JFAChE
Journal of Fundamentals and Applications of Chemical Engineering

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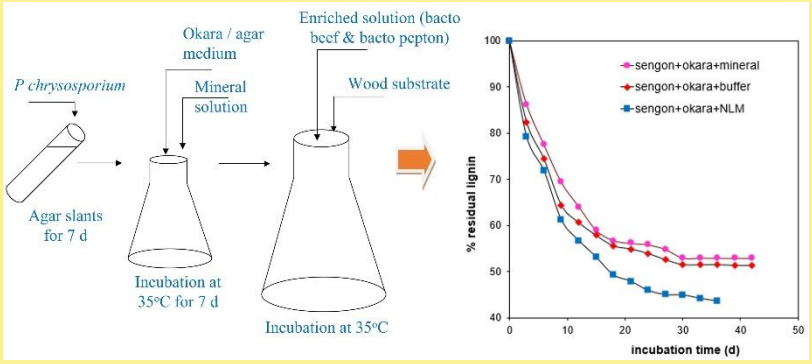
Biodelignification of Sengon (*Paraserianthes falcataria*) and Pine (*Pinus merkusii*) Using White-Rot Fungus *Phanerochaete chrysosporium*

Arief Widjaja[1*], Dwina Moentamaria[2], Hanny F. Sangian[3]

Received May 28, 2022; Revised July 15, 2022; Accepted August 08, 2022
DOI: 10.12962/j2964710X.v3i1.13043

Abstract— Indonesia with its huge area of tropical forest is one of the biggest wood producing countries, thereby potential to become a big pulp and paper producing country. However, the present pulping/bleaching process uses chlorine that is very toxic to the environment. Biopulping process which reduce or eliminate the use of chlorine is therefore, a very promising method. This research performs the biodelignification process using white-rot fungus *Phanerochaete chrysosporium* on Sengon (*Paraserianthes falcataria*) and Pine (*Pinus merkusii*) especially studies the effect of using different medium during incubation. Agar and okara were used for the base media in which the effect of important parameters such as mineral additive, buffering system, and nitrogen content of the media were investigated. The highest degradation of lignin was 55% achieved on Sengon incubated for 30th days using buffered okara with nitrogen limited media. The research also shows that Sengon gives better results than Pine on the degree of its lignin degradation. The results also show that degradation of lignin always occurred together with the degradation of cellulose and hemicellulose, although the degradation of the latter two materials is less than degradation of lignin. It was concluded from the experiment that more complete addition of nutrient with appropriate composition, appropriate adjustment of pH, water content, appropriate incubation time and temperature, would increase the degree of delignification and in the same time will decrease the degree of polysaccharide degradation.



Keywords— *Biodelignification, Hard wood, White-rot fungus, Pulp and paper*

I. INTRODUCTION

Indonesia with its huge area of tropical forest is one of the biggest wood producing countries. Since wood is a cellulosic material that is very potential to be converted to pulp and paper, Indonesia has high potential to become a big pulp and paper producing country. Together with an ever-increasing market demand for pulp and paper, Indonesian government has set several policies to support the development of this forest sector such as developing so called Forest Product for Industry Program [1].

A major part of pulping process is to obtain cellulose fiber from wood or any other lignocellulosic material such as bagasse, rice straw or wheat straw. Prior to this process, lignin which functions is to bind cellulose and hemicellulose must be removed or reduced first. There are several conventional delignification processes such as mechanical delignification, chemical delignification, or combination of the two processes [2]. Mechanical delignification gives low degree of pulp strength. Chemical delignification gives high degree of pulp strength, but it requires high production cost and furthermore, this process uses chlorine which is very toxic to the environment [3]. Although conventional process gives high productivity due to its high speed of process, more and more pulp and paper industries are beginning to avoid this process due to increasing pressures from

[1] Departement of Chemical Engineering, Institut Teknologi Sepuluh Nopember, Surabaya 60111, Indonesia. *E-mail: arief_w@chem-eng.its.ac.id

[2] Departement of Chemical Engineering, State Polytechnic of Malang, Soekarno Hatta 09 Malang 65141, Indonesia.

