

The Kinetic of Biodegradation Lignin in Water Hyacinth (*Eichhornia Crassipes*) by *Phanerochaete Chrysosporium* using Solid State Fermentation (SSF) Method for Bioethanol Production, Indonesia

Eka Sari, Siti Syamsiah, Hary Sulisty, and Muslikhin

Abstract—Lignocellulosic materials are considered the most abundant renewable resource available for the Bioethanol Production. Water Hyacinth is one of potential raw material of the world's worst aquatic plant as a feedstock to produce Bioethanol. The purposed this research is obtain reduced of matter for biodegradation lignin in Biological pretreatment with White Rot Fungi eg. *Phanerochaete Chrysosporium* using Solid state Fermentation methods. *Phanerochaete Chrysosporium* is known to have the best ability to degraded lignin, but simultaneously it can also degraded cellulose and hemicellulose. During 8 weeks incubation, water hyacinth occurred loss of weight reached 34,67%, while loss of lignin reached 67,21%, loss of cellulose reached 11,01% and loss of hemicellulose reached 36,56%. The kinetic of losses lignin using regression linear plot, the results is obtained constant rate (k) of reduction lignin is -0.1053 and the equation of reduction of lignin is $y = w_0 - 0,1.53 x$

Keywords—Biodegradation, lignin, *Phanerochaete Chrysosporium*, SSF, Water Hyacinth, Bioethanol

I. INTRODUCTION

THE development of alternative energy technology is critically important because of rising energy consumption, depletion of fossil fuel and environment issues such as global warming and air pollution. The most widely used biofuel is bioethanol. Bioethanol can be produced from e.g. sugars, starch and various lignocellulosic materials such as straw, wood and waste[1]. The one of potential material for bioethanol production feedstock is water Hyacinth. It has high containing cellulose and hemicellulose for based bioethanol production^[4]. Water hyacinth is a free floating aquatic weed which is considered the world's worst aquatic plant because of its rapid spreading ability in the lakes and ponds[2]. It is responsible to disrupt the aquatic system by creating low oxygen conditions beneath water hyacinth mats. The water hyacinth would, there fore have a great potential as raw material or feedstock for Bioethanol Production[2]. The ethanol production from biomass enzyme hydrolysis, fermentation and product separation. Pretreatments are necessary to improve the

digestibility of the lignocellulosic biomass. The main effects are dissolving hemicellulose and alteration of lignin structure by providing an improved accessibility of the cellulose for hydrolytic enzymes[3]. Biological treatment using white-rot fungi is a safe and environmentally-friendly method is increasingly being advocated as a process which does not require high energy for lignin removal from a lignocellulosic biomass^[6] in spite of long lignin degradation^[7]. The Biodegradation of lignin in water hyacinth using *Phanerochaete Chrysosporium* using Solid state fermentation (SSF) method. *Phanerochaete Chrysosporium* is a group of white-rot fungi, it is the most potential lignin degrading microorganism^[5]. The Solid state fermentation (SSF) is defined as the fermentation of solids in the absence of free water^[8], however the substrate must posses enough moisture to support the growth and metabolism of microorganism^[9]. The technology has been investigated for the development of bioprocesses, such as bioremediation and biodegradation process^[10]. The solid state fermentation provides environmental condition under which white-rot fungi grow in nature, thus making it ideal for *Phanerochaete Chrysosporium*^[11]. Considering these merits applying solid state fermentation technology to pretreat lignocellulosic biomass may lead to better efficiency and reduce pretreatment cost^[12]. Application of Solid state fermentation of *Phanerochaete Chrysosporium* for pretreatment of lignocellulosic materials, especially to meet the need for generation of sugar platform for bioethanol production^[13]. Pilot scale fungal pretreatment facilities have been developed and tested for biopulping of wood chips^{[14][15]}. In this study, the biological pretreatment Water Hyacinth for Bioethanol production. During Incubation in Fungi, occur biodegradation process. However Lignin, cellulose and hemicellulose was degraded during Incubation with *Phanerochaete Chrysosporium*.

The various types to determine the kinetic profile based on absolute biomass concentrations that have found in the SSF system were the presented in equation 1-4. The bioreactor model should be able to predict the relative biomass concentration, in order to allow comparison between the prediction and experimental results obtained in the bioreactor, which are typically obtained in term of relative biomass concentrations^[17].

E Sari, Siti Syamsiah, Hary Sulisty, Muslikhin are with Chemical Engineering Department, Gadjah Mada University, Yogyakarta, Indonesia (e-mail: ekasari_gt@yahoo.com)

E Sari is with the Chemical Engineering Department, Sultan Ageng Tirtayasa University, Serang, Indonesia.

