International Journal of Applied Science and Engineering Review

ISSN: 2582-6271

Vol. 4, Issue.1, Jan-Feb 2023, page no. 32-41

To cite this article: Hargono Hargono, Noer Abyor Handayani, Aji Prasetyaningrum and Muhamad Ichsan H.P. (2023). SIMULTANEOUS HYDROLYSIS AND FERMENTATION OF RICE STRAW: EFFECT OF LIGNIN PRETREATMENT ON GLUCOSE AND ETHANOL CONCENTRATIONS, International Journal of Applied Science and Engineering Review (IJASER) 4 (1): 32-41

SIMULTANEOUS HYDROLYSIS AND FERMENTATION OF RICE STRAW: EFFECT OF LIGNIN PRETREATMENT ON GLUCOSE AND ETHANOL CONCENTRATIONS

Hargono Hargono, Noer Abyor Handayani, Aji Prasetyaningrum and Muhamad Ichsan H.P.

Chemical Engineering Department, Faculty of Engineering, Diponegoro University
Jl. Prof. Soedarto, SH, Tembalang, Semarang, Central Java 50275, Indonesia

DOI: http://dx.doi.org/10.52267/IJASER.2023.4103

ABSTRACT

Lignin is a component of lignocellulosic materials which is relatively difficult to degrade. Its presence limits the use of lignocellulosic materials when they are converted to glucose and ethanol. This objective of the studi to reduce concentration of lignin contained in rice straw, carried out enzymatic hydrolysis and fermentation Pretreatment was carried out at a temperature of 100-140ºC and a NaOH concentration of 4-10% for 16 hours. Hydrolysis and fermentation separately with the addition of cellulase enzymes and Saccharomyces cereviceae during fermentation. The nutrients used for bacterial growth included $(NH4)2HPO4$ 0.5 g/L, $MgSO4.7H2O$ 0.025 g/L. The media then incubated for 15 hours in a shaker at 30°C. at 80 rpm. The results of the study achieved a decrease in lignin during 16 hours the percentage reduction in lignin concentration for variations in NaOH concentrations of 4, 6, 8 and 10% were 33.76, 44.96, 55.73 and 31.41%. The best conditions were achieved during the delignification time of 16 hours at a concentration of 8% NaOH, which was a 44.96% reduction in deligtnification. Hydrolysis and fermentation of rice straw that for 6 to 48 hours, resulted the percentage of increasing glucose and ethanol concentrations before and after pretreatment was 32.99% and 39.99%.

KEYWORDS: rice straw, lignin, delignification, glucose, ethanol

1. INTRODUCTION

Lignocellulosic based hydrolysis which will be converted into glucose will be hampered due to the presence of lignin contained in lignocellulose. The form of lignin bonds is bound in polysaccharides, so that the presence of lignin is very difficult to be degraded by enzymes. Sudiyani et al. (2010) reported the results of their research that lignocellulosic can be converted using bioenzymes that produce sugar as a source of raw material for product of ethanol. This research is also supported by Kim at al. (2013) and (Muryanto et al. (2012). They stated that lignocellulosic as a constituent of biomass can be converted into glucose using enzymes, acids or bases. Lignocellulose which is abundant and never runs out has great potential to be processed into glucose and ethanol. Lignin compounds will interfere in the manufacture of paper, which causes a brown color, thereby reducing the economic value of paper. Lignocellulose is composed of lignin, cellulose and hemicellulose, to reduce lignin a delignification process is carried out. This aims to increase the cellulose content so that the product glucose which is desired to increase the yield. The delignification process is generally carried out using a strong base (NaOH) or strong acid (H2SO4).

Chemical treatment includes using HCl (Handayani et al., 2018), NaOH (Muryanto et al., 2016), and ionic ionic solutions (Domínguez de María et al., 2014; Sen et al., 2012). Of the various recommended strong bases, NaOH is the best base for degrading lignin. The characterization of hemicellulose shows that it is amorphous and porous, so this condition allows NaOH to reduce lignin by extraction process (Julfana, 2012).

The delignification process physically by heating at temperatures above 100C can optimally reduce lignin. The results of Zhao et al. (2013) that delignification results can cause decomposition of β-O-4' and ether with an acid catalyst.NaOH solution can attack and damage the lignin structure in crystals and amorphous parts and separate some hemicelluloses. OH ions from NaOH will break the bond from the basic structure of lignin, while Na+ ions will bind to lignin to form sodium phenolate. This phenolic salt is soluble. Dissolved lignin is marked with a black color in a solution called black liquor (Safaria et al.,2013). Alkali delignification to reduce the lignin content in lignocellulosic in agricultural waste is considered an effective method (Grimaldi et al., 2015; Kim et al., 2005).