[3] Departement of Physics, Sam Ratulangi University, Manado 95115, Indonesia.

environmentally concerned organization to conduct benign process which minimize the production of waste. On the other hand, bioprocess offers a new alternative to process which minimize energy consumption, improving quality of pulp while reducing the waste. Biodelignification which degrades lignin biologically by using white rot fungus or enzymatically by using enzyme secreted from several fungi has attracted much attention of recent researches [4,5]. Koduri and Tien utilized *P. chrysosporium* to oxidize guaiacol [6]. Nakamura and Sawada worked in vitro process by adding expensive mediator agent 1 mM veratryl alcohol to see the effect in increasing enzyme activity [7]. Wong and Yu investigated a white-rot fungi *T. versicolor* for the reduction of synthetic dyes like anthraquinon, azo dan indigo [8]. Almost no research conducted so far concern with the in vivo but simple and inexpensive biodelignification of sengon and pine tree.

This research aims to study the effect of using different medium during incubation of white rot fungus *Phanerochaete crhysosporium* in the biodelignification on Sengon (*Paraserianthes falcataria*) and Pine (*Pinus merkusii*). Agar and okara were used for the base media in which the effect of important parameters such as mineral additive, buffering system and nitrogen content of the media were investigated.

II. Experimental Method

Using two kinds of wood as described above, the experiments were conducted in the following steps:

A. The pure culture of *P. chrysosporium* obtained from Biotechnology Research Center of Gadjah Mada University was grown on Potato Dextrose Agar (PDA) for about 7 days at 30 ^{o}C until all surface of medium was full with fungus.

B. Rich medium for growth consisted of 0.067 g $CuSO_4.5H_2O$ (0.0067% w/v), $ZnSO_4.7H_2O$ 0.67 g (0.067% w/v) and $FeSO_4.7H_2O$ 0.67 g (0.067% w/v) in 1000 ml of pure water. After ten times dilution, 35 ml from this solution was mixed with 50 g dry agar and sterilized in autoclave (121 ^{o}C, 15 min). The strain of *P. chrysosporium* from step A was cultivated on this medium for 7 days at 35 °C.

C. Rich solution for substrate (wood as substrate for growth) was made by mixing 3 g bacto beef substrate (0.3 % w/v) and 5 g bacto pepton (0.5% w/v) in 1000 ml pure water. 56 ml of this solution was mixed with 80 g wood fibers (40/60 mesh) and sterilized in autoclave. 10% (w/w) of the starter from step B was added to this mixture and cultivated at 35 ^{o}C. The concentration of lignin, cellulose and hemicellulose was analyzed every 3 days until lignin concentration attained relatively constant value.

The concentration of lignin, cellulose, hemicellulose and extractives were analyzed according to the *Annual Book of ASTM Standards part 22: Wood, Adhesives, D 1106-56, 1981*.

III. Results and Discussion

Experimental results of lignin degradation on Pine and Sengon tree enriched with bacto beef and bacto pepton solution using okara or agar media enriched with mineral solution are given in Figures 1 and 2.

Figures 1 and 2 show that lignin biodegradation occurred until 21st day tree on agar + mineral media in which degradation of lignin for Pine and Sengon attained 38.08% and 41.15%, respectively. Biodegradation using okara without mineral addition lasted until 30th day in which lignin was degraded 40.85% and 45.04% for Pine and Sengon, respectively. By adding mineral in okara media, better lignin degradation was obtained, in example 43.26% and 46.98% for Pine and Sengon, respectively. Figures 1 and 2 also show that biodelignification rate was higher during the first 15 days. One of the reasons is the decrease of nutrition. Another reason is accumulation of product of biodegradation which inhibits the growing of the fungi. Degradation products such as siringingaldehyde, vanilin and other acids that can reduce the pH value may be responsible for the deactivation of the fungi. There was a

