

# Enhance Enzymatic Digestibility of Rice Straw by Ammonia Modification Process at Ambient Condition

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**Abstract**— As a lignocellulosic biomass material, rice straw consists of cellulose, hemicelluloses, and lignin. Lignin might cover cellulose and hemicelluloses from chemical or biological treatment. Therefore, delignification process is needed to remove lignin from lignocellulosic biomass structures and increase the productivity of cellulose and hemicelluloses conversion. Many researchers were trying to have a better method for delignification by using many chemical in various conditions, such as sulphuric acid, NaOH, Ca(OH)<sub>2</sub>, NH<sub>4</sub>OH, NMMO, etc. In this study, delignification was carried out in mixture solution from ammonia and sodium hydroxide at ambient temperature for various time of pretreatment (0.5, 1, 2, 5, 24, 48 hrs, 7 and 14 days). Rice straw is mainly consisting of 33 wt% cellulose, 26 wt% hemicelluloses, and 18 wt% lignin, respectively. The results indicate that about 90 wt% lignin was removed with the mix solution in 24 hrs, compared to 28% with water, 55% with sodium hydroxide and 69% with ammonia only. As a linear correlation, the sodium hydroxide and ammonia mixture solution shown the better result also for enzymatic digestibility compare to use them separately.

**Keywords**— delignification process, rice straw, ambient temperature, mixture solution, enzymatic digestibility

## I. INTRODUCTION

One of the potential and available lignocellulosic biomass feedstock for energy source is rice straw. As per BPS (Statistic Centre Agency) statistics, Indonesia was about 77 million tons of rice yields every year. It will be produce 77 – 115 million tons of rice straw. The rice straw has not been used widely as a useful product, such as bioethanol. It can cause water and air pollution; and consequently affects public health. As a lignocellulosic biomass, rice straw contains approximately 32 – 47% cellulose, 19 – 27% hemicelluloses, and 5 – 24% lignin (Saha, 2003).

Cellulose and hemicelluloses (known by carbohydrate component) can be hydrolyzed into simple sugar, and then fermented into bioethanol by microorganism. Unfortunately, cellulose and hemicelluloses were covered by lignin from the chemical or biological interference. Lignin might cover the carbohydrate component chains further decreasing their accessibility for the enzymes and microorganism, together with the high crystallinity of cellulose (Kim and Lee, 2006). Therefore, delignification process (also known by pretreatment process) is needed to remove lignin content and

enhance the productivity of cellulose and hemicelluloses conversion.

The pretreatment step is required, so that the following enzymatic conversion to monomeric sugar can be performed faster with greater yield (Mosier et al., 2005). Several studies support chemical pretreatments such as treatment with dilute-acid (Hsu et al., 2010; Saha et al., 2005 ); sodium hydroxide and lime (Kaar and Holtzapple, 2000; Kim and Holtzapple, 2005); or aqueous ammonia (Jung et al., 2011; Salvi et al., 2010). Each pretreatment reagent has the advantages and disadvantage. Base reagents will be selective for lignin removal than acid. Acid pretreatment has a better result for separating cellulose from lignin and hemicellulose.

The hypothesis of this research is that lignin containing in rice straw will be more soluble in mixture solution between NaOH and ammonia (NH<sub>4</sub>OH), which might improve the effect of the pretreatment compared with that of aqueous ammonia, NaOH, and water treatment at ambient temperature. The present work was also aimed to investigate the synergistic combination effect when using NaOH-ammonia mix-solution for the consuming time of pretreatment process, and the effect of NaOH-ammonia mix solution to enzymatic digestibility.

## II. MATERIAL

Rice straw was kindly supplied from rice farm in Padang Pariaman, Sumatera Barat Province. It was cut into 1 cm length, washed, and oven dried at 40°C overnight to achieve a moisture content of 5 – 10% weight. The dried material was kept on plastic bag and stored at 4°C until further use. Enzyme cocktail was obtained from Novozyme. All chemicals were obtained from Sigma-Aldrich.