Previous studies on alkaline pretreatment have demonstrated its effectiveness. Most of the acetyl groups and about 10-52% lignin have been removed, increasing enzymatic saccharification in bagasse, switchgrass, corn stover, poplar wood, wheat straw (Guilherme et al., 2015; Chang et al., 1998). Although this pretreatment has been extensively studied on various types of lignocellulosic biomass, there have not been many studies on the delignification of rice husks for conversion to ethanol.

The objective of this study was to reduce the lignin content in rice straw by chemical and physical processes, to hydrolyze and ferment the treated rice husks to obtain glucose and ethanol.

ISSN: 2582-6271

Vol. 4, Issue.1, Jan-Feb 2023, page no. 32-41

2. MATERIALS AND METHODS

2.1. Materials: rice straw obtained from Candirejo village, Semarang districs , Central Java, whereas potassium sodium tartrate tetrahydrate and 3,5-dinitrosalicylic acid (Sigma-Aldrich Indonesia), glucose (99.5%, Sigma-Aldrich Indonesia), H2SO4 (98.5 %, Merck), NaOH (98 %, Merck), distilled water (from the supplier Indrasari, Semarang-Indonesia), Cellulase enzym was produced Aspergillus niger FNU 6018 and Saccharomyces cerevisiae were obtained from the microbiology laboratory, Chemical Engineering Department-Universitas Diponegoro Indonesia.

2.2. Preparation of rice straw materials

Five kg of rice straw which is still mixed with dirt and other materials is sorted and selected for the rice straw, then washed with water, dried in the sun for 48 h. Furthermore, the rice straw was reduced in size using a knife mill until it was about 1 cm in size. Pieces of rice straw stored in a desiccator as a research sample.

2.3. Analysis of lignin

Rice Straw powder delignification was carried out based on research by Richana et al. (2007), using 1% sodium hypochlorite (NaOCl) solvent, the delignification process was carried out for 5 hours at room temperature. The result is then washed until the smell of NaOCl is gone, then a filtering process is carried out to remove water containing lignin. The straw powder was then dried at 50 ºC for 48 hours. The results of the delignification were analyzed proximately and the fiber components were returned to obtain the content of cellulose, hemicellulose and lignin after delignification.

2.4. Effect of temperature delignification and NaOH concentration on lignin concentration

Erlenmeyer 500 mL as a delignification reaction tool is filled with 50 g of rice straw mixed with 250 mL of water and added NaOH at various 4,6,8 and 10%, stirred using a magnetic stirrer at 100 rpm. Then the mixture is heated to temperature 100, 120, 130 and 140 ºC using an autoclave for 12 hours. Furthermore, the straw-liquid solids were separated using a centrifuge (Oregon Centrifuge LC-04R, China). The rice straw solids was dried in an oven at 110ºC, then the materials analyzed for lignin content. To calculate the percentage increase in lignin concentrations based on in eq. 1.

% decreasing of lignin

$$= \frac{lignin\ concentration\ before\ pretreatment - lignin\ concentration\ after\ pretreatment}{lignin\ concentration\ before\ pretreatment} \times 100 \ldots\ldots\ldots(1)$$

2.5. Effect of time delignification and NaOH concentration on lignin concentration

Erlenmeyer 500 mL as a delignification reaction tool is filled with 50 g of rice straw mixed with 250 mL of water and added NaOH at various 4,6,8 and 10%, stirred using a magnetic stirrer at 100 rpm at various time 4, 8, 12, 16 and 20 hours, after that the mixture is heated to temperature 120 ºC using an autoclave for 12 hours

2.6. Hydrolisis Process

Erlenmeyer 1000 mL was used for hydrolysis of 50 grams of pretreated rice straw in 500 mL of water at pH 5 (controlled with citrate buffer solution), then added cellulase enzymes Trichoderma reesei and Aspergillus niger 1% (v/v), heated over a shaker at temperature 50ºC for 72 hours, stirring speed 75 rpm. The samples were taken for period of 6, 12, 18 to 72 hours and centrifuged it for 4 minutes at 100 rpm. The samples were filtered using Whatman CAT 40 filter paper No.1440-125 mm, in order to obtain a clear filtrate, then the filtrate was analyzed to determine for glucose concentration using measuring absorbance at 540 nm by a UV/visible spectrophotometer (UV-160A, SHIMADZU, Kyoto, Japan). (Hargono et al., 2017) The best result of this hydrolysis process that produces maximum glucose. Furthermore, glucose produced from these best conditions is used as raw material for the fermentation process to produce ethanol.