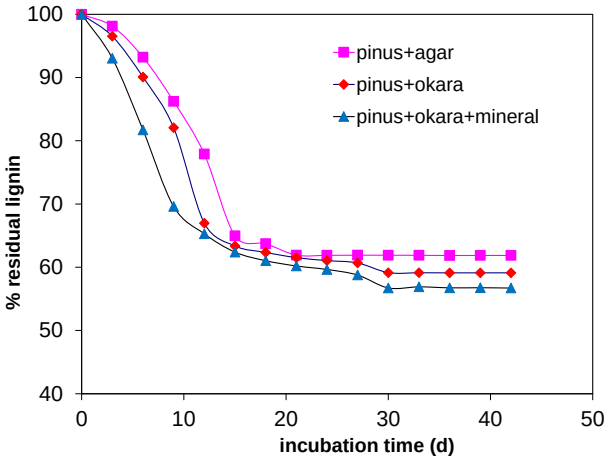


Figure 1. % Residual lignin vs. incubation time during biodegradation of Pine tree by *P. chrysosporium* at 35 ^{o}C in various media.

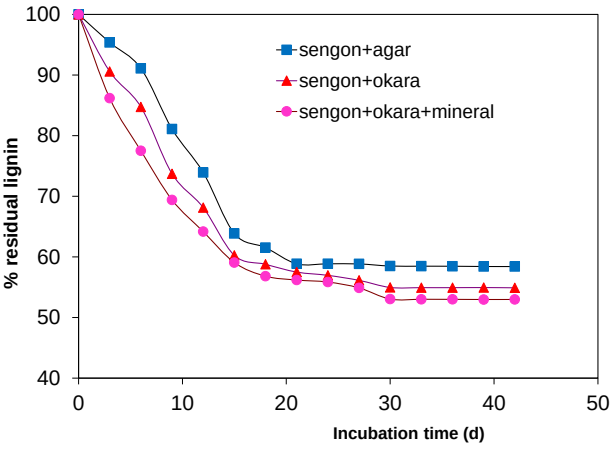


Figure 2. % Residual lignin vs. incubation time during biodegradation of Sengon tree by *P. chrysosporium* at 35 ^{o}C in various media.

reduction of pH value from the beginning of the process compared to the final (data not shown). According to Kirk, lignin degradation by *P. chrysosporium* show that the final metabolic product CO_2 mostly comes from metoxyl moiety, side chain carbon and aromatic ring [4]. Low oxygen concentration and high CO_2 concentration will reduce the degradation ability of fungi compare to natural equillibrium condition [9]. The above figures show that *P. chrysosporium* performed better lignin degradation using okara medium supplemented with mineral solution of $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, and $FeSO_4.7H_2O$. This shows the important role of these mineral solutions as active agent for ligninolitic enzymes works in vivo inside *P. chrysosporium* cells [9].

Biodegradation of lignin can be carried out if white rot fungi produce extracellular lignin degrading enzymes with appropriate environmental condition and availability of lignin as the main substrate [10]. According to Nishida et al., enzymes involved in lignin biodegradation are laccase, lignin peroxidase (LiP), and manganese peroxidase (MnP) generally produced by Basidiomycetes fungi [11]. Kirk showed that these three enzymes work by oxydizing phenolic groups in lignin [4]. As most lignins are distributed in the outer side of cell wall covering hemicellulose and cellulose, this component will be consumed first by white rot fungi. The above experimental results show that lignin degradation on Sengon was better than that on Pine tree. This is due to some toxic materials contained in Pine tree such as resin, tannin, and gum. Furthermore, Pine tree contains much sticky sap which also inhibits the growing of microorganism [12]. On the contrary, Sengon tree has simpler cell morphology compared to that of Pine tree and contains higher percentage of easily degraded wood. This is because there is more wood located on the outer side of stem which is the living part of wood functions as reservoir of amylum and carbohydrate [13]. Wood located in the inner part of stem is usually a dead wood which produces organic deposits such as resin, phenolic substances and pigments [2]. Biodegradation of lignin using *P. chrysosporium* is affected by many factors such as the structure and ultrastructure of wood, specific gravity, type and amount of lignin and contact surface area between wood and fungi [14].

