cellulose, and hemicellulose by the pretreatments are analyzed individually, and the pretreated biomass-reagent mixture is submitted to the downstream process of anaerobic digestion to investigate the lignocellulose degradability and its effect on methane production potential.

## 2. Materials and methods

An overview of the experimental procedure applied in the current study is depicted in Fig. 1. The procedure is described in detail in the following.

### 2.1. Preparation and analysis of substrate and inoculum

Rice straw was obtained from Korea Rural Development Administration (Seoul, Korea). The raw material received was cut to small pieces and ground using a mortar and pestle. The ground material was then sieved to obtain < 2 mm size and dried at 60 °C for 24 h. Digested sludge was obtained from Jungnang Wastewater Treatment Plant (Seoul, Korea). The sludge was sieved with 0.5 mm openings and stored at 4 °C until use as a microorganism seed. The total solids (TS)

and volatile solids (VS) contents, and lignin, cellulose, and hemicellulose contents of the rice straw were reported in a previous study [11] as given in Table 1.

### 2.2. Pretreatment methods

Three different thermal and/or chemical pretreatment methods, hot water, acid, and alkali pretreatment, were applied to rice straw following the procedures suggested in the literature [1,8,13,31,34,41,42] with slight modifications. For each pretreatment method, the reaction time, temperature, pressure, and concentration of chemical agent were varied strategically to observe the effect of the pretreatment conditions. In tests designed to determine the changes in lignin, cellulose, and hemicellulose contents after the pretreatment, 50 g dry weight rice straw and 500 mL liquid agent were added to a 1 L bottle for initial solid to liquid (SL) ratio of 1:10. For preparing samples for batch anaerobic digestion tests, 0.50 g VS of rice straw (0.576 g in dry weight) and 5.76 mL liquid agent were added (SL ratio of 1:10) to a 600 mL serum bottle.

For hot water pretreatment, deionized (DI) water was used as a liquid agent. The prepared bottles were placed in an oil bath set at 100 °C with the caps slightly open for hot water pretreatment without pressure control. Two different reaction time periods, 30 and 60 min, were tested. Autoclaving at 121 °C for 60 min. under 1.45 atm is applied for an additional set to identify the effect of pressurization. Acid pretreatment was also applied both without pressure control and with controlled pressurization. For acid pretreatment without pressure control, 0.01% and 0.2% H<sub>2</sub>SO<sub>4</sub> solutions in DI water were used as liquid agents, and the bottles were heated in an oil bath set at 120 °C for 60 min. An extreme condition of 2% H<sub>2</sub>SO<sub>4</sub> solution as an agent and autoclaving at 121 °C for 60 min. under 1.45 atm was used as pressurized acid pretreatment following the suggestion in the literature [1,2]. Ammonia (NH<sub>3</sub>) solutions at concentrations of 1% and 5% were used for alkali pretreatment. The mixture of rice straw and the alkaline agent was heated to 35 °C for 24 h.

The volume of the liquid agents was reduced due to water evaporation during the pretreatment procedure. However, the pretreated samples still contained more than 1/3 of the initially added liquid agent volume.

### 2.3. Batch digester operation

Batch tests were carried out to investigate the potential of methane production and lignocellulose degradability for the pretreated rice straw samples. The mixture of rice straw and liquid agent after pretreatment was added to 600 mL bottles. A set of bottles containing 0.50 g VS of rice straw without any thermal and/or chemical pretreatment (i.e., untreated rice straw) was also prepared as controls. Quadruplicate samples were prepared for each treatment. In each bottle, 100 mL of the seed and 400 mL nutrient solution was added, leaving approximately 100 mL as head space. The composition of the nutrient solution followed that commonly used for biological methane potential (BMP) tests [29]. The nutrient solution consisted of a phosphate buffer (KH<sub>2</sub>PO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub>), primary nutrients (NH<sub>4</sub>Cl, CaCl<sub>2</sub>·H<sub>2</sub>O, MgCl<sub>2</sub>·6H<sub>2</sub>O, and FeCl<sub>2</sub>·4H<sub>2</sub>O), and trace elements (MnCl<sub>2</sub>·4H<sub>2</sub>O, H<sub>3</sub>BO<sub>3</sub>, ZnCl<sub>2</sub>, CuCl<sub>2</sub>, NaMo<sub>4</sub>·2H<sub>2</sub>O, CoCl<sub>2</sub>·6H<sub>2</sub>O, NiCl<sub>2</sub>·6H<sub>2</sub>O, and Na<sub>2</sub>SeO<sub>3</sub>). After purging with nitrogen gas, the bottles were capped with a rubber stopper and an aluminum cap to maintain anaerobic state. The digester bottles were incubated in a 35 °C room for mesophilic anaerobic digestion with agitation at 50 rpm using a magnetic stirrer for 80 days.