2.7. Fermentation

The best glucose concentration from hydrolysis is used for fermentation feed. Glucose fermentation was carried out in a 1200 mL reactor at 30ºC and pH 4.5 (controlled using 3 M NaOH solution). The nutrients used for bacterial growth included (NH4)2HPO4 0.5 g/L, MgSO4.7H2O 0.025 g/L, and yeast extract 1g/L added to the media then incubated for 15 hours in a shaker at 30°C. speed 80 rpm. Then 5 g/L dry yeast Saccharomyches cerevisiae was added and incubated for 78 hours. Sampling was carried out every 6 hours to analyze of ethanol content (Hargono et al., 2015). To calculate the percentage increase in glucose and ethanol concentrations based on eq. 2 and eq.3.

$$\%\ \text{increasing of glucose} = \frac{glucose\ concentration\ after\ pretreatment\ - glucose\ concentration\ before\ pretreatment}{glucose\ concentration\ after\ pretreatment} \times 100 \quad \ldots\ldots \quad (2)$$

$$\%\ \text{increasing of ethanol} = \frac{ethanol\ concentration\ after\ pretreatment\ - etanol\ concentration\ before\ pretreatment}{ethanol\ concentration\ after\ pretreatment} \times 100 \quad \ldots\ldots \quad (3)$$

3. RESULT AND DISCUSSION

The result of chemical component of rice straw before and after pretreatment as shown in Table 1.

Table 1. Analysis of chemical components contained in rice straw

Materials	Cellulose, %	Hemicellulose, %	Lignin, % %	Ash, %
Native Rice Straw (RS)	30.62	27.42	18.75	11.53
Pretreatment RS	46.32	-	8.58	

3.1. Effect of time delignification and NaOH concentration on lignin concentration

As shown in Fig.1, the effect of delignification time on lignin concentration is significant

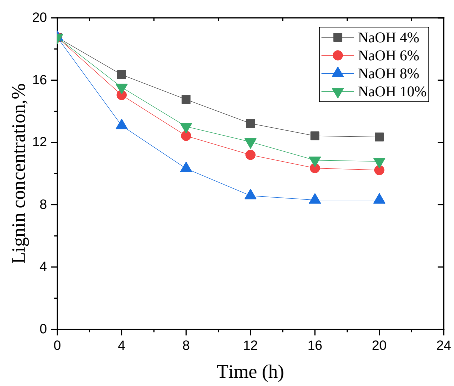

Figure 1. Effect of delignification time and NaOH concentration on lignin concentration

The delignification experiment was carried out for 4-20 hours at 4-10% NaOH concentration. During 16 hours, the lignin concentration decreased significantly for 4-10% NaOH concentration, but after 16 hours the lignin concentration tended to be constant. During 16 hours the percentage reduction in lignin concentration (based on Eq.1) for variations in NaOH concentrations of 4, 6, 8 and 10%, respectively were 33.76, 44.96, 55.73 and 31.41%. The best conditions were achieved during the delignification time of 16 hours at a concentration of 8% NaOH, which was a 44.96% reduction in deligtnification. Process of delignification using 2% (w/v) NaOH from rice husks, a decrease in lignin concentration from 9.22% to 3.78% (Rahnama et.al., 2014). Zhang et al. (2008) reported treatment of rice husk using 2% NaOH, there

ISSN: 2582-6271

Vol. 4, Issue.1, Jan-Feb 2023, page no. 32-41

was a decrease in lignin from 14.9% to 9.5%. The chemical treatment of the delignification process is up to the use of NaOH because this chemical compound can remove lignin while extracting hemicellulose, as shown in Fig.2. NaOH solution can attack and damage the lignin structure in the crystalline and amorphous parts and separate some hemicelluloses. OH ions from NaOH will break bonds from the basic structure of lignin, while Na+ ions will bind to lignin to form sodium phenolate. This phenolic salt is soluble. Dissolved lignin is marked with black color in the solution, which is called black leachate (Permata et al., 2021).

