Hydrothermal Pretreatment of Rice Straw with Alkaline Addition for Enhancing Biogas Production in Semicontinuous Anaerobic Digester

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Abstract. This study presents the results of hydrothermal pretreatment of rice straw on its ability to improve the biogas production process in anaerobic digesters. Hydrothermal treatment on rice straw biomass was carried out with the addition of 0%, 3% and 5% NaOH (w/w rice straw) for one hour at a temperature of 140 °C. This study showed that hydrothermal and alkaline hydrothermal pretreatments were able to increase organic degradation of rice straw as indicated by an increase in the dissolution of lignin and hemicellulose from rice straw. Temperature and NaOH worked synergistically to dissolve lignocellulose in the hydrothermal pretreatment process. In the semicontinuous digester fed with pretreated rice straw, NaOH content in the pretreatment stage was found to give significant effect in enhancing biogas production. Average daily biogas production by the untreated rice straw, hydrothermally treated rice straw with 3% NaOH and 5% NaOH was 23.9, 57.1, 95.8 and 108.8 L/kg rice straw, respectively.

1. Introduction
Consumption of energy derived from fossil fuels continues to increase, causing an energy crisis, increasing greenhouse gases and other environmental problems. Research to obtain renewable energy sources from biomass as a substitute for fossil fuels is important for the future energy provision. Biomass lignocellulosic material is very widely available throughout the world so its utilization as a source of energy and chemicals is very attractive [1].

Lignocellulosic constituents are mainly composed of lignin, cellulose and hemicellulose. Cellulose is a crystalline polymer mainly composed of glucose; hemicelluloses are interconnecting materials which are branched heteropolymers, while lignin is heteropolymer which are combination of phenolic subunits providing tightness to the cell wall [2]. This complex structure in plants causes lignocellulose resistant to be degraded by enzymes from microorganism and thus contributing difficulties in production of bioethanol, biogas or biohydrogen by fermentation route. Due to the difficulty of decomposition of lignocellulose, it is necessary to develop pretreatment technology to break the compact structure of lignocellulosic material so that it will increase enzymatic digestibility by microorganisms [3]. Among these pretreatments, hydrothermal treatment is a process that could be further developed because it is an environmentally friendly process, gives less corrosion and provides high energy efficiency [4,5]. The hydrothermal treatment process can increase the biomass surface area and thus improve the accessibility of enzymes [6]. Previous study showed that temperature of hydrothermal process has been found to be the major effect on the pretreatment effectiveness towards producing highly digestible substrate [7]. Hydrothermal pretreatment is also a promising effort to increase biogas production. Chandra et al.[8] conducted a laboratory scale work and obtained an
improvement of 225% increase in biogas production. However, poisonous derivatives, such as phenolic compounds, furfural and HMF may be released during hydrothermal process and induce inhibitions in the anaerobic digester [9]. Previous research on a laboratory scale conducted by Wang et al. [10] showed that hydrothermal pretreatment of rice straw at 90 -120 °C for 10 min resulted an increase in biogas production only 3% higher than using untreated rice straw.

In this study, the hydrothermal pretreatment was utilized to treat lignocellulosic material, rice straw, to produce biogas. Hydrothermal temperature was set at 140 °C. Previous study showed that higher temperature would lower the biogas production [11] and lower temperature would lower dissolution of organic compound from rice straw [12], thus lower the biogas production. The process was modified using alkaline condition by adding NaOH solution directly during hydrothermal process. It is well known that addition of NaOH will accelerate the degradation of lignin [13]. Degradation of lignin may help solubilization of cellulose and hemicellulose and reduce the production of poisonous substances [7]. Enhancing lignin degradation may also lead in conducting the hydrothermal process at lower temperatures with smaller energy requirements. In this way, it was expected that the volume of biogas produced from rice straw will be increased even further. After each hydrothermal process, measurements of the ingredients which play a role in the fermentation process such as cellulose, lignin, and hemicellulose were also determined. This study would characterize the effect of biomass composition on the next process of fermentation to produce biogas.

2. Material and Method

2.1. Raw Material
Rice straw was collected from a local rice field in Sidoarjo district Indonesia. The biomass was sun dried and cut into pieces in size of 5 cm then stored in plastic bags without any additional treatment. The processed biomass rice straw was then used as a material for experimental studies. Finer size of rice straw was not required because in a previous study [14], mechanical pretreatment to reduce rice straw size to 5, 2, 0.5, and 0.2 cm, did not show any significant increases in biogas yield. The native rice straw used in this work mainly contained of 25.4% lignin, 34.8% cellulose and 26.5% hemicellulose with ash content of 15.0%.