### 2.4. Sampling and analysis

Biogas was collected from the samples under incubation using a glass syringe. The biogas volume produced at each sampling time was

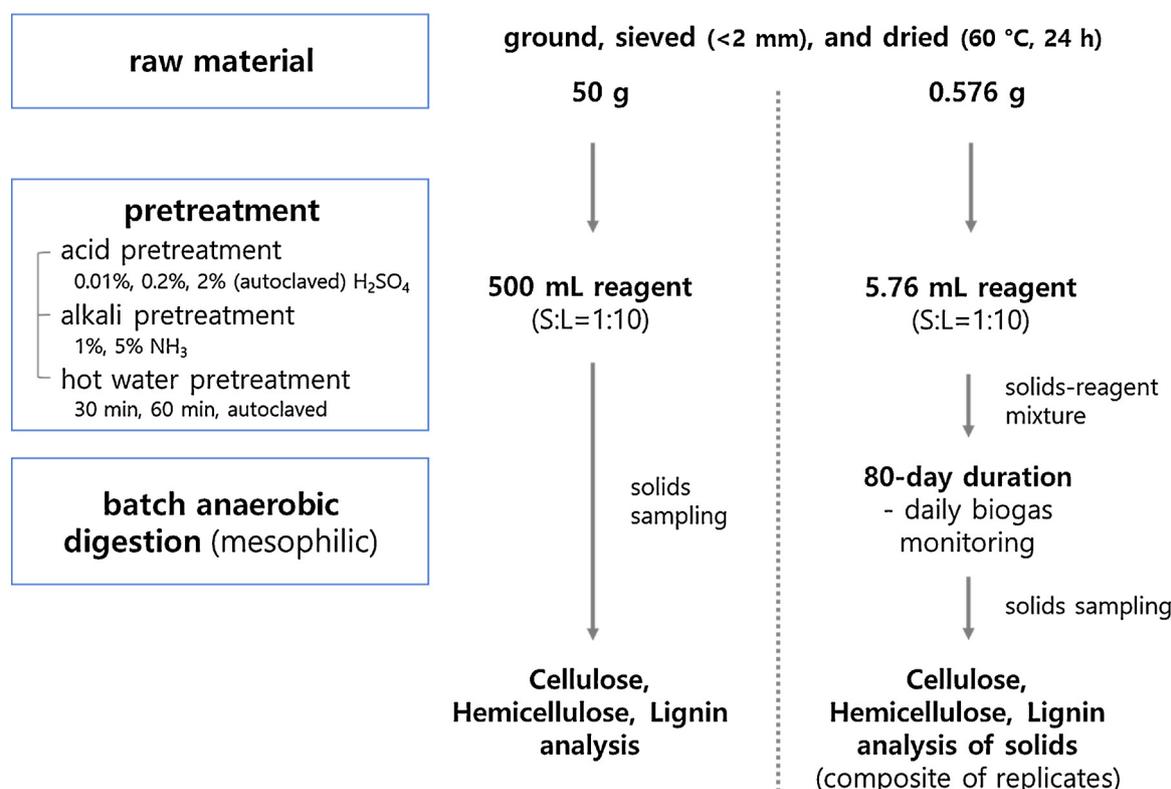


Fig. 1. The flowchart of overall process including pretreatment and anaerobic digestion in this study.

Table 1

Composition of rice straw used in this study [17]. All data are on weight basis.