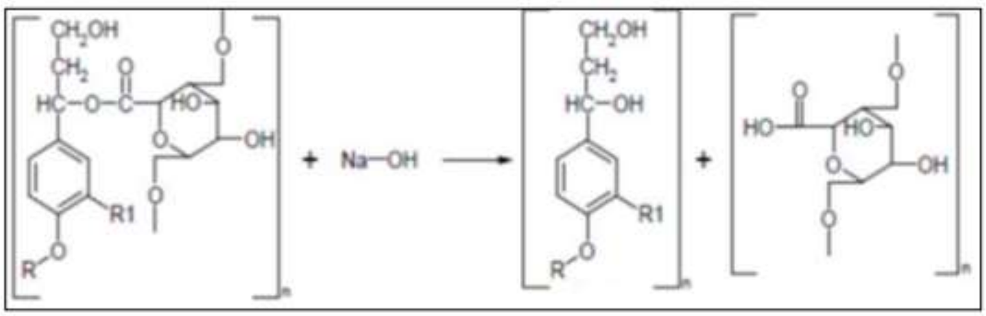

Figure 2. Chemical treatments include using NaOH to lignin and cellulose (Yu-Shen Cheng et al., 2010).

3.2. Effect of delignification temperature and NaOH concentration on lignin concentration during 16 hours

The effect of temperature treatment, especially at temperatures of 100 and 120ºC on lignin concentrations showed significant changes, as shown in Fig.3. The concentration of lignin in native rice straw (18.75%) after the temperature was increased to 100 and 120ºC would decrease the lignin concentration for each treatment with NaOH concentration 4, 6, 8 and 10%. The greatest decrease occurred at an increase in temperature of 120ºC and 8% NaOH concentration, namely at 8.58% lignin concentration (the percentage decrease in lignin concentration reached 54.24% (based on eq. 1). Under the same conditions, when the delignification temperature was increased to 130ºC, the decrease in lignin concentration became 11.35% (the percentage decrease in delignification lignin concentration was 39.63%). The best conditions for reducing lignin concentration occurred at a temperature of 120ºC and treatment with 8% NaOH concentration.

International Journal of Applied Science and Engineering Review

ISSN: 2582-6271

Vol. 4, Issue.1, Jan-Feb 2023, page no. 32-41

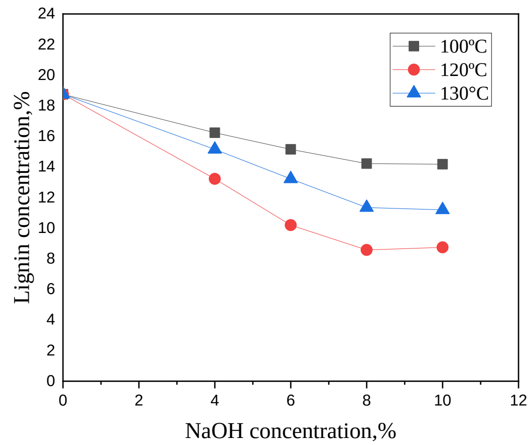

Figure 3. Effect of delignification temperature and NaOH concentration on lignin concentration for 16 hours.

The effect of temperature treatment, especially at temperatures of 100 and 120ºC on lignin concentrations showed significant changes, as shown in Fig.3. The concentration of lignin in native rice straw (18.75%) after the temperature was increased to 100 and 120C would decrease the lignin concentration for each treatment with NaOH concentration 4-10%. The greatest decrease occurred at an increase in temperature of 120ºC and 8% NaOH concentration, namely at 8.58% lignin concentration (the percentage decrease in lignin concentration reached 54.24%, according to equation 1). Under the same conditions, when the delignification temperature was increased to 130ºC, the decrease in lignin concentration became 11.35% (the percentage decrease in delignification lignin concentration was 39.63%). The best conditions for reducing lignin concentration occurred at a temperature of 120C and treatment with 8% NaOH concentration. From previous research conducted by Cheng et al. (2010) at 55°C with 4% NaOH for 3 hours was generated delignification of 23.1%. While research is conducted by Imman et al. (2014) at 140 °C with 0.25% NaOH (w/v) and a pressure of 25 bar decreased lignin from 24.4 to 11.3% with delignification of 53.68%. When compared in this study, the lignin dissolution was smaller. While research conducted by Menardo et al. (2011) on 5 cm rice straw with pretreatment hydrothermal at 120 °C lignin dissolution occurs with delignification 9.63%, smaller than this study using 3% NaOH at the same temperature that is the case delignification of 16.91%. It shows that the addition of NaOH in the hydrothermal process has an effect to greater lignin solubility.