## III. EXPERIMENTAL

20 grams of rice straw was soaked into 0.1M NaOH – 5% w/w Ammonia (1:1) mix solution for various time at room condition. 0.1M NaOH, deionized (DI) water, and 5% w/w NH<sub>4</sub>OH were used as delignification reagent as same as mix solution to find the comparison between a single alkaline and mix solution. When the time of delignification was reached, the samples were filtered and washed with DI water until the normal pH ( $\pm$  7) could be achieved. The treated rice straw was then oven dried at 40°C overnight. The dried material was kept on plastic bag and stored at 4°C.

Furthermore, the enzymatic digestibility was determined by NREL laboratory analytical procedure (Brown and Torget, 2004). A 5 grams pretreated and untreated rice straw were load in 250 mL erlenmeyer flask and mixed with 0.1 mL enzyme and citrate buffer solution (0.05M) until 5% final concentration of rice straw. Hydrolysis reaction was carried out at 50 °C, pH 5.0, and 120 rpm in a water-bath shaker. Glucose concentration was monitored by glucometer (Accu-Chek Active, Germany) periodically.

#### IV. RESULT AND DISCUSSION

NaOH or KOH pretreatment can be performed at low or high temperatures but need an extreme condition, such as in high concentration of alkaline, long time process, etc. (Binod et al., 2010). Ammonia pretreatment also can be performed at high temperature for the effective process, but consumed high energy to the system. In this study, pretreatment was carried out at room temperature. This temperature was chosen since no heat input required may decrease supply energy to the system and will reduce processing cost.

The typical of alkaline pretreatment on biomass conversion is generally has a stronger effect on lignin than cellulose and hemicelluloses (Sharma et al., 2002). Based on this statement, the effect of lignin removal from various chemicals (NaOH, deionized water, NH<sub>4</sub>OH, and NaOH-Ammonia mix-solution) was shown in Table 1.

As seen in Table 1, there are two variable condition of alkaline pretreatment in the recent study. First, the pretreatment time was fixed at 24 hours with several kind of delignification reagent base. DI water pretreatment shown only 28% w/w of lignin from rice straw component could be removed by water. This is significantly influenced by the previously process (physical treatment (rice straws were cutting in to 1 cm length of material). The cutting process was break the lignin bonds at the end of lignin-carbohydrate chains and make it easily removed by water.

TABLE I  
THE EFFECT OF VARIOUS ALKALINE PRETREATMENT ON LIGNIN REMOVAL

Solution	Time (h)	Lignin Removal (%)
NaOH	24	55.56
NH <sub>4</sub> OH	24	69.44
DI water	24	27.78
NaOH-Ammonia	0.5	34.72
	1	48.61
	2	48.61
	5	76.39
	24	90.28
	48	90.28
	168	100
	336	100

When ammonia was used as a pretreatment reagent, the degree of lignin removal was increased compare to NaOH, 55% w/w to 69% w/w. Zhang and Cai (2008) carried out of rice straw by 2% NaOH with 20% solid loading at 85°C for 1 h and the data shown 36% lignin was removed by the process.

Ko et al. (2009) evaluated the effect of temperature; time and concentration of ammonia on remove lignin content from rice straw and obtained the optimum condition for the highest lignin removal was 60.6±0.3 at 70°C, 12 h, and 20% w/w of sample. The result of previous study is almost similar with the result in this recent study. Furthermore, this result was proven ammonia has high selectivity for reaction with lignin than other alkaline. However, NaOH or alkaline pretreatment appears to be the most effective method in pulp process. NaOH solubilized the lignin fragment as well as carbohydrate. NaOH cleavage lignin bond into small fragment and dissolve it. Ammonia pretreatment effectively remove lignin in different way by hydrolyzing the linkages between lignin and carbohydrate.

When the pretreatment reagent (NaOH and ammonia) was mixed into 1:1 ratio, the lignin removal was increasing to 90% w/w at the same condition (24 h, room temperature). The synergy between NaOH and ammonia reaction is significantly enhanced the degree of delignification lignocellulosic biomass. Thus, adding NaOH in ammonia process will decrease the processing cost, compare to NaOH or ammonia as a single pretreatment reagent.