TS (% total weight)	VS (% total weight)	Lignocellulosic material content (% VS)		
		Lignin	Cellulose	Hemicellulose
98.65 ± 0.21	86.76 ± 0.65	3.8	26.0	24.0

TS: total solids; VS: volatile solids.

recorded. Methane, carbon dioxide, and nitrogen contents in the biogas were analyzed using a gas chromatograph (GC; 6000 Series, ACME 6100, Seoul, Korea) with a thermal conductivity detector (TCD). A Porapak N 80/100 column was used for separation with helium as a carrier gas. The oven temperature was set initially at 80 °C and held for 2.5 min, and heated at a rate of 15 °C/min–120 °C, which was held for 1.5 min [15].

Lignin, cellulose, and hemicellulose contents were analyzed for the tests designed for determining the changes in lignocellulose contents after pretreatment and for the batch anaerobic digestion experiments. Firstly, the samples were centrifuged at 4000g for 10 min to harvest solids. For samples to observe the changes by the pretreatment, the harvested solids were washed several times using DI water. For samples to determine the lignocellulosic material contents after anaerobic digestion, the solids in quadruplicate bottles for each condition were combined because at least 10 g dry weight was needed for analysis. The combined solids were then washed with DI water with gentle abrasion on a 0.1 mm opening sieve to remove microorganisms as described in previous study [11]. The digested rice straw maintained its physical structure after the anaerobic digestion and was visibly distinguishable from other types of reactor solids. The color of the digested rice straw changed from blackish to brownish indicating the removal of microorganisms and organic residue attached. After applying the solid harvesting and washing procedure, the solids were sent to the Korea Feed Ingredients Association (Daejeon, Korea) for lignin, acid detergent fiber (ADF), and neutral detergent fiber (NDF) content analysis as described

in detail in the previous study [11]. The cellulose contents were determined as the difference between the ADF and lignin contents, and the hemicellulose contents as the difference between the NDF and ADF contents [11].

### 2.5. Statistical analysis

The results of the measurements from quadruplicate reactor runs were analyzed and expressed as mean ± standard deviation. One-way ANOVA was applied to identify statistical differences of cumulative methane production for different pretreatment methods and conditions. SPSS 15.0 software (SPSS Inc., Chicago, USA) was used for all statistical analyses.

## 3. Results and discussion

### 3.1. Effect of pretreatment on lignocellulose contents of rice straw

In terms of decomposition of the lignocellulosic materials of rice straw, acid pretreatment generally shows the highest efficiency among the three pretreatment methods (Table 2). The overall lignocellulose decomposition efficiency by pretreatment ranges 47.8%–65.4% for acid pretreatment while hot water and alkali pretreatments exhibit overall decomposition efficiency of 17.0%–50.4% and 26.8%–42.9%, respectively. The 2% H<sub>2</sub>SO<sub>4</sub> autoclaved sample shows the highest overall decomposition efficiency of 65.4% among all pretreatment methods

**Table 2**  
Decomposition efficiency of the lignocellulosic materials by pretreatment.

Experimental conditions		Decomposition efficiency for each component (%) <sup>a</sup>			Overall decomposition efficiency (%) <sup>a</sup>
		Lignin	Cellulose	Hemicellulose	
Hot water pretreatment	DI water, 30 min (100 °C)	13.6	14.7	20.1	17.0
	DI water 60 min (100 °C)	19.4	23.2	22.6	22.7
	DI water autoclaved (121 °C, 6 atm, 60 min)	45.9	50.3	51.2	50.4
Acid pretreatment	0.01% H <sub>2</sub> SO <sub>4</sub> (120 °C, 60 min)	31.6	32.9	66.5	47.8
	0.2% H <sub>2</sub> SO <sub>4</sub> (120 °C, 60 min)	36.2	37.1	64.1	49.1
	2% H <sub>2</sub> SO <sub>4</sub> , autoclaved (121 °C, 6 atm, 60 min)	46.6	48.8	86.2	65.4
Alkali pretreatment	1% NH <sub>3</sub> (35 °C, 24 h)	19.3	35.2	18.8	26.8
	5% NH <sub>3</sub> (35 °C, 24 h)	32.3	49.3	37.7	42.9

<sup>a</sup> Decomposition efficiency for each lignocellulose component is determined by  $\left[1 - \frac{(M_j)_p}{(M_j)_i}\right] \times 100$  (%) and overall decomposition efficiency by  $\left[1 - \frac{\sum_j (M_j)_p}{\sum_j (M_j)_i}\right] \times 100$  (%) where  $(M_j)_i$  is the initial mass added for pretreatment and  $(M_j)_p$  is the mass remaining after pretreatment ( $j$  = lignin, cellulose, or hemicellulose).

applied.

