World Academy of Science, Engineering and Technology
International Journal of Chemical and Molecular Engineering
Vol:5, No:6, 2011

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 Sulistyo, 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 hemicelulose. 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 $y = w_o - 0, 1.53\ x$

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 bioetanol 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 Bioetanol 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 environmently-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 Chysosporium*[11]. Considering these merits applying solid state fermentation technology to pretreat lignocellulosic biomass may lead to better efficiency and reduce pretreatment cost[12]. Aplication of Solid state fermentation of *Phanerochaete Chrysosporium* for pretreatment of lignocellulosic materials, especially to meet the need for generation of sugar platform for bioetanol 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 Bioetahanol production. During Incubation in Fungi, occur biodegradation process. However Lignin, cellulose and hemicelulose was degraded during Incubation with *Phanerochaete Chrysosporium.*

The various types to determine the kinetic profile based on absolute biomass consentrations 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 consentration, in order to allow comparison between the prediction and experimental results obtained in the bioreactor, which are typically obtained in term of relative biomass consentrations[17].

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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 detemined 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 fermen*tation, Water Hyacinth occurred loss of weight. The analysis of water hyacinth after the 8-week biodegradation process are show in Fig 1.

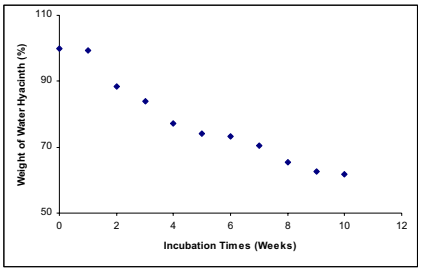

Fig 1 Loss of weight of water hyacint sample during incubation by *Phanerochaete chrysosporium*

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 agitatation 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 consentration. The overall reduction of all substances in the water Hyacinth can be seen in the Fig 2 below :

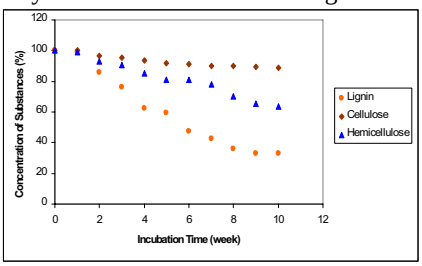

Fig 2 Reduction of lignin, cellulose and Hemicellulose during Incubation by *Phanerochaete Chrysosporium*

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 occured, 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 loses 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 :

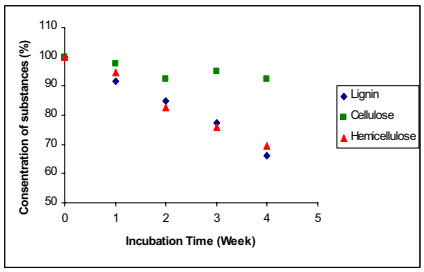

Fig 3 Reduction of lignin, cellulose and Hemicellulose during Incubation by combination of Fungi

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

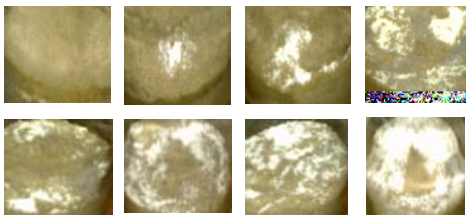

Fig 4 Fungal growth on the surface of the substrate

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 hemicelulose. 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 :

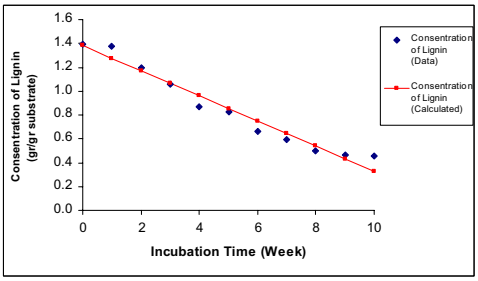

Fig 5 The resulting differences in liglin reduction experimental and calculated result

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 :

$$y = w_o - 0, 1.53\ x$$

where is : y is the number of lignin at times of t, w_o 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 simultanously 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 $y = w_o - 0,1053\ x$.

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