

Pretreatment of *Marasmius* sp. on Biopulping of Oil Palm Empty Fruit Bunches

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Received: May 4, 2015

Accepted: June 5, 2015

Online Published: June 30, 2015

doi:10.5539/mas.v9n7p1

URL: <http://dx.doi.org/10.5539/mas.v9n7p1>

Abstract

White rot fungi have an ability to degrade lignin by employing lignin-degrading enzymes i.e Lignin Peroxidase, Manganese Peroxidase and Laccase. Therefore, the fungi can be utilized on the pretreatment of biomass in pulp making (biopulping) and biobleaching. In this study, the pretreatment using White Rot Fungi of *Marasmius* sp. has been conducted on the the Oil Palm Empty Fruit Bunches (EFBs). *Marasmius* sp. has been grown on EFBs for 30 days. The results showed that the lignin content could be removed by 35.94%. However, cellulose and hemicelluloses relatively did not show any changes in the EFBs. From the pulping process, the pretreatment exhibited the Kappa Number of 31.10. Compared to no pretreatment of white rot fungi, the Kappa Number obtained was 38.63. This result demonstrated a promising process for a green pulp making.

Keywords: biopulping, Empty Fruit Bunches, lignin, Kappa Number, *Marasmius* sp.

1. Introduction

In the past few decades, there are intensive research on biopulping, a process where wood chips or other lignocellulosics materials are inoculated with naturally lignin-degrading fungus which possess the ability to selectively degrade woody materials prior to pulping (Kirk *et al.*, 1992; Scott *et al.* 2001; Wan dan Li, 2012). These fungi originally produce enzymes (biological catalysts) which degrade lignin and fragment wood fibers to forms more readily digested. Lignin peroxidase, manganese peroxidase and laccase were reported as results from biopulping process on oil palm trunk using white rot fungus *Trametes versicolor* (Singh *et al.*, 2013). The utilization of white rot fungus have several advantages such as energy-efficient, less chlorine consumption on bleaching process, and reduce the pollution to the environment (Yadav *et al.*, 2010). A promising white rot fungus *Marasmius* sp. secreted extracellular laccase enzyme that degrade lignin in black liquor and unbleached kraft pulp (Risdianto *et al.*, 2007; Risdianto *et al.*, 2011). In addition, *Eucalyptus grandis* wood chips treated by *Ceriporiopsis subvermispora* on a 50-tonne pilot-plant demonstrated an energy saving in laboratory and mill-scale biopulping (Ferraz *et al.*, 2008).

Indonesia is the largest of palm oil producer in the world. Crude Palm Oil (CPO) is the main product of palm oil industries. In 2012, Indonesia has produced 26.5 milion metric tonnes of CPO (www.indonesia-investments.com). From that production, the EFBs generated was around to 27.7 million metric tonnes (Visvanathan *et al.*, 2009). Biomass waste of empty fruit bunches is yielded from a conversion of Fresh Fruit Bunches (FFBs) into CPO. The EFB is in large amount and usually incinerated at the plant site which could causes air pollution. EFBs of palm oil contains cellulose (37.26 – 40.79%), hemicellulose (14.62 – 29.4%), lignin (22.67 – 31.68%), and extractives (1.34 – 3.30%) (Erwinsyah *et al.*, 2012; Sudiyani *et al.*, 2013). The fiber length of EFBs is approximately 1.11 mm and classified as a short fiber. The EFBs pulp can be mixed with old corugated containers (OCC) to produce kraft liner and corrugating medium paper (Erwinsyah *et al.*, 2012)

Due to its content and amount, the EFBs has a great potential as a material for pulp and papermaking. The challenges of the utilization of this nonwood materials for paper making are raised (Daud and Law, 2011). It is also triggered by the fact that increase of demand for paper production and limited wood resources hence there are efforts to find a new resources of non-wood materials for pulp and paper making. EFBs is one of the promising non-wood materials in Indonesia for pulp and paper making. This paper focused on the biopulping process of a promising non-wood material Empty Fruit Bunches employing *Marasmius* sp. as a lignin-degrading fungus.