Among the three lignocellulose components (i.e., lignin, cellulose, and hemicellulose), the effect of acid pretreatment is most pronounced in decomposing hemicellulose. For 2% H<sub>2</sub>SO<sub>4</sub> autoclaved, 86.2% of rice straw hemicellulose is decomposed while the decomposition efficiency for lignin and cellulose is slightly less than 50%. For hot water and alkali pretreatments, the decomposition efficiency for hemicellulose ranges from 18.8% to 51.2%. These results indicate the efficiency of acid pretreatment in decomposing and solubilizing the hemicellulose component. The highest hemicellulose decomposition efficiency of 86.2% in this study agrees well with the highest efficiencies of 77%–93% recorded in the literature at the most extreme acid pretreatment conditions they tested using corn residues as a substrate [20,28,35].

The decomposition of lignocellulose by alkali treatment is more efficient at higher NH<sub>3</sub> concentration. By increasing the NH<sub>3</sub> concentration from 1% to 5%, the overall decomposition efficiency increases from 26.8% to 42.9%. A consistent increase in the lignocellulose decomposition efficiency of rice straw in the NH<sub>3</sub> concentration range of 1%–4% was reported [31] although their reported values (3.7% to 22.9%) are smaller than those in the current study. Considering the decomposition efficiency of 60%–80% for corn residue and 65%–85% for switchgrass by NH<sub>3</sub> pretreatment reported in another study [9], the current study results are within the literature range. On the other hand, the effect of acid concentration on the decomposition efficiency is relatively unclear. Increasing the H<sub>2</sub>SO<sub>4</sub> concentration from 0.01% to 0.2% does not improve the decomposition efficiency. The overall decomposition efficiency is higher for 2% H<sub>2</sub>SO<sub>4</sub> autoclaved than 0.2% H<sub>2</sub>SO<sub>4</sub>, but pressurization (1.45 atm for autoclaved) may also contribute to the improvement.

Hot water pretreatment exhibits decomposition efficiencies similar to those for alkali pretreatment. The overall decomposition efficiency slightly increases by extending the pretreatment duration (from 30 min to 60 min) and substantially increases by the combined effect of temperature increase and pressurization (compare DI water 60 min and DI water autoclaved in Table 2; the same pretreatment duration of 60 min is applied for the two conditions). Consequently, the DI water autoclaved sample shows the highest decomposition efficiency of 50.4% among the hot water pretreatment conditions applied.

### 3.2. Effect of pretreatments on methane production

The total amount of methane produced in the digesters is greater for the pretreated samples than the untreated control except for 2% H<sub>2</sub>SO<sub>4</sub> autoclaved sample. The total methane production is comparable for all alkali and hot water pretreatments with a descending order of 1% NH<sub>3</sub> (831.9 ± 1.77 mL CH<sub>4</sub>/g total carbon (TC)), 5% NH<sub>3</sub> (827.3 ± 0.94 mL CH<sub>4</sub>/g TC), DI water 30 min (824.2 ± 0.54 mL CH<sub>4</sub>/g TC), DI water autoclaved (806.8 ± 1.06 mL CH<sub>4</sub>/g TC), and DI water 60 min (805.8 ± 0.96 mL CH<sub>4</sub>/g TC). The methane production does not show clear correlation with the severity of the alkali and hot water pretreatments. The alkali and hot water pretreatments enhance the methane production by a factor of 2.1 compared to the untreated control. The highest methane production of 831.9 mL/g TC is close to the value of 962 mL/g TS [17] obtained by thermal pretreatment of rice straw.