2.2. Hydrothermal pretreatment
The hydrothermal treatment process was performed in an autoclave reactor made of 8 mm thick stainless steel, 20 cm in diameter and 65 cm in height with total volume of 13 L. The reactor vessel was provided with thermocouple connected to PID temperature controller to control the temperature inside the vessel. The reactor contained 1 kg of dry rice straw and NaOH of 0%, 3% and 5% (w/w dry rice straw). NaOH crystal was dissolved in water and the solution was added to 1 kg of dry rice straw to attain 12.5% total solid biomass concentration. The substrate was put into the autoclave reactor, sealed tightly and ready for the hydrothermal process. The reaction temperature was co-heated to desired temperature for 50–60 min and maintain at temperature of 160 °C for 60 min.

After hydrothermal process, a small portion of solid fraction was taken, washed with distilled water and dried for further analytical test. The liquid fraction was also separated for determination of sugar content and furfural content as inhibitory by-product of hydrothermal process.

2.3. Anaerobic digester for biogas production
Bench-scale anaerobic digesters made of PVC plastic with 15.5 cm in diameter and 85 cm in height (a total volume of 19 L) and working volume of 14 L were used in all the experiments. Figure 1 shows the equipment scheme of the anaerobic digester operation for biogas production. The digester was operated at mesophilic temperature semicontinuously, i.e. feeds from straw pretreatment were fed every day with solid concentration of 5% and a hydraulic retention time (HRT) of 20 d, and the gas produced was collected in the gas holder. Volume of biogas was measured daily by measuring the height displacement of the gas holder upper cylinder.
2.4. Analytical methods

Characterization of feedstock is one of the most significant steps in the biogas production process. Determining the general composition of the substrate (input feed) is essential for calculating the quantity and composition of the generated biogas. The total solids, volatile solids and fixed solids were determined by standard method [15].

The composition of raw rice straw and pretreated rice straw was measured using DE method [16] for cellulose and hemicellulose content and TAPPI T222 method [17] for lignin content. The DE method is a gravimetric analysis of lignocellulosic constituent after being hydrolyzed or dissolved. The steps were: first, eliminating extractive content, then dissolution of lignin by sodium chlorite (NaClO₂) resulting solid holocellulose. Further dissolution using dilute hydrochloric acid would remove hemicelluloses and leave solid cellulose.

The profile of reducing sugar in the hydrothermal liquor product were analyzed using DNS method [18] and the inhibitory by-products (furfural) in the liquid fractions from hydrothermal treatment were determined on a high performance liquid chromatograph, equipped with UV-Vis Detector using an Prominence (CTO-20A) Purospher® STAR C18 (250 mm x 4,0 mm, 5 µm) column operating at 30 °C with Acetonitrile : Water (5:95 v/v) as the mobile phase at a flow rate of 1 mL/min, injection volume of 10 µL and running time of 40 min.

3. Result and Discussion.

3.1. Influence of Hydrothermal treatment on rice straw solid composition

Figure 2 shows the liquid product and solid product composition of rice straw after the hydrothermal process. Liquid yield was calculated as weight of initial rice straw minus weight of solid product of hydrothermal process in dry basis. The liquid product shows that during the hydrothermal process some of the components in the straw were dissolved into water so that the amount of solid product becomes lower. Figure 2 also shows that the temperature and NaOH levels during the hydrothermal process play an important role in dissolving the components of rice straw biomass. The higher the temperature and NaOH content in the hydrothermal process, the more components were dissolved into the water. The lowest solid yield, 70.5%, was obtained at hydrothermal process of 140 °C and 5% NaOH by weight of dry rice straw. This temperature and NaOH content is the highest content (29.5%) of organic dissolved into the water during hydrothermal pretreatment.

![Figure 1. Anaerobic digester experimental setup.](image-url)
The chemical compositions after hydrothermal process of rice straw biomass such as cellulose, hemicellulose and lignin are also presented in Fig. 2. As the temperature increased, pH values decreased only slightly but still in the range of neutral pH (data not shown). The reason of the decreasing of pH may be the acetyl groups in lignin and hemicelluloses undergoes a hydration reaction led to the acidification of the liquor [19] during the hydrothermal pretreatment. Previous work reported that 5% NaOH was added to maintain appropriate pH of the substrate suitable for fermentation process[8]. In the present study, however, the pH of treated rice straw by hydrothermal process was kept to neutral by adding alkali solution directly before hydrothermal processes. NaOH would react with organic acid released in hydration reaction and thus neutralize the pH. Cellulose content of rice straw was originally 35% before treatment, which increased to around 57% after hydrothermal treatment at NaOH content of 5% and temperature of 140 ºC. In contrast, as the treatment temperature and NaOH content were increased, the hemicellulose content of rice straw decreased, which is in line with the works of Zhou et al. [20], especially at NaOH content of 3% and 5%.

