# The Alkaline-Ozonolysis Pretreatment and Simultaneous Saccharification and Fermentation (SSF) for the Production of Bioethanol from Rice Straw

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## Abstract
The utilization of rice straw to produce bioenergy such as bioethanol is one of the most strategies for overcoming fossil fuel crisis. The mixture between the highly purity bioethanol and gasoline (gasohol) leads the increasing of octan number. Rice straw contains cellulose which is the raw material of the second generation of bioethanol. In this research, the straw was pretreated with ozonolysis-alkaline pretreatment. The ozonolysis were carried out in fixed bed reactor after the alkaline pretreatment process. The particle size of biomass on the ozonolysis-alkaline pretreatment showed the significant effects on the degradation of lignin. Additionally, the ozonolysis-alkaline pretreatment experiments evidenced a degradation of lignin. The ethanol production from rice straw was investigated by using the simultaneous saccharification and fermentation (SSF) with cellulase enzyme from the *aspergilus niger* and *Saccharomyces cerevisiae*. The results showed that the highest ethanol concentration is about 110,97 mg/l which is obtained at the SSF time of 120 hours dan the yeast concentration of 20 mL.
**Key words:** alkaline pretreatment, bioethanol, rice straw, SSF, ozonolysis

## Introduction
The crisis of petroleum and climate change has caused the interest of renewable energies in many countries such as Indonesia significantly increases. The conversion of lignocellulosic biomass to biofuels especially bioethanol as transportation fuels gives the best optional to decrease the greenhouse emission and supply the energy security. Rice straw is one of the abundant lignocellulosic waste materials that can be utilized to become renewable energy. The utilization of rice straw in Indonesia is still limited to the animal food and fertilizer. Meanwhile, the rice straw has the huge potential to be converted to the second generation of bioethanol. It has high cellulose and hemicelluloses content that can be readily hydrolysed into fermentable sugars. The rice straw predominantly contains cellulose (41.06 %), hemicellulose (13.88 %) and lignin (25.33%) (Chang, Thitikorn-amorn, J., & Ou, 2011).

The production of bioethanol second generation is more difficult compared than the first generation of bioethanol. The lignin content in lignocellulosic biomass must be removed. Therefore, a pretreatment process is very essential in order to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials (Karimi, Emtiazi, & Taherzadeh, 2006). Pretreatment is required to change the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier, et al., 2005). The cellulose and hemicellulose fractions can be enzymatic hydrolysed to monomeric sugars after a pre-treatment process. The pretreatment process is highly cost, so the good pretreatment can decrease the amount of enzyme for enzymatic hydrolysed (Wyman, 2007). Pretreatment enhanced the yield of glucose. Without pretreatment results less than 20% of glucose, meanwhile with the pretreatment process produces the yield of glucose is about 90% (Hamelinck, van Hooijdonk, & Faaij, 2005). Several methods for the pretreatment of ligncellulosic materials to enhance saccharification have been developed in laboratories by using steam explosion and alkaline peroxide (Cara, Ruiz, Ballesteros, Negro, & Castro, 2006), ozonolysis pretreatment (García-Cubero, González-Benito, Indacoechea, Coca, & Silvia, 2009; Lee, Jameel, & Venditti, 2010); alkaline pretreatment (Mcintosh & Vancov, 2010; Harun, Jason, Cherrington, & Danquah, 2010); and hydrothermal pretreatment (Thomsen, Thygese, & Thomsen, 2008). These technologies are usually implemented under severe reaction conditions with a large capital investment, high processing costs and great investment risks (Li, Kim, Jiang, Kang, & Chang, 2009). Some of these methods produce strong acidic, toxic residues in the treated lignocellulosic and inhibit compounds for saccharification and subsequent ethanol fermentation. Moreover, the use of strong acids has significant environmental risks. Meanwhile, alkaline pretreatment was shown to be more effective on agricultural residues than on wood materials (Taherzadeh & Karimi, 2008). Ozonolysis can effectively degrade lignin and part of hemicellulose. The pretreatment is usually carried out at room temperature, and does not lead to inhibitory compounds (Taherzadeh & Karimi, 2008). Based on the advantages of alkaline and ozonolysis pretreatment, this research studied the combination of alkaline-ozonolysis pretreatment to remove the lignin content in the rice straw. Pretreatment is the most expensive process in lignocellulosic biomass to be converted to fuels, but it has the huge potential for improving the efficiency and reducing of the costs through further research and development.