Despite the higher lignocellulose decomposition efficiency by the pretreatment, samples with acid pretreatments show lower methane production potential than samples with the other two pretreatment methods. The total methane production for the acid pretreated samples is the highest for the most dilute acid treatment (i.e., 0.01% H<sub>2</sub>SO<sub>4</sub>) and the lowest for the most concentrated acid treatment (i.e., 2% H<sub>2</sub>SO<sub>4</sub>, autoclaved) indicating the inhibitory effect of the sulfuric acid addition on methane production. Although the pH after the acid pretreatment is moderate to strongly acidic (Table 3), the pH is maintained to be in the range of 6.5–7.0 after adding the inoculum and the nutrient solution, suggesting that pH is not a major inhibitory factor. A more plausible culprit for the reduced methane production potential is the anionic component of sulfuric acid, in other words, sulfate ion. Sulfate ion can limit methane production via consumption of hydrogen and acetic acid, the most important methane precursors, by sulfate reducing bacteria (SRB) [6,21,22]. The inhibitory effect of sulfate to methanogenesis is largely dependent on the molar ratio of organic substrates and sulfate rather than the absolute sulfate concentration [22]. Therefore, as the soluble substrate is gradually depleted over time in the batch reactors containing the sulfuric acid pretreated rice straw, the inhibitory effect of sulfate is likely to be exhibited at some point, with earlier occurrence for the reactor with higher initial sulfate concentration. The initial sulfate concentration is 1.02, 20.4, and 206 mM for the batch reactors containing rice straw pretreated by 0.01, 0.02, and 2% H<sub>2</sub>SO<sub>4</sub>, respectively. In an oligotrophic lake sediment, the inhibitory effect was

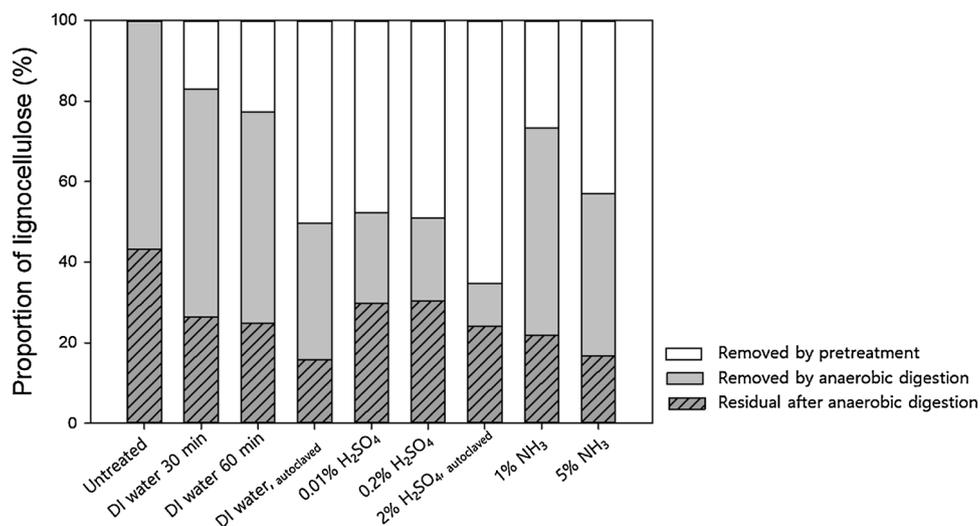
**Table 3**  
pH after pretreatment (mixture of rice straw and reagent) and after mixing the mixture with inoculum and nutrient medium.

Experimental conditions		pH after pretreatment	mixed with inoculum and medium
Hot water pretreatment	DI water, 30 min (100 °C)	7.2 ± 0.6	7.3 ± 0.3
	DI water 60 min (100 °C)	7.2 ± 0.3	7.4 ± 0.3
	DI water autoclaved (121 °C, 6 atm, 60 min)	7.0 ± 0.4	7.4 ± 0.2
Acid pretreatment	0.01% H <sub>2</sub> SO <sub>4</sub> (120 °C, 60 min)	6.1 ± 0.9	7.0 ± 0.3
	0.2% H <sub>2</sub> SO <sub>4</sub> (120 °C, 60 min)	5.3 ± 0.2	6.9 ± 0.2
	2% H <sub>2</sub> SO <sub>4</sub> , autoclaved (121 °C, 6 atm, 60 min)	1.0 ± 0.2	6.5 ± 0.1
Alkali pretreatment	1% NH <sub>3</sub> (35 °C, 24 h)	8.1 ± 0.1	7.6 ± 0.1
	5% NH <sub>3</sub> (35 °C, 24 h)	8.4 ± 0.3	7.7 ± 0.2