Figures 3 and 4 show the experimental results of biodegradation of cellulose on Sengon and Pine tree using *P. chrysosporium* on agar or okara media enriched with bacto beef and bacto pepton solution and mineral. The most important substance for the pulping process is cellulose. The more cellulose present the better the quality of the raw material for pulping process. The use of fungi for degradation of lignin is expected to degrade only lignin not cellulose so that the product of degradation will contain high concentration of cellulose. Figures 3 and 4 show that biodegradation still continues until the 21st day using agar media enriched with mineral in which degradation of cellulose attained 13.33% and 16.06% for Pine and Sengon tree, respectively. Using okara as the media, degradation continues until 30th day in which 12.52% and 13.02% cellulose degradation was obtained for Pine and Sengon tree, respectively. Biodegradation using okara enriched with mineral continues for 30th days giving 11.01% and 11.1% cellulose degradation for Pine and Sengon tree, respectively. The difference in cellulose degradation using okara media and agar media is due to the fact that okara contained more additional nutrients than agar. The minimum requirement for the growth of fungi is the availability of C, H, O, N, S, and P of which all these substances present in okara. Figures 1 to 4 show that appropriate nutrients added to the media will accelerate lignin degradation and on the same time will decelerate cellulose degradation. These results coincide with that of Kashino et al. who mentioned that additional nutrients are very important for the growth of white-rot fungi in which this will accelerate lignin degradation, improve the growth of fungi, reduce cellulose degradation and improve strength properties of the pulp [3].

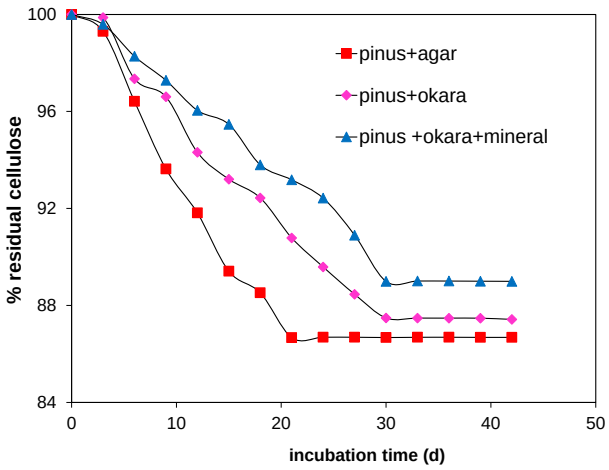


Figure 3. Residual cellulose vs. incubation time during biodegradation of Pine tree by *P. chrysosporium* at 35 ºC in various media.

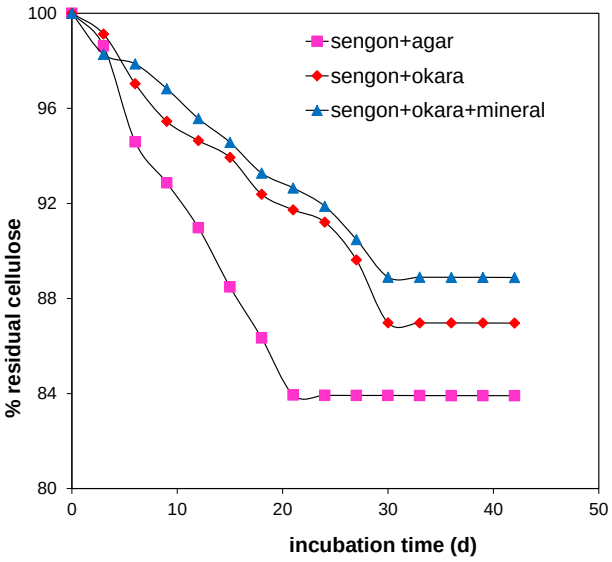


Figure 4. Residual cellulose vs. incubation time during biodegradation of Sengon tree by *P. chrysosporium* at 35 ºC in various media.

Figures 5 and 6 show hemicellulose degradation in Pine and Sengon tree enriched with bacto beef and bacto pepton using okara or agar media enriched with mineral solution. These figures show that hemicellulose degradation continues until the 21st day giving 14.4% and 22.5% hemicellulose degradation for Pine and Sengon tree, respectively. By using okara as the media, hemicelulose degradation still continue until the 30th day giving 16.9% and 19.7% hemicellulose degradation for Pine and Sengon tree, respectively. If mineral solution is added to the okara media, the degradation was reduced to give 14.7% and 18.8% hemicellulose degradation for Pine and Sengon tree, respectively.

