Biological Delignification by *Phanerochaete Chrysosporium* with Addition of Mineral Mn and Its Effect on Nutrient Content of Oil Palm Frond

Dewi Febrina[1], Novirman Jamarun[2], Mardiati Zain[2], Khasrad[2] and Rini Mariani[2]
[1]Faculty Agriculture and Animal Sciences, State Islamic University Sultan Syarif Kasim, Riau, Indonesia [2]Faculty of Animal Science, University of Andalas, Padang, Indonesia
Corresponding email: hanna_suska@yahoo.com

ABSTRACT

The experiment was conducted to study the interaction between mineral Mn and time of fermentation with *P.chrysosporium* on nutrient and fiber components of Oil Palm Frond (OPF). This research was done based on Completely Randomized Design with 2 factor as treatments where every treatment is repeated for three times. The first factors were Mn doses (0, 100, 200 and 300 ppm), while the second factors were time of fermentation (10, 15 and 20 days). Result of research showed there were interaction between mineral doses with time of fermentation on dry matter, organic matter, crude fiber, cellulose, hemiselulose, ADF, NDF and lignin contents. There was no interaction between mineral doses with time of fermentation on crude protein and crude fat content. The results indicated that the best combination is 15 days time of fermentation and 100 ppm Mn dose due to low lignin content.

Key Word: Biological delignification, Oil palm frond, Nutrients content, *Phanerochaete chrysosporium*

INTRODUCTION

An Oil Palm Frond (OPF) was available in large quantities at all times. Utilization of OPF as feedstuff was very limited because of high lignin content (30.18%). Lignocellulose is a macromolecular complex consisting of lignin, cellulose, and hemicellulose. The degradation of lignin is the key step to lignocellulose transformation (Zeng et al., 2013).

White-rot fungus *Phanerochaete chrysosporium* is the most active lignolytic organism. *P. chrysosporium* and its extracellular lignin-degrading enzymes have been investigated intensively because of their great delignifying potential (Arora et al., 2002). The presence of manganese seems to be crucial for the function of Mn (Baldrian et al, 2005). The purpose of this studi was to evaluate the effect of Mn minerals doses and time of fermentation of OPF fermented by *P.chrysosporium* on nutrient content.

MATERIALS AND METHODS

Substrate used in this research was OPF that has been cut and dried, and then finely milled. Source of Mn minerals was in $MnSO_4.H_2O$. *Phanerochaete chrysosporium* was maintained on Potato Dextrose Agar (PDA) slants at 4^0C and then transferred to PDA plates at 37^0C for 6 days, subsequently grown on rice bran. The fermentation process begins by adding water to the OPF so that the water level reaches 65% (Kerem et al., 1992), then added Mn according to treatment.

The proximate components were determined according to AOAC (1995) using Foss equipments (Sweden).The predominantly fiber residues (hemicelluloses, cellulose, and lignin) was determined by the method of Van Soest (1991) using Foss Fibertec 2010 (Sweden). The research used Completely Randomized Design 3 x 4 factorial pattern of each repeated 3 times. The first factor is the time of fermentation (10, 15 and 20 days), the second factor is the levels of Mn (0, 100, 200 and 300 ppm). The differences between treatments were tested by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Dry matter and organic matter. The addition of 100 ppm Mn with 10 days fermentation resulted the highest of dry matter (DM) and organic matter (OM) content. The cycle of availibility nutrient during the fermentation process will change DM due to the degradation process and the utilization of nutrients by the fungus. Moisture content significantly affected lignin degradation. Lignin was degraded 6% higher at 75% and 80% moisture than of 65% after 14 days (Shi et al., 2008).