**Table 4**  
Degradability of the lignocellulosic materials by pretreatment and subsequent anaerobic digestion.

Experimental conditions		Degradability for each component (%) <sup>a</sup>			Overall lignocellulose degradability (%) <sup>a</sup>
		Lignin	Cellulose	Hemicellulose	
Untreated		4.6	28.0	96.4	56.9
Hot water pretreatment	DI water, 30 min (100 °C)	14.6	60.9	96.6	73.6
	DI water 60 min (100 °C)	19.2	65.7	94.0	75.1
	DI water autoclaved (121 °C, 6 atm, 60 min)	46.8	77.5	97.6	84.4
Acid pretreatment	0.01% H <sub>2</sub> SO <sub>4</sub> (120 °C, 60 min)	32.2	58.0	89.5	70.3
	0.2% H <sub>2</sub> SO <sub>4</sub> (120 °C, 60 min)	37.2	58.3	86.9	69.6
	2% H <sub>2</sub> SO <sub>4</sub> , autoclaved (121 °C, 6 atm, 60 min)	46.5	71.2	85.7	76.0
Alkali pretreatment	1% NH <sub>3</sub> (35 °C, 24 h)	19.3	70.4	96.0	78.3
	5% NH <sub>3</sub> (35 °C, 24 h)	33.6	77.4	97.5	83.3

<sup>a</sup> Degradability for each lignocellulose component is determined by  $\left[1 - \frac{(M_j)_f}{(M_j)_i}\right] \times 100$  (%) and overall degradability by  $\left[1 - \frac{\sum_j (M_j)_f}{\sum_j (M_j)_i}\right] \times 100$  (%) where  $(M_j)_i$  is the initial mass added for pretreatment and  $(M_j)_f$  is the mass remaining after pretreatment and subsequent anaerobic digestion ( $j$  = lignin, cellulose, or hemicellulose).



**Fig. 2.** Proportion of lignocellulose (i.e., sum of lignin, cellulose, and hemicellulose) removed by the pretreatment and the anaerobic digestion, and residual after the anaerobic digestion.

observed at sulfate concentrations in the range down to 0.1 mM [21]. In sum, the literature suggests that sulfate ion added during the pretreatment step reduces the methane production potential with the greater influence for the higher sulfuric acid concentration used, and removal of sulfate ion may be needed after the acid pretreatment to improve the methane production potential. This challenges the economic feasibility of using sulfuric acid for rice straw pretreatment.

Lignocellulose degradability, which indicates the combined effect of rice straw pretreatment and subsequent anaerobic digestion, is higher for all pretreatments applied than the untreated control (Table 4). The benefit of the pretreatments on degradability is significant for lignin and cellulose components. On the other hand, hemicellulose degradability for the untreated rice straw is near completion (i.e., 96.4%), showing that hemicellulose can be eventually degraded by long-term operation of anaerobic digestion under optimal conditions as achieved in the batch digesters in the current work.

A substantial proportion of the lignocellulosic materials is degraded by anaerobic digestion for hot water and alkali pretreatments while for acid pretreatments, the reduced amount is relatively smaller (Fig. 2). As a result, the lignocellulose degradability for hot water and alkali pretreatments is substantially increased (i.e., by 29–48%) compared to the untreated control. The pretreatment step accounts for 17–50% reduction relative to the initial lignocellulose mass and the anaerobic digestion step another 34–57%, giving the summed value of 74–84% (cf. 57% for the untreated control). As described in the Introduction section, alkali pretreatment removes the crosslinks between hemicellulose and other components and reduces cellulose crystallinity [32]. Hydrothermal pretreatment can change the crystalline structure of cellulose by disrupting hydrogen bonds [37]. The results shown in Fig. 2 suggest that these structural changes are not fully addressed by the chemical analysis for lignocellulose content determination employed in this study, but leads to the improved degradability and methane production in the subsequent biological process (i.e., anaerobic digestion). In contrast to the hot water and alkali pretreatments, acid pretreatment is likely to work mainly on decomposing the lignocellulosic materials considering the relatively low additional lignocellulose degradation by anaerobic digestion.