Enzymatic hydrolysis has several advantages (1) reduce the negative impact to environmental (2) the enzymatic hydrolysis can be performed together with the fermentation, instead of subsequent to the enzymatic hydrolysis (Olofsson, Bertilsson, & Lidén, 2008). This is called as Simultaneous Saccharification and Fermentation (SSF). Enzymatic hydrolysis occurs in two steps which are degradation of cellulose to cellobiose by endo-β-1,4-glukanase and ekso-β-1,4 glukanase, subsequent by degradation of cellobiose by β-1,4 glukosidase (Martins, Kolling, Camassola, Dillon, & Ramos, 2008; Ahamed & Vermette, 2008). *Aspergillus niger* produce high content of β-glukosidase and low content of endo-β-1,4-glukanase and ekso-β-1,4-glukanase. Saccharification is carried out by adding cellulase enzym and β-glukosidase. The research of bioethanol from lignocellulosic biomass has been studied by several researchers (Adsul, et al., 2005); (Cara, Ruiz, Ballesteros, Negro, & Castro, 2006); (Cerveró, Skovgaard, C, Sørensen, & Jørgensen, 2010); (Harun, Jason, Cherrington, & Danquah, 2010); (Mcintosh & Vancov, 2010); (García-Cubero, González-Benito, Indacoechea, Coca, & Silvia, 2009) (Erdei, Barta, Sipos, Reczey, Galbe, & Zacchi, 2010); (Lee, Jameel, & Venditti, 2010). This research studied the simultaneous saccharification and fermentation (SSF) of pretreated rice straw for the production of bioethanol.

## Materials and Methods

### *Raw Material*

Rice straw was collected from the location of Musi 2 Bridge (Keramasan) in the city of Palembang, South Sumatera, Indonesia. The rice straw was then chopped, air-dried and stored at room temperature. The rice straw were milled and sieved. The fractions were varied between a 80-mesh screen and a 20-mesh screen and were collected for further use. Determination of the structural lignin followed standard analytical methods of Kappa Number.

### *Lignocellulose Pretreatment*

Lignocellulosic materials were pretreated with the following procedures. 50 gram supply of each dry material was placed in a 500 mL glass beaker and mixed well with 500 ml of NaOH solution with the concentration of 5%. Ratio rice straw and NaOH solution was (1:10 w/v).

The slurry was incubated in a rotary air bath at 120 rpm and 85 $^oC$ for 1 h. After the reaction, the slurry was poured into 1.2 L of pre-cold acetone and mixed thoroughly. The mixture was centrifuged at 8000 rpm for 10 min, and the supernatant was collected. After that, the solid residue was washed again in 1.2 L of distilled water and centrifuged. The lignin content after alkaline pretreatment was analysed by standard methods of Kappa Number.

The alkaline pretreated samples were continued by ozonolysis pretreatment to enhance the removal of lignin content in the rice straw. The ozonolysis pretreatment of rice straw was performed in a fixed bed reactor under room conditions. At the beginning of each test, the ground rice straw was hydrated to the required value of 10 % moisture content. The raw material fed into the reactor until total reaction volume was achieved and then exposed to the oxygen/ozone gas stream in the fixed bed reactor. The production of ozone from the generator was controlled by varying either the oxygen flow rate with the fixed electrical power supply. The concentration of ozone in the gas phase was measured following the iodometric method (Clesceri, Greenberg, & Eaton, 1999). The outlet gas flow of reactor was passed by a 2% of KI solution to remove any unreacted ozone from the gas stream. The lignin content after ozonolysis pretreatment was analysed by standard methods of Kappa Number. The resulting ozone-treated substrate was dried in an oven at 45 $^oC$, stored in a freezer before be used for Simultaneous Saccharification and Fermentation (SSF) and for analysis of lignin content.