Second variable is the effect of time on lignin removal. 55% w/w removal lignin was achieved in 1 or 2 hours by mix-solution, compare to 24 hours by single NaOH. Table 1 shown the similar result for single ammonia reagent, which is 69% w/w was reached after 24 hours compare to 5 hours by mix-solution. It is noteworthy that the NaOH-ammonia mix-solution reduces the time of delignification process in a significantly number. Increasing the time process above 24 hrs is not necessary to enhance the lignin removal.

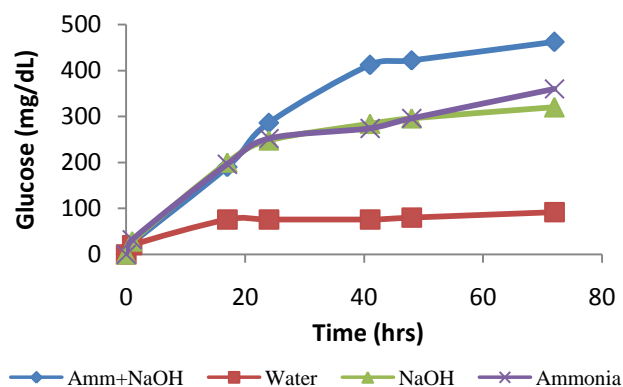


Fig. 1 Glucose production of enzymatic digestion for various pretreatment reagent

The main purpose of pretreatment step is to provide the cellulose or carbohydrate content of lignocellulosic biomass to be attacked by enzyme or other chemical. The ability of enzyme in converting of cellulose into monomeric sugar, such as glucose, will be assessed by monitoring glucose concentration periodically. Fig. 1 had shown the concentration of glucose after the enzymatic digestion for various pretreatment reagents. After 72 hrs saccharification processes,

mix-solution of ammonia and NaOH reagent showed the highest result of concentration of glucose production compare to other single reagent. It was enhanced from 92 mg/dL by DI water, 320 mg/dL by NaOH, 360 mg/dL by ammonia, to 462 mg/dL by mix-solution of NaOH-Ammonia. These result prove that the synergetic effect of mixture of NaOH and ammonia could increase the enzymatic digestibility, due to its ability to remove lignin in biomass. In other word, the lignin presence in the lignocellulosic biomass material, positively affected to the enzymatic hydrolysis.

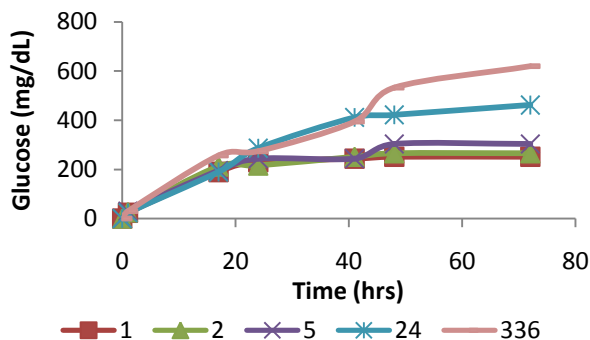


Fig. 2 Time effect of pretreatment process to enzymatic hydrolysis by NaOH-Ammonia solution

Fig. 2 present the effect of time consuming of pretreatment process to the enzymatic digestibility. It was enhanced from 462 mg/dL after 24 hrs to 620 mg/dL of glucose production with 336 hrs (14 days) of process. It is not worthy; because it was increased 30% of glucose concentration only, compare to add 13 days for the pretreatment. However, the 14 days of pretreatment may find a little interest in industry. The 24 hrs reaction is more effective than others.

#### V. CONCLUSIONS

In alkaline pretreatment, NaOH and ammonia are generally used as a pretreatment reagent for removing lignin content in lignocellulosic biomass. At room condition, the NaOH and ammonia process consume much time (24 h) for achieve above 50% w/w of lignin removal. Combination (NaOH-ammonia mix-solution) has proved to be a selected delignification reagent for maximum efficacy of the process in this paper. Due to the selectivity of lignin removal, this mixture solution appears to have the best performance as a pretreatment reagent for enzymatic digestibility. The mix-solution will reduce the time consuming, enhance the degree of lignin removal, and increase the accessibility of enzyme to convert cellulose into glucose.

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