2. Method

Microorganism

The white rot fungus *Marasmius* sp. was obtained from Laboratory of Microbiology and Bioprocess Technology, Department of Chemical Engineering, Institut Teknologi Bandung, Indonesia. The strain was maintained on Potato Dextrose Agar (PDA) medium in the Petri dish and incubated for 7 days at 28°C. The seven days age of stock culture was stored at 4°C until it was used.

Lignocellulosic material

The empty fruit bunches was obtained from plantation in South Sumatera. The EFBs was chopped in length around 5 cm.

Biopulping

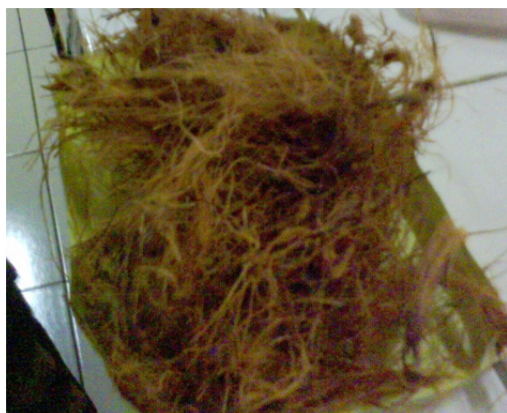
A 250 grams of EFB chopped was impregnated by 250 mL of modified Kirk medium in heat resistant plastic bag, consisting of glucose 10 g/L, KH_2PO_4 1.7 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.4 g/L, CaCl_2 0.09 g/L, sodium acetate 2.3 g/L, diammonium tartrate 0.4 g/L, MnCl_2 0.02 g/L, yeast extract 0.3 g/L, $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g/L, H_2MoO_4 0.007 g/L, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.01 g/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.006 g/L and $\text{Fe}_2(\text{SO}_4)_3$ 0.007 g/L. The EFBs and medium in plastic bag were steam sterilised in autoclave at 120°C for 20 minutes. Then, the EFBs and medium were allowed to cool down. The inoculum size of 1.5 cm x 1.5 cm in total area of 63 cm² was transferred aseptically to the sterilised EFBs. Incubation was performed in room temperature (\pm 28°C) for 30 days. Every 10 days, sample was taken from a bag of cultures to analyse lignin, hemicellulose and total cellulose.

Pulp making

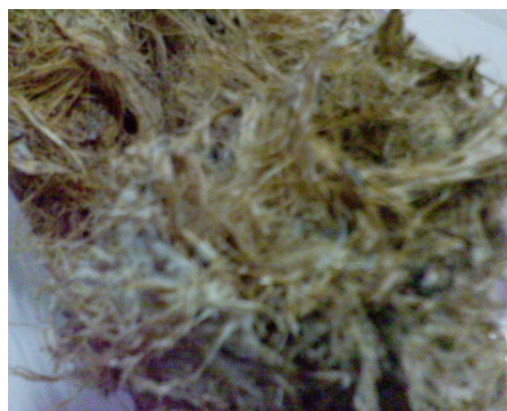
The treated EFBs was pulped by soda process in a digester with the following conditions: (a) Active Alkali was 12% and 18%, solid to liquor ratio was 1:4, temperature was 140°C, and total time was 3 hours (the to time and at time was 1.5 hours, respectively). The brown stock from digester was defiberated in fiber separator for 20 minutes, and then washed by water. The stock was then filtered by using flat screen and pressed to determine water content and the Kappa number. To evaluate the effectiveness of biopulping process, pulp was also made from EFBs in similar pulp making condition but no fungus treatment.

3. Results and Discussion

Marasmius sp. was well visible sign of growth on Empty Fruit Bunches as shown in Fig. 1. It is indicated that fungus can uptake the medium in substrate (EFBs) and bind it. Biopulping process is similar to the Solid state culture/fermentation. *Marasmius* sp. also was assisted by chopped EFBs due to the enhancement of surface area. Niladevi *et al.* (2007) revealed that surface area was one of the physical properties of substrate that influence the growth of fungus in Solid State Fermentation (SSF). This biopulping process was conducted at room temperature (\pm 28°C). This process is similar to Solid State Fermentation (SSF) process. Pandey *et al.* (2008) and Risdianto *et al.* (2010), reported that SSF usually was performed by mesophilic microorganism at temperature range of 20 - 50°C. Fungus pretreated on biomass makes it porous and soft (Singhal, 2008).