- a. Linear $\frac{dC_{XA}}{dt} = k$
- b. Exponential $\frac{dC_{XA}}{dt} = \mu C_{XA}$

II. MATERIALS AND METHODS

A. Material

The water hyacinth (*Eichhornia Crassipes*) leaves and stalk were collected, it from Cilegon Banten Indonesia . It chopped and sun-dried for 4-6 days, and was open dried at 70°C. It was grinding in Ball mill and ground to pass through a 40 mesh pore size screen. The powder water hycinth as a substrate 15 g was weighed and added to glass bottle of volume 500 ml

The reactor was filled with water hyacinth and then water is added according to the desired ratio is dry weight and water is 1 : 3. The bottle is wrapped with heat resistant plastic and autoclaved for 20 minutes at 121°C and cooled to room temperature prior to inoculation. The fungi was used *Phanerochaete Chrysosporium* from Chemical Department in Indonesia of science and research Institute (LIPI). Preparation of Fungi prior to inoculation was used slant and liquid medium. The Incubation in slant is optimal growth of fungi for 7 days. Then the *Phanerochaete Chrysosporium* cultures in slant were transferred into the liquid medium on erlenmeyer 1 liter and incubated for 10 days at room temperature. It is preparing prior inoculation in water hyacinth substrate. After 10 days incubation in liquid medium, *Phanerochaete Chrysosporium* is blend into mixed solution.

B. Solid State Fermentation

The reactor was filled with powdered water hyacinth such as 70% of its volume was left as headspace. The bottles were subsequently with thermoplastic and autoclaved for 20 minutes and 121°C and cooled to room temperature prior inoculation. Inculation was added *Phanerochaete Chrysosporium* solution 10% of dry weight water hyacinth substrate and were incubated at room temperature for a set period of time. Its Inbucated for 8 weeks and analysis is used every week.

The reduction of weight, lignin, cellulose, and hemicellulose were determined by Chesson Metods (Datta, 1981).

III. RESULT AND DISCUSSION

Based on our preliminary experiment, it was found that the yield resulted by extraction time of 30 minutes and 60 minutes were no different. Therefore, we run the extraction time for 45 minutes in this present work.

a. Reduction of Weight

During incubation in *Phanerochaete Chrysosporium* using solid state fermentation, Water Hyacinth occurred loss of weight. The analysis of water hyacinth after the 8-week biodegradation process are show in Fig 1.

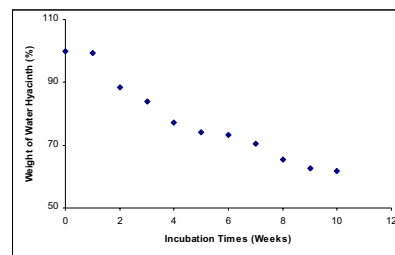


Fig 1 Loss of weight of water hyacinth sample during incubation by *Phanerochaete chrysosporium*

Beyond week 4, reduced of weight was continously degraded following linear trends at average rate of 5,72% per week. Reduced of weight rate slow down in week 6 and 7, But the final reduced of weight on week 9 with the reduced of weight reached 37, 4% and week 10 reduced of weight is more stagnant with only 0,85 % from week 9. On the week 5 until 7 occur decreased rate of weight losses, propably The growth area degrading biomass by fungi more much and the hypa of fungi should be access more inside substrate in bioreactor. During week 5 until 7, we think hypa of fungi have find new area for continue degraded substrate. We have sampling only the first layer on the third section of totally weight of substrate in bioreactor. The first section is growth many hypa of *Phanerochaete Chrysosporium* (PC). For the optimally degradation of substrate, needed agitation substrate for periodic times so the growth of fungi evenly to every part in bioreactor.

b. Reduction of lignin, Cellulose and Hemicellulose

During incubation by *Phanerochaete Chrysosporium* using solid state fermentation, Substances of water hyacinth occurred decrease concentration. The overall reduction of all substances in the water Hyacinth can be seen in the Fig 2 below :

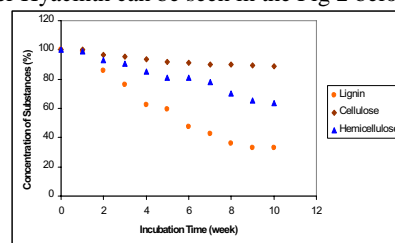


Fig 2 Reduction of lignin, cellulose and Hemicellulose during Incubation by *Phanerochaete Chrysosporium*