3.2. Effect of fermentation time on glucose and ethanol concentration

International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 4, Issue.1, Jan-Feb 2023, page no. 32-41

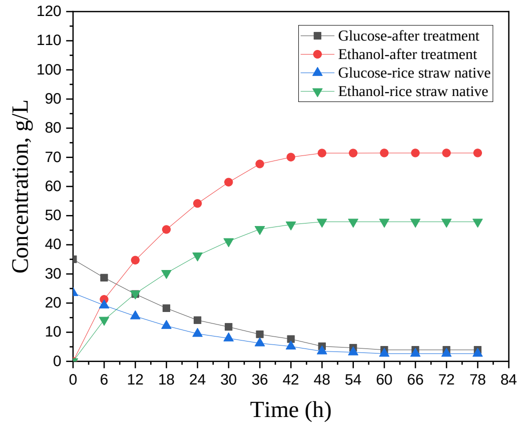

Figure 4. Effect of time fermentation on glucose and ethanol concentration before and after pretreatment rice straw

Hydrolysis and fermentation of rice straw that have undergone delignification treatment for 78 hours produce glucose and ethanol as shown in Fig.4. During 6 to 48 hours, the effect of pretreatment of lignin on glucose and ethanol concentrations as shown in Table 2. The delignification process will reduce the lignin concentration and simultaneously increase the concentration of cellulose (see Table 2), as a result the concentrations of glucose and ethanol will increase significantly. The following are the results of a study on the effect of lignin pretreatment on glucose and ethanol concentrations during SHF, as shown in Table 2.

Table 2. Analysis of chemical components before and after pretreatmen of rice straw

Material	Celullose,%	Lignin,%	Glucose, g/L	Ethanol, g/L
Native Rice Straw (RS)	30.62	18.75	23.46	42.88
After Pretreatment of RS	46.32	8.58	35.01	71.45

Percentage of increasing glucose and ethanol concentrations before and after pretreatment was 32.99% and 39.99%. (based on eq. 2 and eq.3). This is in accordance with the rule that during hydrolysis and fermentation a substrate will form glucose and ethanol (Hargono et.al., 2017). Rahnama et al. (2014) reported research on the hydrolysis of rice straw pretreated using various concentrations of NaOH was subjected to enzymatic hydrolysis. The saccharification of rice straw pretreated with 2% (w/v) NaOH using crude cellulase from local T. harzianum SNRS3 resulted in the production of 29.87 g/L reducing sugar and a yield of 0.6 g/g substrate.

CONCLUSION

Reducing lignin of rice straw through delignification processes via pretreatment by physics and chemistry has an effect on decreasing of lignin 54.24% and affect the increase in cellulose concentration 33.90%, as well as this will have an impact on increasing glucose concentrations and ethanol concentrations during SHF. The best conditions were achieved during the delignification time of 16 hours at a concentration of 8% NaOH, which was a 44.96%. Percentage of increasing glucose and ethanol concentrations before and after pretreatment was 32.99% and 39.99%, therefore lignocellulosic based biomass is feasible to be converted into ethanol.

REFERENCES

Chang, V.S., Nagwani, M. and Holtzapple, M.T., "Lime pretreatment of crop residues bagasse and wheat straw". Appl Biochem Biotechnol, 74(3), pp. 135-159, 1998.

Domínguez de María P., "Recent trends in (ligno)cellulose dissolution using neoteric solvents: switchable, distillable and bio- based ionic liquids," J. Chem. Technol. Biotechnol., 89 pp.11–18., 2014.

Grimaldi, M.P., Marques, M.P., Laluce, C., Cilli, E.M. and Sponchiado, S.R.P., "Evaluation of lime and hydrothermal pretreatments for efficient enzymatic hydrolysis of raw sugarcane bagasse". Biotechnology for Biofuels, 8(1), pp. 205, 2015.

Guilherme, A.A., Dantas, P.V.F., Santos, E.S., Fernandes, F.a.N., Macedo, GR. and Guilherme, AA., "Evaluation of composition, characterization, and enzymatic hydrolysis of pretreated sugar cane bagasse" Brazilian Journal of Chemical Engineering, 32(1): pp. 23-33, 2015.