(A) initial condition



(B) day 30

Figure 1. The growth of *Marasmius* sp. on Empty Fruit Bunches

The efficacy of *Marasmius* sp on biopulping was indicated by the reduction of lignin content during the incubation. As shown in Fig. 2, lignin content was linearly decrease. The initial lignin content was 24.25% and at the end of incubation time (day of 30) exhibited at value of 15.54%. It means that *Marasmius* sp. employed in this study has a great ability to degrade lignin in EFBs. *Marasmius* sp. degraded lignin by secreting enzymes such as lignin peroxidase, manganese peroxidase and laccase. Yadav *et al.* (2010) revealed that the reduction of lignin content as a result of the action of lignin-degrading enzymes secreted by the fungi. The lignin biodegradation mechanism has been clearly described by Martinez *et al.* (2005). Laccase production in the cultures of *Marasmius* sp. began on day 3 (51.04 U/L) and reached maximum activity at day 8 (330.07 U/L) on the EFBs substrate (Risdiyanto *et al.*, 2012). Glucose in the medium was consumed to growth and produce laccase for lignin degradation (Risdiyanto *et al.*, 2010). Laccase secreted by white rot fungi degrade non phenolic lignin compound in absence of mediator (Octavio *et al.*, 2006; Camamero *et al.*, 2007). The lignin degradation is oxidative in nature and occurred in aerobic process. For biopulping process, selective lignin degradation is required. Thus, fungi having robust ligninase system and deficient cellulolytic activity are suitable for biopulping (Singhal, 2008).

The incubation time for the fungal treatment was 30 days, and this is relatively a long period for a process at industrial scale. However, the fungal treatment gave several advantages such as energy-efficient by reducing about 30% in refining process (Scott *et al.*, 1998), less chlorine consumption on bleaching process, and reduces the pollution to the environment (Yadav *et al.*, 2010). To achieve that advantages, Scott *et al.* (1998) has proposed a biopulping process consist of decontamination, cooling, inoculation and incubation unit prior to pulping process.

Reducing lignin content give a great advantage for the pulp making due to lowering the cooking chemicals. The fungus treatment of lignocellulosic materials usually followed by the cooking using chemicals to obtained an unbleached pulp. From this study, the reduction of lignin was 35.94% of initial lignin content. An in line study on biopulping conducted by Singh *et al.* (2013) resulted the lignin content in Oil Palm Trunk (OPT) chips was determined to be 20.55% but was reduced by fungal treatments to the value of 9.35% after 28 days of incubation.