During thermal treatment, hydronium ions were released due to autoionization and this caused hydrolysis of the biomass material. Hydrolysis process could lead to lignocellulosic decomposition and thus alter the lignocellulosic chemical constituent [21], converting the material into simpler compounds. Hemicellulose is the constituent which has the weakest bonding compared to lignin and cellulose so that it is widely reduced from rice straw solids during the hydrothermal process. Mechanism of hemicellulose solubilisation was given by Liu [19].

The content of lignin in the rice straw did not show a definite tendency of increasing or decreasing along with the operating conditions of the hydrothermal process. This shows that degradation during the hydrothermal process occurs more in hemicellulose. The effect of the temperature is more apparent at 5% NaOH where it was the lowest content of lignin.

The reaction with NaOH caused delignification of rice straw. This tendency can be seen more clearly at higher NaOH concentration. However, since the heating during the hydrothermal process dissolved a lot of hemicellulose into the water, lignin content resulted an increase in the solids of the hydrothermally processed rice straw.
Some studies reported the degradation of lignin during hydrothermal process, by which lignin is broken down into water-soluble products. This dissolution process is accelerated by the reaction between phenol hydroxyl groups in lignin and NaOH [22] so that the remaining solids will contain more cellulose. Ran et al. [23] obtained a 43% increase of cellulose due to hydrothermal treatment of lignocellulosic biomass at 160 °C.

3.2. Influence of Hydrothermal treatment on rice straw on liquid product composition

The result of reducing sugar and furfural content in hydrothermal liquid product is shown in Fig. 3 and Fig. 4 respectively. The properties of solid product of thermally treated biomass indicate that thermal pretreatment of rice straw leads to production of sugar or low molecular constituent as a result of hemicelluloses and lignin depolimerization and consequently increases its digestibility for microorganism. During hydrothermal process glucose yield in the filtrate resulting from the pretreatment nearly constant until NaOH content of 5% due to further formation of furfural [7]. Glucose yield end to decreases in with increasing NaOH content. The highest reducing sugar yield was obtained at 5% NaOH concentration with reducing sugar yield of 1.3%. This studies showed that furfural concentrations were still below 2 mg / mL, which is the inhibitory threshold for ethanologens [24]. Therefore, direct fermentation from pre-hydrolyzates is feasible for further fermentation routes for biogas production.

Comparing with previous studies conducted by Imman et. al. [7] with operating conditions of 140 °C, 25 bar and 0.25% NaOH (b/v) for 5, 10 and 20 minutes resulted a glucose yield relationship with furfural. Glucose yield was decreasing with increasing time and oppositely furfural was increasing with increasing hydrothermal time operation. The higher concentration of reducing sugars formed, the more easily digested substance by biogas-forming microorganisms, while furfural formation during hydrothermal pretreatment causes a decrease in reducing sugar yield and could affect the biogas yield. The pretreatment results showed the degradation of sugar in the product of liquid fraction and formed furfural. Higher furfural formation was observed at higher pretreatment temperatures, which corresponds to the conditions with the highest yield observed in depolymerization of sugar. Imman et.al. [7] found that glucose and pentose were released as the main sugars from hydrolysis of cellulose and hemicellulose in pretreated rice straw. Elevating NaOH content was observed to reduce glucose yield. Greater NaOH can cause loss of sugar from hydrolysis of glucan to monomeric sugar and subsequent conversion to furan and other degradation products. The trend of furan formation from sugar degradation was observed from further degradation of sugar monomers under acidic environments [25], the concentration of furan accumulation in the alkaline pretreatment process is higher than that done without NaOH.

Figure 3. Reducing sugar content of liquid hydrothermally pretreated rice straw

Figure 4. Furfural content of liquid product of product of hydrothermally pretreated
Increasing the temperature of pretreatment resulted in increased hydrolysis of cellulose with concurrent glucose higher in the liquid phase. The result of an increase in sugar levels with the temperature coincides with the increase in accumulation of inhibiting products. The highest furfural content (0.063 mg/mL) was observed at NaOH content of 5%. Lower concentrations of sugar degradation are found in the presence of lower bases.

3.3. Biogas Production
The result of anaerobic fermentation to produce biogas is shown in Fig. 5. Data in these figures were taken at steady state operation of digester feeding or more than 7 days after first feeding of rice straw. This Figure shows daily biogas production of the digester operated at mesophilic temperature fed with hydrothermally pretreated rice straw with NaOH content of 5%, 3% and 0%. This Figure also shows that biogas production fluctuates day by day and this such fluctuations are very common in anaerobic digester in producing biogas. Fluctuations are common due to changes in the composition of organic matter digested by bacteria.