There was a lag period until week 2 before significant lignin degradation was observed. It is estimated that at the beginning of incubation, the fungus to adapt to the substrate. and still consume glucose existing in the liquid medium is added into the bioreactor together with the fungus or the fungi still consume other substances that have a short chain in the substrate. Beyond week 3, lignin was continously degraded following linear trends an average rate of 5,41% per week. The significant increase of lignin losses occurred between week 3 until week 8. The final of degradation occurred loss of lignin

reached 67,23%. During incubation in *Phanerochaete Chrysosporium*, water hyacinth as a substrate having loss of cellulose reached 11,01% for 10 weeks of degradation. At the beginning of incubation, loss of cellulose has not occurred, because the fungus has not been able to access the cellulose, probably the fungus still degraded lignin or other substances. At the second week, cellulose degraded not significant only about 3%, but at week 4 to week 9 starting cellulose continuously degraded following linear trends at an average rate of 0.89% per week but the week 9 until week 10 occurred slowly degraded of cellulose. Hemicellulose occurred significant losses during incubation by *Phanerochaete Chrysosporium* reached 36,57% for 10 weeks incubation. The same of lignin and cellulose, there was lag period for 2 week before significant cellulose degradation was observed. At the week 4 until week 5, hemicellulose degraded following linear trends an average rate of 1,72% per week. But at the week 5 until 7 occurred decreasing rate of hemicellulose losses and then week 8 until the final degradation occurred increase hemicellulose degraded following linear trends an average rate of 2,26 % per week. If we compared incubation with combination of fungi using mixed *Pleurotus Osterotus* (PO) and *Phanerochaete Chrysosporium* (PC) have resulted reduction lignin, cellulose and hemicellulose can be expressed Fig 3 below :

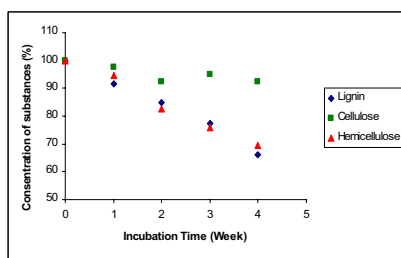


Fig 3 Reduction of lignin, cellulose and Hemicellulose during Incubation by combination of Fungi

The stages of fungal growth on the surface of the substrate at the first week until the eighth weeks shows fig 4 below :

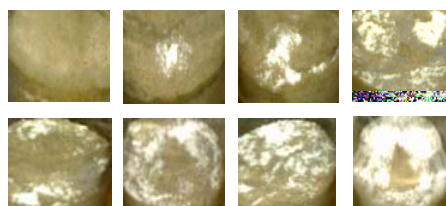


Fig 4 Fungal growth on the surface of the substrate

c. The kinetic of Lignin Degrading

The Degradation of water hyacinth by *Phanerochaete Chrysosporium* using solid state fermentation (SSF) occurred reduced of weight, loss of lignin, loss of cellulose and loss of hemicellulose. if we compare in the fig 3, The lignin occurred more significant than cellulose and hemicellulose.

Phanerochaete Chrysosporium is known to have the best ability to degraded lignin, but simultaneously it can also degraded cellulose and hemicellulose. It means The *Phanerochaete Chrysosporium* can produced cellulase and hemicellulase enzyme in addition to the main producing lignin peroxidase and manganesse peroxidase enzyme. For the purposes of bioethanol production from lignocellulose, which is expected to glucose and then can be fermented into ethanol. It should be on optimizing the conversion of glucose from the fermentation with this fungus. Future studies need to develop and optimize solid state Fermentation process so that the fungus can optimally and the significant to produce of glucose. The kinetic of lignin degrading determine using equation (1) and (2). Considering the average reduction of lignin and the relative error of calculation. Considering the average mass of lignin decreased, and the relative error calculation. For linear equations : $\frac{dC_{XA}}{dt} = k$

Using relative error method, the resulting differences in lignin reduction experimental results with calculated data can be expressed Fig 5 below :

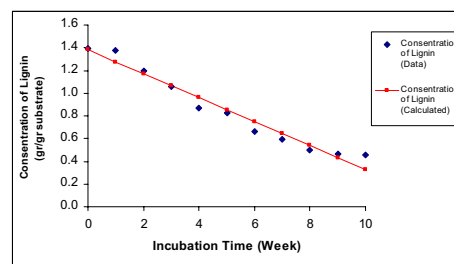


Fig 5 The resulting differences in lignin reduction experimental and calculated result

For the calculation, the corrected relative to a linear equation is generated relative error reaches 8.86%, while for the exponential equation $\frac{dC_{XA}}{dt} = \mu C_{XA}$ obtained reaches 16,41%.