***Simultaneous Saccharification and Fermentation***

A 50 grams of rice straw pretreated were put into 1000 ml Erlenmeyer, then it was added the solution of media (yeast extract of 5 g / L, $(NH_4)\,_2SO_4$ 7.5 g / L; $K_2HPO_4$ 3, 5 g / L; $MgSO_47H_2O$ 0.75 g / L and $CaCl_22H_2O$ 1 g / L) with a ratio of 1: 10 (w / v) and be adjusted the pH of ± 5. Then it was sterilized in the autoclave at 121 ° C for 60 min (Li et al, 2009). Once in the autoclave, rice straw pulp is allowed to cool and then added with a cellulase enzyme concentration of 20 ml. The fermentation time was varied between 72 hours to 168 hours, while the treatment of the yeast Saccharomyces cereviseae concentration of 5 ml to 20 ml. Furthermore Erlenmeyer shut with a cork. Then placed on a rotary shaker at 120 rpm for each fermentation time. Ethanol fermentation Pycnometer method and analysed by spectrophotometer method.

**Results and Discussions**

The results showed that the rice straw has the potential to be utilized to sugars especially glucose and bioethanol. Initial pretreatment of rice straw aims to assist saccharification process.

***Effect of the Particle Size on the Reducing of Lignin Content of the Alkaline-Ozonolysis Pretreatment***

The Pretreatment were conducted by chemical method which is the combination of alkaline-ozonolysis. Alkaline pretreatment was shown to be more effective on agricultural residues than on wood materials (Taherzadeh & Karimi, 2008). Ozonolysis can effectively degrade lignin and part of hemicellulose. The pretreatment is usually carried out at room temperature, and does not lead to inhibitory compounds (Taherzadeh & Karimi, 2008). Ozone as strong oxydator degraded the lignin content in the rice straw, so it released the cellulose that can be hydrolysed to glucose.

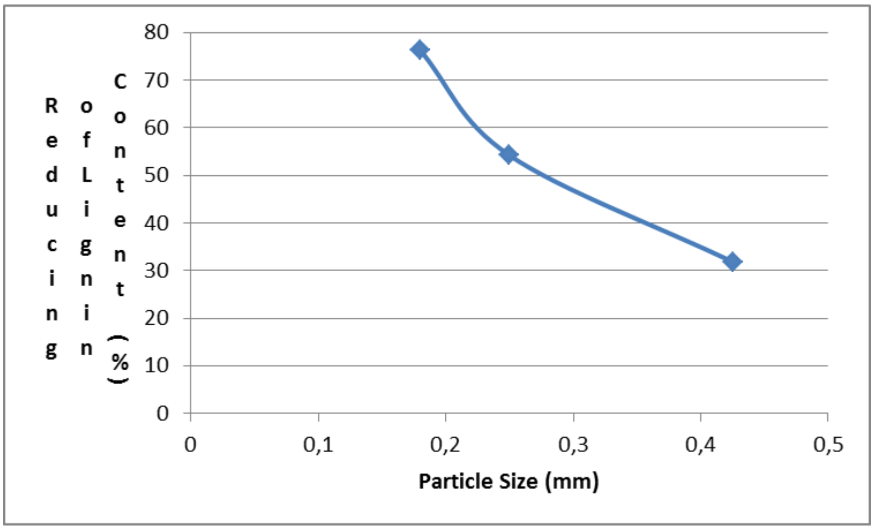


**Figure 1.** Effect of particle size on the reducing of lignin content of alkaline-ozonolysis pretreatment

Figure 1 shows that the lignin content was effected by the biomass particle size. The lower size of particle leads the reducing of lignin content increase significantly. The reducing of lignin content is caused by the alkaline-ozonolisis pretreatment. The analytical method of Kappa Number indicated that the reducing of lignin content is about 31,82 % for the size particle of +0,425-0,850 mm. On the other hand, the size particle of +0,250-0,425 mm resulted is about 54,17 % of the decreasing of lignin content. The reducing of lignin content for the size particle of +0,180-0,250 mm is 76,19 %.