The methane production pattern in the digesters over time further supports the conclusion on the effect of each pretreatment on anaerobic digestion (Fig. 3). For 2%  $\text{H}_2\text{SO}_4$  autoclaved sample, the methane

production is almost ceased within 30 days. On the other hand, for hot water and alkali pretreated samples, methane is produced slowly but continuously between 30–80 days of operation. This suggests that the severe acid pretreatment partially decomposes the lignocellulosic materials, making them relatively readily biodegradable, while the remaining materials are relatively stable. The hot water and alkali pretreatments may produce both decomposed organics and lignocellulose materials with looser structure or less crystallinity, which are slowly but eventually biodegradable.

Considering methane production potential, lignocellulose degradability, and possibility of microbial inhibition and the cost associated with chemical addition, hot water pretreatment is likely to be the most appropriate pretreatment method among those tested. Autoclaving rice straw in DI water increases the methane production potential by a factor of 2.1 compared to the rice straw without pretreatment. The methane production potential is comparable to those for the alkali pretreated samples and substantially higher than those for the acid pretreated samples. An overall lignocellulose degradability of 84.4% is achieved by the pretreatment of autoclaving in DI water and subsequent anaerobic digestion, which is higher than those with the alkali and acid pretreatments.

#### 4. Conclusions

This study demonstrates the effect of various pretreatments on lignocellulosic material composition in rice straw and anaerobic biogas production. The benefit of acid pretreatments on methane production is limited despite the highest performance of 65.4% in decomposing lignocellulose, presumably because of sulfate ion inhibition. On the other hand, although the decomposition efficiency for hot water and alkali pretreatments is relatively low (i.e., 50.4% and 42.9%), the rice straw biodegradability is substantially enhanced by the pretreatments, leading to methane production of > 800 mL  $\text{CH}_4/\text{g TC}$  or 2.1-fold increase compared to the control with no pretreatment. Hot water pretreatments show relatively high methane production and lignocellulose degradability, suggesting their potential as an efficient, chemical-free biological methane production method for rice straw. We suggest future studies on rice straw pretreatment to be focused on optimizing the hot water application including the duration, temperature, and the necessity and degree of pressurization.

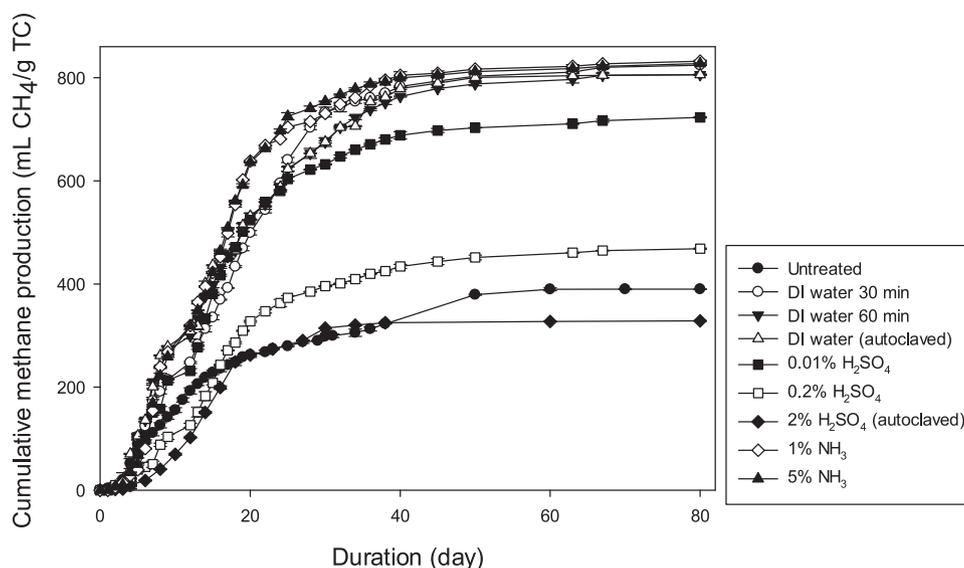


Fig. 3. Cumulative methane production measured in the batch reactors over time for different pretreatment methods and conditions.

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