Considering the relative error more than less 10% then the equation is suitable for the reduction of lignin in the water hyacinth is following a linear equation. Using Regression linear plot, we can have constant velocity for lignin reduction is $k = -0.1053$. The equation reduction of lignin follows the equation :

$$y = w_0 - 0,153 x$$

where is : y is the number of lignin at times of t, w_0 is the initial amount of lignin, x is the constant velocity reduction of lignin.

IV. CONCLUSION

In conclusion, During incubation with *Phanerochaete Chrysosporium* using solid state fermentation, water hyacinth can be degraded. The most significant matter can be degraded is lignin, but simultaneously cellulose and hemicellulose degraded too, reached 11,01%, 36,57% is respectively.

The result of kinetic degrading lignin have The constant rate of reduction lignin is $k = -0,1053$ and the equation of lignin degradation is $y = w_0 - 0,1053 x$.

REFERENCES

- [1] Jacques K, Lyons TP, Kelsall DR. "The alcohol textbook", 3rd ed. Trowbridge, Wiltshire: Redwood Books; 1999.
- [2] Gunarson, C.C., Petersen, C.M., 2006, "Water hyacinth as a resource in agriculture and energy production : Literatur review", Waste management 27 (2007) 117-129
- [3] Bong J.P., and Yun, H.S., "Biodegradation of lignin", Dept. of Biological Engineering, Inha University, Incheon, Republic of Korea, Journal of Bioscience and Bioengineering 108 (2009) S41-S56
- [4] Hronich, J.E., Martin, L., Plawsky, J., Bungay, H.R., 2008, "Potential of *Eichhornia crassipes* for biomass refining", Ind Microbiol Biotechnol 35: 393 – 402
- [5] Martin, H., 2002, "Review :lignin conversion by manganese peroxidase", Enz.mic.Technol 30,454-466
- [6] Lee J.W., Gwak K.S., Park J.Y., Park M.J., "Biological Pretreatment of Softwood *Pinus densiflora* by Three White Rot fungi", The journal of Microbiology, December 2007, p.485-491, the Microbiological Society of Korea.
- [7] Okano, K., M. Kitagawa, Y. Sasaki, and T. Wanabe. 2005. "Conversion of Japanese red cedar (*Cryptomeria japonica*) into a feed for ruminant by white rot *Basidiomycetes*". Animal Feed Sci. Technol 120, 235-243
- [8] Pandey A., 1992. "Recent process developments in solid state fermentation". Process Biochem. 27, 109-117
- [9] Ashok, P., 2003, "Solid state fermentation". Biochem. Eng. J. 13, 81-84
- [10] Pandey A., Carlos, R.S., David, M., 2000. "New Development in solid state fermentation: I-bioprocesses and product". Process Biochem. 35, 1153-116
- [11] Datta, A., Bettermann, A., Kirk, T.K., 1991. "Identification of a specific manganese peroxidase among lignolytic enzymes secreted by *Phanerochaete chrysosporium* during wood decay". Appl. environ. Microb. 57, 1453-1460
- [12] Shi, J., Chinn, M.S., Ratna, R., Shivappa, S., "Microbial pretreatment of cotton stalks by solid state cultivation of *Phanerochaete chrysosporium*". Bioresources Technology 99 (2008) 6556-6564
- [13] Lee, J., 1997. "Biological conversion of lignocellulosic biomass to ethanol". J. biotechnol. 56, 1-30
- [14] Akhtar, M., Scott, G.M., Swaney, R.E., Kirk, T.K., 1998. "Overview of biomechanical and biochemical pulping research". In : Enzyme Application in Fiber Processing. American Chemical Society, John Wiley & Sons, Inc, New York, NY, pp 2-16, Chapter 2
- [15] Hatakka, A., Maijala, P., Hakala, T., Hauhio, L., Ellmen, J., 2003. "A new white-rot fungus and its use in the pretreatment of wood". Finish Patent, FL 112248
- [16] Chesson, A. 1981. "Effects of sodium hydroxide on cereal straws in relation to the enhanced Degradation of structural polysaccharides by rumen microorganisms". J. Sci. Food Agric. 32:745-758
- [17] Mitchel, D.A., Krieger, N., Berovit, M., 2006, "Solid-State Fermentation Bioreactor: Fundamental of Design and Operation", Springer, Germany., 16:220-223
- [18] Lee, J.W., Gwak, K.S., Park, J.Y., Park, M.J., Choi, D.H., 2007, "Biological Pretreatment of Softwood *Pinus densiflora* by Three White Rot Fungi", Journal of Microbiology p 485 – 491
- [19] Kumar, A.G., Sekaran, G., Krishnamoorthy, 2005, "Solid state fermentation of *Achras zapota* lignocellulose by *Phanerochaete chrysosporium*", Bioresources Technology 97:1521 -1528