Table 1 shows a summary of biogas production results obtained from hydrothermal pretreatment of rice straw. The concentration of methane in biogas showed a change in concentration, but the fluctuation changes were negligible due to little deviation (data not shown).

Table 1 shows that biogas production from digester fed with hydrothermally pretreated rice straw with the addition of 3% NaOH has a higher average biogas production than that of with the addition of 5% NaOH. The higher the addition of NaOH concentration does not means higher correlation with the production of methane. The degradation of lignin in straw during the hydrothermal process has a role in the production of biogas, the more lignin dissolved in water due to addition of NaOH would become an inhibitor in biogas production.

The Lowest biogas production results were obtained from anaerobic digester fed with rice straw hydrothermally processed without the addition of NaOH. Biogas production from digester fed with rice straw was about 1.9 L per day. For raw untreated rice straw, only about 0.8 L of biogas is produced per day, as shown in Table 1. Higher biogas production was shown using digester fed with hydrothermally pretreated rice straw with addition of NaOH. These results indicate that hydrothermal operations under alkaline conditions, by which lignin degradation and dissolution of components took place, have a significant role in biogas production. The more lignocellulosic components dissolved in water will be.

Figure 5. Biogas Production from alkaline hydrothermally treated rice straw at different NaOH content
Table 1. Summary of biogas production from anaerobic digester fed with hydrothermally pretreated rice straw at various NaOH content.

| NaOH content of hydrothermal process | Average biogas daily production (L/day) | Standard deviation of biogas production | Methane concentration of biogas (%) | Average biogas production potential (L/kg rice straw) |
|-------------------------------------|----------------------------------------|----------------------------------------|------------------------------------|-----------------------------------------------------|
| untreated                           | 0.79                                   | 0.34                                   | 68.39                              | 23.90                                               |
| NaOH 0%                             | 1.89                                   | 0.71                                   | 67.23                              | 57.10                                               |
| NaOH 3%                             | 3.51                                   | 0.97                                   | 68.75                              | 95.84                                               |
| NaOH 5%                             | 3.59                                   | 0.94                                   | 68.39                              | 108.81                                              |
| NaOH 3%*                            | 3.53                                   | 0.39                                   | 68.39                              | 97.96                                               |

*repeated experiment

Driving force in increasing biogas production. In addition, hydrothermal operations without addition of NaOH would produce more inhibitor materials, as stated by Wang et. al. [10]. He and coworkers’ studies resulted higher hydrothermal operating temperature, i.e. at a temperature of 190 – 210 °C, biogas production only increased 3% compared with biogas from raw rice straw without any pretreatment. The presence of NaOH would reduce the inhibitor material so that the performance of microorganisms in producing biogas becomes better.

Sequential biogas production from the lowest to the highest were straightforward with severity factor during hydrothermal processes. Biogas production from an anaerobic digester fed with untreated rice straw has the lowest result, and the higher result was digester fed with rice straw hydrothermally pretreated without addition of NaOH. Digester fed with rice straw hydrothermally pretreated with 3% NaOH addition resulted higher than without NaOH addition and the highest result was digester fed with rice straw hydrothermally pretreated with 5% NaOH addition.

The results of biogas production from anaerobic digester pointed out that hydrothermal pretreatment in rice straw significantly increased biogas production from 23.9 L/kg straw for biogas production from untreated rice straw to 57.1 L/kg rice straw which hydrothermally pretreated at 140 °C without addition of NaOH or an increase of 139%. The results of biogas production from the digester also prove that hydrothermal pretreatment in rice straw significantly increases biogas production. Higher biogas production result were obtained 95.8 L/kg rice straw which was the digester with hydrothermally pretreated rice straw at 140 °C with the addition of 3% NaOH or an increase of 300% and also an increase of 355% at NaOH content of 5%.

The highest biogas production, obtained from a thermophilic digester which was fed with hydrothermally pretreated rice straw at 140 °C with the addition of 5% NaOH which had a daily production potential of 108.8 L per kg of dry straw.

4. Conclusion
In this study, effects of alkaline addition in hydrothermal treatment of rice straw on the biogas production were studied. It was found that the presence of NaOH on hydrothermal treatment has effectively promoted the production of biogas. This works showed the advantage of alkaline hydrothermal treatment of rice straw for the enhancement of the further biomass conversion to produce biogas. Increasing NaOH content resulted more significant effect in increasing biogas production compared with increasing pretreatment temperature. The highest production of biogas was 108.8 L per kg rice straw from anaerobic digester using rice straw from hydrothermally pretreated at NaOH content of 5%.
5. References

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