***The Effect of Fermentation Time on the Ethanol Concentration for Various Yeast Concentration***

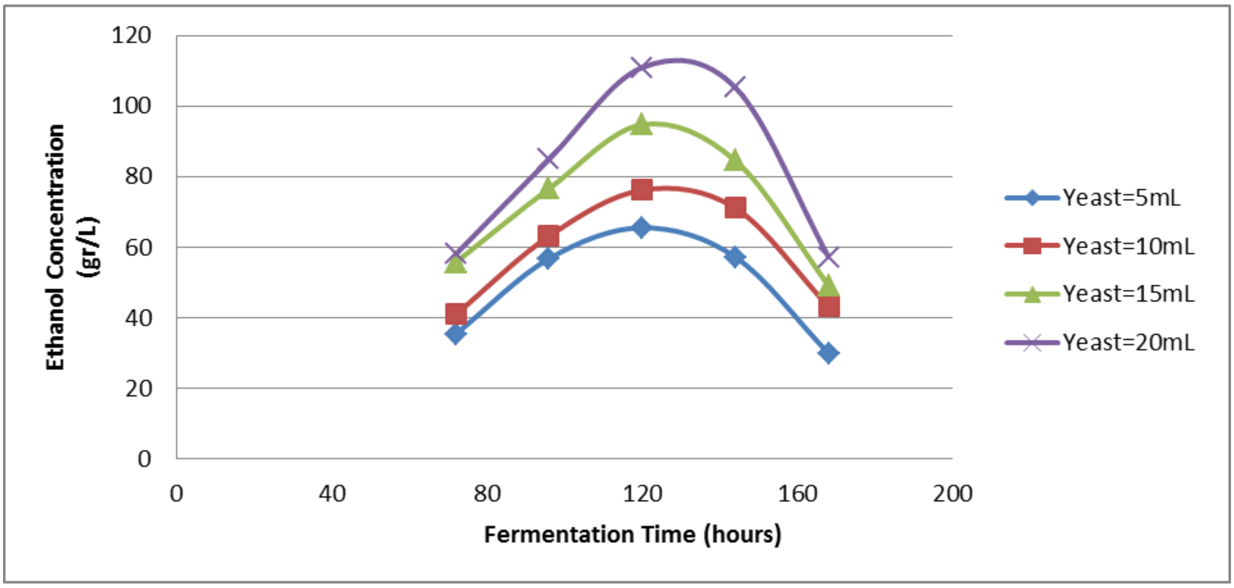


**Figure 3.** The effect of SSF time on the ethanol concentration for various yeast concentration.

The parameters used to study the effect of SSF time on the ethanol concentration are the SSF time (72 hours to 168 hours) and the yeast concentration of 5 ml to 20 ml. The effect of SSF time on the ethanol concentration for various yeast concentrations can be seen in figure 3. The highest concentration of ethanol is about 110,97 gr/l which is obtained at the SSF time of 120 hours and the yeast concentration of 20 mL. The figure also showed that the concentration of ethanol increase with the raising of SSF time. However after 120 hours, the ethanol concentration decreased. This is because the time of SSF relate to microorganism growth curve. The microorganism growth consists of six phases which are the adaptation phase, initiation growth phase, quick growth phase, constant phase (stationer) and the death phases.

**Conslusions**

It has been made clear that efficient pretreatment of rice straw is possible using combination of alkaline-ozonolyisis pretreatment. This can be attributed to the fact that the ozonolysis affects the cellulosic region and the further alkaline solution removes of the re-localized lignin and hence facilitates the enzyme treatment. The result showed that the lower size of particle leads the higher reducing of lignin content. The highest lignin content decreasing is about 76,19% for the size of +0,180-0,250 mm. The highest concentration of ethanol is about 110,97 gr/l which is obtained at the SSF time of 120 hours and the yeast concentration of 20 mL.

**Acknowledgement**

The authors acknowledge the DP2M-DIKTI of Indonesia for funding the project (Hibah Strategis Nasional). The authors are also thank to Dr. Muhammad Faizal for giving the ideas in the research and thank to Mr. Arif Nurahman and Miss. Selvia Aprilyanti for research assistance.

**References**

[1] Adsul, M., Ghule, J., Shaikh, H., Singh, R., Bastawde, K., Gokhale, D., et al. (2005). Enzymatic Hydrolysis of Delignified Bagasse Polysaccharides. *Carbohydrate Polymers*, 62, 6–10.

[2] Ahamed, A., & Vermette, P. (2008). Culture-based Strategies to Enhance Cellulase Enzyme Production from Trichoderma reesei RUT-C30 in Bioreactor Culture Conditions. *Biochemical Engineering Journal*, 40, 399-407.