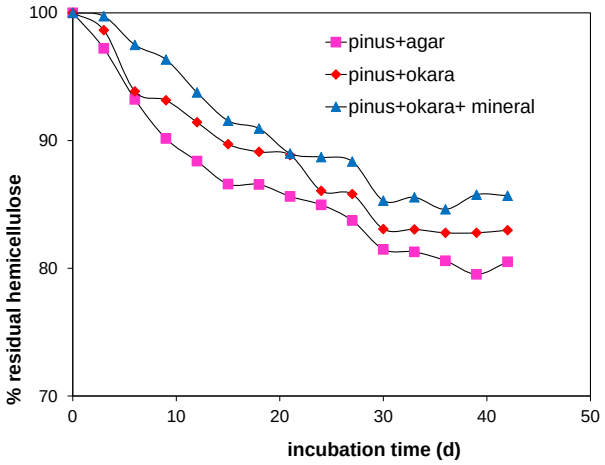


Figure 5. Residual hemicellulose vs. incubation time during biodegradation of Pine tree by *P. chrysosporium* at 35 °C in various media.

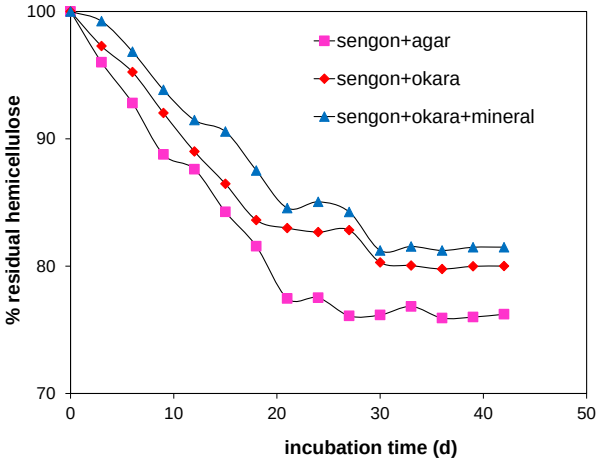


Figure 6. Residual hemicellulose vs. incubation time during biodegradation of Sengon tree by *P. chrysosporium* at 35 °C in various media.

According to Zabell and Morrell, hemicellulose is the cell wall component that is often degraded first by white-rot fungi [15]. This is probably due to the fact that hemicellulose has shorter chain and lower molecular weight compared to cellulose, higher ability to dissolve and locate at open side around cellulose microfibril. Lignin can be degraded without loss in cellulose; however, hemicellulose will be simultaneously degraded. This fact implies that white-rot fungi need carbon source that is relatively easy to be metabolized. To prevent degradation of polysaccharide in wood, white-rot fungi require carbon source as additional energy whether in glucose form, malt extract, agar or other nutrition source. With this energy source, the fungi will be able to degrade lignin optimally in wood or any other lignocellulosic materials [16].

Figure 7 shows lignin degradation on Sengon tree enriched with bacto beef and bacto pepton using buffered or unbuffered okara media enriched with mineral solution. It is clear from this figure that lignin degradation still continues until the 30th day. When pH of media was adjusted by using buffered solution a 48 % degradation of lignin was obtained, and when pH of the media was not controlled by buffered solution, a lower lignin degradation of 47 % was obtained. This result show that an optimal growth of fungi that leads to optimal lignin degradation needs pH controlling by using buffered solution.

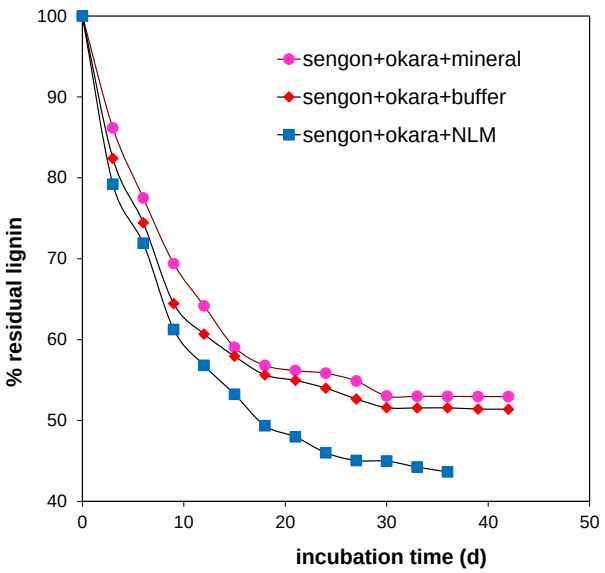


Figure 7. Residual lignin vs. incubation time during biodegradation of Pine tree by *P. chrysosporium* at 35 °C in buffered and unbuffered media including the Nitrogen Limited Media (NLM).