Handayani SS, Tarnanda R, Rahayu BA, Amrullah, "The process of delignification of lignin in tobacco stem waste as preparation for ethanol production", J.Oijar MIPA, 13(2): 140–146, 2018.

Hargono, Kumoro, A. C., & Jos, B. (2015, December). Comparative study on the conventional and non thermal simultaneous saccharification and fermentation of Manihot glaziovii root starch. In AIP Conference Proceedings Vol. 1699, No. 1, p. 030013, 2017.

Hargono, H., Jos, B., Kumoro, A.C., "Kinetics of the Enzymatic Hydrolysis of Sweet Cassava Starch, Bitter Cassava, and Gadung (Dioscorea hispida Dennst) Flours at Low Temperature", Bulletin of Chemical Reaction Engineering & Catalysis, 12 (2), pp. 256-262, 2017.

Imman, S., Arnthong, J., Burapatana, V., Champreda, V., dan Laosiripojana, N., "Effects of Acid and Alkali Promoters on Compressed Liquid Hot Water Pretreatment of Rice Straw", Bioresource Technology, 171, pp. 29–36, 2014.

Julfana R,. "Enzymatic hydrolysis of cellulose from sago dregs using a mixture of cellulases from Trichoderma reesei and Aspergillus niger" J. Kim. Katulistiwa, 2(1), pp. 52–57, 2012.

Kim S, Kim C.H., "Bioethanol production using the sequential acid/alkali-pretreated empty palm fruit bunch fiber", Renew. Energy, 54, pp.150–155, 2013.

ISSN: 2582-6271

Vol. 4, Issue.1, Jan-Feb 2023, page no. 32-41

Kim, S. and Holtzapple, M.T.," Lime pretreatment and enzymatic hydrolysis of corn stover". Bioresource Technology, 96(18), pp. 1994-2006, 2005.

Menardo, S., Airoldi, G., dan Balsari, P., "The Effect of Particle Size and Thermal Pre-Treatment on The Methane Yield of Four Agricultural By-Products", Bioresource Technology, 104, pp.708–714, 2011.

Muryanto M, Sudiyani Y, Abimanyu H., "Optimization of the NaOH pre-treatment process of oil palm empty fruit bunches to become bioethanol", J. Kim. Terap. Indonesia., 18(01) pp. 27–35, 2016.

Muryanto, Sahlan M, Sudiyani Y., "Simultaneous saccharification and fermentation of oil palm empty fruit bunch for bioethanol production by Rhizopus oryzae", Int. J. Environ. Bioenergy, 3(2), pp. 111–120, 2012.

Rahnama, N., Foo, H.L., Abdul Rahman, N.A., "Saccharification of rice straw by cellulase from a local *Trichoderma harzianum* SNRS3 for biobutanol production". *BMC Biotechnol*, 14, p.103, 2014.

Richana, N., Irawadi, MA., Nur, MA., Sailah, I., Syamsu, K., and Arkenan, Y., 2007, "Ekstraksi xilan dari tongkol jagung". J. Pascapanen. 4(1) pp.38-43, 2007.

Safaria S., "The effectiveness of cellulase enzyme mixture from Aspergillus niger and Trichoderma reesei in hydrolyzing coconut coir substrate", J. Kim. Khatulistiwa, 2(1) pp. 46–51, 2013.

Sen S, Binder J, Raines R, Maravelias C., "Conversion of biomass to sugars via ionic liquid hydrolysis: process synthesis and economic evaluation," Biofuels Bioprod. Biorefin, 6, pp. 444–452, 2012.

Sudiyani Y, Heru R, Alawiyah S., "Utilization of lignocellulosic waste biomass for bioethanol as a new renewable energy source", Ecolab, 4(1), pp. 1–54, 2010.

Yu-Shen Cheng, Y.S., Zheng, Y., Yu, C.W., Dooley, T.M., Jenkin, B.M., Vandeer Gheynst, J.S., "Evaluation of High Solids Alkaline Pretreatment of Rice Straw", Appl Biochem Biotechnol, 162, pp. 1768–1784, 2010.

Zhang, Q.Z and Cai, W.,"Enzymatic hydrolysis of alkali-pretreated rice straw by Trichoderma reesei ZM4-F3", Biomass and Bioenergy, (32), pp. 1130-1135, 2008.

Zhao J, Quan C, Fan S., "Role of lignin in bio-ethanol production from lignocellulosic biomass", J. Biobased Mater. Bioenergy, 7(5), pp. 533–540, 2013.