[3] Cara, C., Ruiz, E., Ballesteros, I., Negro, M. J., & Castro, E. (2006). Enhanced Enzymatic Hydrolysis Of Olive Tree Wood By Steam Explosion And Alkaline Peroxide Delignification. *Process Biochemistry*, 41, 423–429.

[4] Cerveró, J. M., Skovgaard, P. A., C, F., Sørensen, H. R., & Jørgensen, H. (2010). Enzymatic Hydrolysis And Fermentation Of Palm Kernel Press Cake For Production Of Bioethanol. *Enzyme and Microbial Technology*, 46, 177–184.

[5] Chang, K., Thitikorn-amorn, J., J., H., & Ou, B. (2011). Enhanced Enzymatic Conversion with Freeze Pretreatment of Rice straw. *Biomass and bioenergy* , 35: 90-95.

[6] Erdei, B., Barta, Z., Sipos, B., Reczey, K., Galbe, M., & Zacchi, G. (2010). Ethanol production from mixtures of wheat straw and wheat meal. *Biotechnology for Biofuels*, 3:16, 1-9.

[7] García-Cubero, T., González-Benito, G., Indacoechea, I., Coca, M., & Silvia, B. (2009). Effect of ozonolysis pretreatment on enzymatic digestibility of wheat and rye straw. *Bioresource Technology*, 100, 1608–1613.

[8] Hamelinck, C., van Hooijdonk, G., & Faaij, A. ( 2005). Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. *Biomass Bioenergy*, 28, 384–410.

[9] Harun, R., Jason, W., Cherrington, T., & Danquah, M. K. (2010). Exploring Alkaline Pre-Treatment Of Microalgal Biomass For Bioethanol Production. *Applied Energy*, 88, 10, 3464-3467.

[10] Karimi, K., Emtiazi, G., & Taherzadeh, M. J. (2006). Ethanol production from dilute-acid pretreated rice straw by simultaneous saccharification and fermentation with Mucor indicus, Rhizopus oryzae, and Saccharomyces cerevisiae. *Enzyme and Microbial Technology* , 40:138–144.

[11] Lee, J. M., Jameel, H., & Venditti, R. A. (2010). Effect of Ozone & Autohydrolysis Pretreatments on Enzymatic Digestibility of Coastal Bermuda Grass. *BioResources*, 5(2), 11084-1101.

[12] Li, H., Kim, N., Jiang, M., Kang, J. W., & Chang, H. N. (2009). Simultaneous saccharification and fermentation of lignocellulosic residues pretreated with phosphoric acid–acetone for bioethanol production. *Bioresource Technology*, 100: 3245–3251.

[13] Martins, L., Kolling, D., Camassola, M., Dillon, A., & Ramos, L. (2008). Comparison of Penicillium echinulatum and Trichoderma reesei Cellulases in Relation to Their Activity Against Various Cellulosic Substrates. *Bioresource Technology*, 99, 1417.

[14] Mcintosh, S., & Vancov, T. (2010). Enhanced Enzyme Saccharification of Shorgum Bicolor Straw Using Dilute Alkali Pretreatment. *Bioresource Technology*, 6718-6722.

[15] Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y., Holtzapple, M., et al. (2005). Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass. *Bioresource Technology*, 96(6):673-686.

[16] Olofsson, K., Bertilsson, M., & Lidén, G. (2008). A short review on SSF – an interesting process option for ethanol. *Biotechnology for Biofuels*, 1:7 doi:10.1186/1754-6834-1-7.

[17] Taherzadeh, M. J., & Karimi, K. (2008). Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. *International Journal of Molecular Sciences*, 9, 1621-1651.

[18] Thomsen, M. H., Thygese, A., & Thomsen, A. B. (2008). Hydrothermal treatment of wheat straw at pilot plant scale using a three-step reactor system aiming at high hemicellulose recovery, high cellulose digestibility and low lignin hydrolysis. *Bioresource Technology*, 99; 4221–4228.

[19] Wyman, C. E. (2007). What is (and is not) vital to advancing cellulosic ethanol Original Research Article. *Trends in Biotechnology*, Volume 25, Issue 4, Pages 153-157.