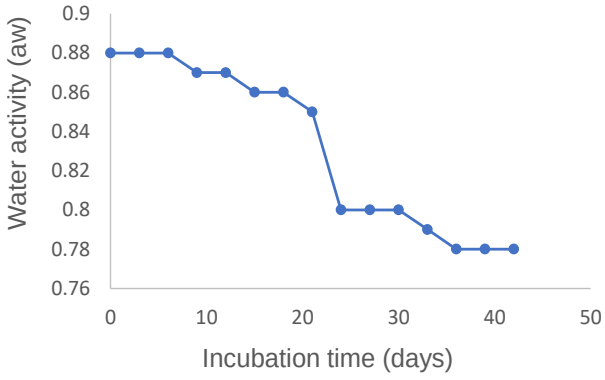


Figure 8. Water activity on Sengon tree using okara media inriched with mineral solution.

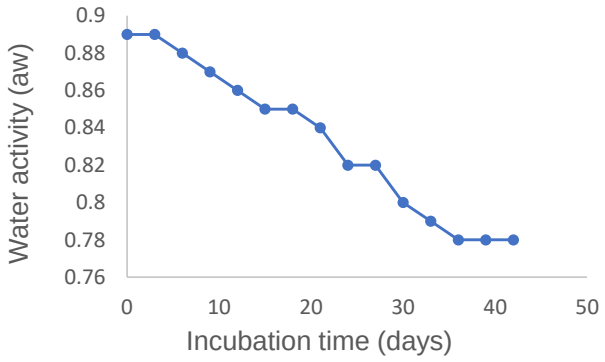


Figure 9. Water activity on Sengon tree using okara media inriched with NLM media.

The use of okara media enriched with limited nitrogen content was aimed to produce extracellular ligninolitic enzymes with optimal activities in *P. chrysosporium* cells. This is because this fungus has ability to reuse nitrogen in the substrate and furthermore, this limited nitogen content enhances the productivity of the ligninolitic enzymes [9]. Figure 7 also shows that on the 6th day the fungi enriched with mineral solution was able to degrade 26 % of lignin, whereas by using buffered system for the media the fungi was able to degrade 28 % of lignin. This result shows that good buffering system and mineral additives gives better lignin degradation in the same period of time.

The water activity was also monitored in this experiment and was shown in Figure 8 and 9. It was noticed that the water activity decreased as the incubation time increased. This probably due to the fact that the fungi require water for their lives and furthermore, the product of metabolism activity such as siringaldehydic acid, vanilic acid may contribute to decreasing the water activity. The decrease in water activity was also responsible for the enzyme activity leading to deceleration of the lignin degradation rate [17].

IV. CONCLUSION

From the experimental results we can coclude some important points:

1. Biodelignification using P. chrysosporium on Sengon tree gave better degree of delignification compared to that on Pine tree.
2. The highest delignification of 55.02% was obtained in the 30th day of incubation on Sengon tree using buffered okara media enriched with nitrogen-limited media (NLM).
3. Biodelignification was always followed by cellulose or hemicellulose degradation although the degradation of the two polysaccharides was not as big as degradation of lignin.
4. More complete addition of nutrient with appropriate composition, appropriate adjustment of pH, water content, appropriate incubation time and temperature, will increase the degree of delignification and in the same time decrease the degree of polysaccharide degradation.

AUTHOR CONTRIBUTIONS

Arief Widjaja as supervisor, Dwina Moentamaria as student working the research, Hanny F. Sangian as student completing the research. All authors have given approval to the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Ministry of Education and Cultural, Directorate of Higher Education for the financial support of the research.

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