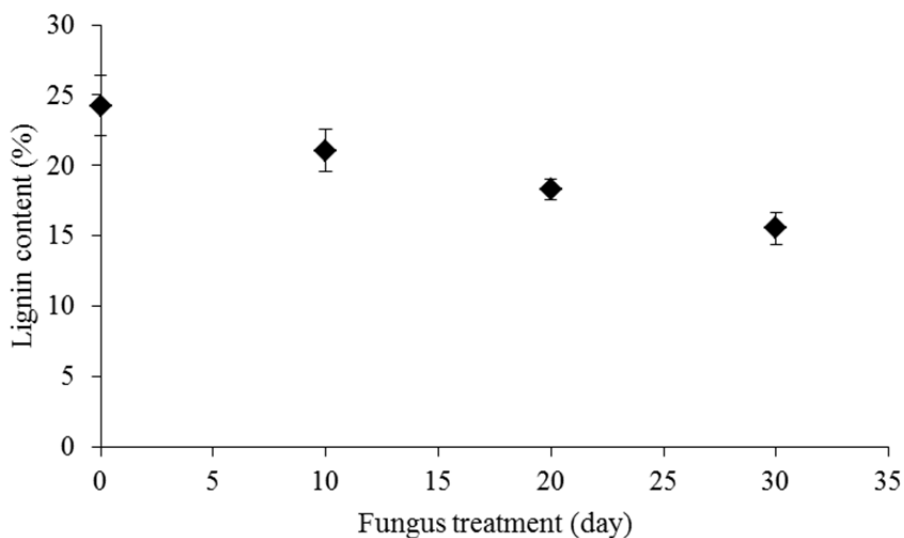


Figure 2. Lignin content in EFBs during treatment by *Marasmius* sp.

The analysis of hemicellulose after 30 days of biopulping process is presented in Fig. 3. The hemicellulose content in EFBs was determined to be 28.53%. During the biopulping process, the hemicellulose content is relatively constant. At the end of biopulping process, hemicellulose content was detected at level 26.95%. By using the ANOVA (Analysis of Variance), there is no difference in significance for the hemicellulose content during biopulping indicated by p value was 0.64 ($p > 0.05$). It means that *Marasmius* sp. selectively degrades lignin in EFBs. Nazarpour *et al.* (2013) found by the FTIR analysis that there were chemical changes during incubation of rubberwood by *Ceriporiopsis subvermispora* i.e. lignin degradation but only a little change on carbohydrate degradation. The high selectivity for lignin degradation suggests that the fungus secretes a higher activity of lignin-degrading enzymes than cellulose and hemicellulose, and is therefore white rot fungus treatment is

suitable for the biopulping process (Singh *et al.*, 2013).

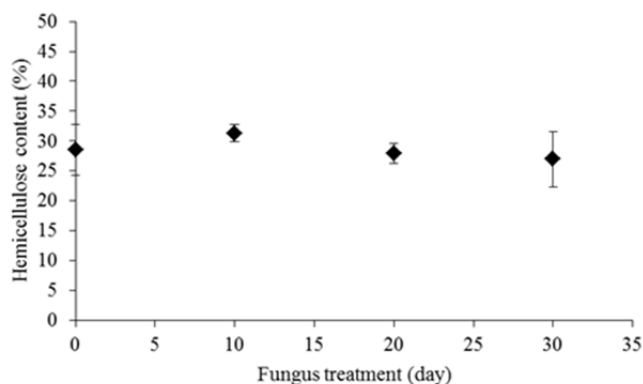


Figure 3. Hemicellulose content in EFBs during treatment by *Marasmius* sp.

The decreasing of lignin content in EFBs gave an important role for the further stage of pulpmaking. Lower lignin content can save the cooking chemicals in pulpmaking and therefore reduce the load of pollutant to be treated in waste water treatment plant. However, cellulose and hemicellulose are the main component that expected in high level due to the strenght of pulp and paper depend on these components. Comparison to other studies shows that the reduction of lignin content in this study is high around 35.94% as presented in Table 1.

Table 1. Comparison to other studies of microorganism pretreated on biomass

Material	Lignin content (%)		% Reduction	Reference
	Initial	After pretreated		
Bagasse pretreated by <i>Ceriporiopsis subvermispora</i> (2 weeks/14 days)	20.1	19.3		Bajpai <i>et al.</i> , 2004
Mixed chips of Eucalyptus and poplar wood pretreated by <i>Ceriporiopsis subvermispora</i> (3 weeks/21 days)	27.70	26.40	4.69	Yadav <i>et al.</i> , 2010
<i>Acacia mangium</i> pretreated by <i>Trametes versicolor</i> (60 days)	-	-	26.9	Liew <i>et al.</i> , 2011
Oil Palm Trunk (OPT) pretreated by <i>Trametes versicolor</i> (28 days)	20.55	9.35	54.50	Singh <i>et al.</i> , 2013
Empty Fruit Bunches pretreated by <i>Marasmius</i> sp. (30 days)	24.26	15.54	35.94	This study

After pretreated by *Marasmius* sp, EFBs was subjected to chemical cooking in digester to enhance the lignin degradation to the desired Kappa Number. Kappa number is an indirect measurement of lignin content in pulp m (Casey,1980). Higher Kappa number means lignin content is at high content. Sodium hydroxide (NaOH) was used as chemical cooking due to it is a powerfull swelling agent for lignocellulosic materials that having low lignin content such as hardwood and nonwood plants. The alkali charge is one factor that gives a high effect in the soda pulping process (Ibrahim *et al.*, 2011). Alkali charge of 20 – 30% was performed in the pulping process of oil-palm frond-fiber (Wanrosli *et al.*, 2004a) and oil palm empty fruit bunch fibres (Wanrosli *et al.*, 2004b). In this study, a lower alkali charge of 12% and 18% was used due to the EFBs has been treated by *Marasmius* sp which can degrade lignin content prior to soda pulping process.

At the active alkali (AA) 18%, the Kappa number of EFBs pulp pretreated by *Marasmius* sp. was detected at value of 31.1. While Kappa number of pulp of control EFBs (no fungus pretreatment) was higher at value of 38.6 as shown in **Figure 4**. A similar results was observed in biopulping of Eucalyptus wood (*Eucalyptus grandis*) by *Cryptococcus albidus*. The Kappa number of pulp of control and treated wood were 21 and 17, respectively (Singhal *et al.*, 2013). When the EFBs pretreated was pulped by using Active Alkali 12% showed an increase Kappa Number of 44.22. The low active alkali resulted higher Kappa number which might be due to slow reactive penetration to the fiber and dissolve lignin (Ibrahim *et al.*, 2011). From this study, pretreatments

with *Marasmius* sp. can reduce the cooking chemical consumption on pulp making that can be used as an alternative pulp manufacturing process more environmentally friendly.

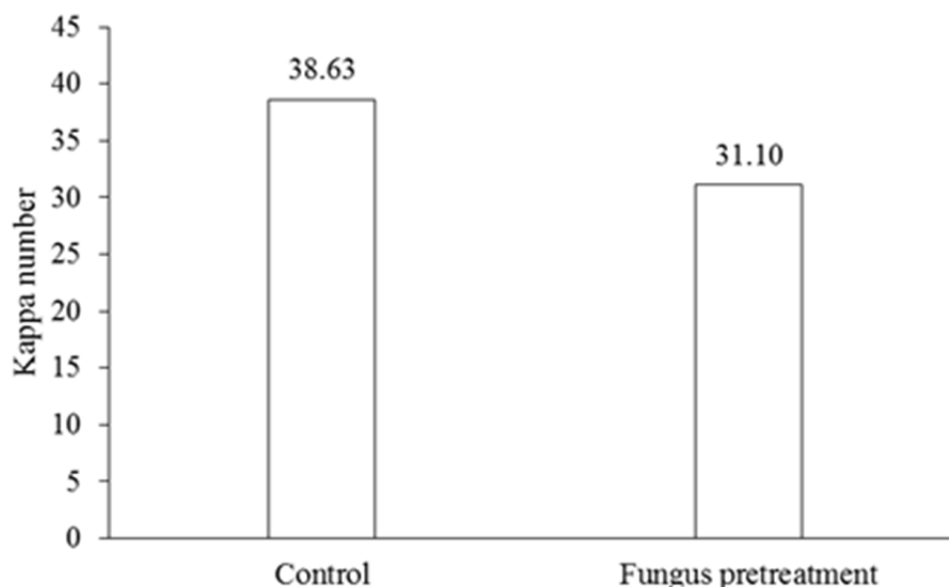


Figure 4. Kappa number of pulp

4. Conclusion

Marasmius sp. grew well in Empty Fruit Bunch for the pretreatment process and reduce lignin of 35.94% for 30 days. The Kappa Number of untreated EFBs by *Marasmius* sp. was 38.63 and for treated was 31.10. Therefore, *Marasmius* sp. has a great potential to be used in a green pulp making process.

Acknowledgments

Authors would like to thank to the Center for Pulp and Paper (CPP) for the funding and our colleagues at CPP for the support and encouragement.

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