Tabel 1. Proximate components of Oil Palm Frond fermented by *P.chrysosporium*

Time	Mineral Doses Mn (ppm)				Mineral Doses Mn (ppm)			
(days)	0	100	200	300	0	100	200	300
	Dry matter				Organic matter			
10	33.249^{ab}	34.571^{a}	33.155^{ab}	32.300^{ab}	94.737^{a}	94.719^{a}	94.719^{a}	94.398^{ab}
15	32.543^{ab}	33.157^{ab}	31.806^{bc}	31.723^{bc}	94.380^{ab}	93.730^{bcd}	93.885^{bcd}	93.751^{bcd}
20	29.560^{c}	31.999^{b}	30.978^{bc}	31.656^{bc}	94.234^{abc}	93.885^{bcd}	93.409^{d}	93.554^{cd}
	Crude Fat				Crude Protein			
10	3.000	3.333	3.000	3.000	2.655	2.626	2.772	2.655
15	3.333	3.000	3.000	3.333	2.801	2.772	2.801	2.889
20	3.333	3.000	3.667	3.000	2.889	2.830	2.772	2.831
	Crude Fiber				Lignin			
10	38.361^{bc}	32.353^{e}	35.527^{d}	39.009^{abc}	25.460^{abcd}	24.982^{abcd}	25.460^{abcd}	24.535^{abcd}
15	39.216^{abc}	36.601^{cd}	40.986^{ab}	40.471^{ab}	24.353^{bcd}	21.159 ^{d}	26.609 ^{abc}	25.472^{abcd}
20	38.614^{bc}	38.032^{bcd}	39.672^{ab}	41.775^{a}	23.228 ^{bcd}	22.436 ^{cd}	26.923 ^{abc}	27.735^{ab}
	Cellulose				Hemicelulose			
10	31.229^{bc}	27.130^{c}	36.333^{ab}	36.337^{ab}	18.469 ^{a}	13.288 ^{bc}	7.061 ^{e}	8.905 ^{de}
15	36.560^{ab}	37.201^{a}	38.977^{a}	38.849^{a}	15.388 ^{abc}	13.440 ^{bc}	12.124 ^{cd}	16.062 ^{abc}
20	39.354^{a}	40.385^{a}	38.462^{a}	38.093^{a}	15.877 ^{abc}	16.524 ^{ab}	15.614 ^{abc}	15.107 ^{abc}
10	31.229^{bc}	27.130^{c}	36.333^{ab}	36.337^{ab}	18.469 ^{a}	13.288 ^{bc}	7.061 ^{e}	8.905 ^{de}
	NDF				ADF			
10	80.890 ^{ab}	70.363 ^{e}	73.366 ^{de}	78.350 ^{bc}	58.599^{d}	52.879^{e}	63.062^{bc}	65.610^{ab}
15	78.878 ^{bc}	75.515 ^{cd}	82.172 ^{ab}	84.204 ^{a}	60.913^{cd}	59.630^{d}	65.586^{ab}	65.602^{ab}
20	81.640 ^{ab}	83.833 ^{a}	84.180 ^{a}	82.822 ^{ab}	63.223^{abc}	64.744^{ab}	66.667^{a}	66.457^{ab}

Superscripts in the same column and row in each component indicated significantly different (P<0,05)

The OM content of OPF before fermentation was 94.5%, changed during the fermentation, ranging from 93.409- 94.737%. Lopez and Garcia (2006) found that the OM loss from chicken litter compost was 9% of the initial OM. This OM loss was similar to that found by Tiquia et al. (2002) during composting of sewage sludge. The mass of OM and total organic carbon also decreased with composting time (Haddadin et al., 2009).

Crude fat and crude protein. Table 1 showed that crude fat content of fermented OPF was not affected by Mn dose, fermentation time and there was no interaction between time of fermentation with doses of minerals (P>0.05). Jonathan et al. (2012) reported no significant changes in the fat content of rice straw fermented with *P. florida* with different time of fermentation, being 1.47-1.82%. Fermentation time of 20 days resulted in the highest crude protein content (2.830%), but not significantly different compared to that of 15 days (2.816%). Increasing doses of Mn had no effect on the CP content of fermented OPF. This is presumably because the concentration of Mn in the substrate has been exceeding the needs of *P. chrysosporium*.

Crude fiber and lignin. Table 1 showed addition of 100 ppm Mn with 10 days fermentation resulted in the lowest crude fiber content (32.353%). Oil palm frond is a waste and has lignified. Breakdown of lignin involves by lignolytic enzyme from *P.chrysosporium* that will decompose lignin to carbon dioxide (CO_2), using the enzyme of lignin peroxidase and manganese peroxidase (Vallie et al., 1992). Addition of 100 ppm Mn with 15 days fermentation resulted in the lowest lignin content (21.159%), indicated that Mn was able to decrease lignin content (30.18 vs 21.159%). However, Mn addition more than 200 ppm with 15 days fermentation was not effectively decreased the lignin content. Biodegradation of cotton stalk by *P. chrysosporium* displayed a higher lignin degradation at higher moisture content, although the results did not show the direct relationship of nutrients on the lignin degradation upon a longer fermentation time (Shi et al., 2008). Most of the lignin degradation occurred during 4–10 days and delignification rates varied over the 14 days pretreatment period due to fungal metabolism as well as that Mn addition can not increase ligninolytic activity (Shi et al., 2009). In this case that excess nutrients, especially Mn^{2+}, may inhibit fungal growth and delignification.

Cellulose and hemicellulose contents. Combinations of 100 ppm Mn addition with 10 days of fermentation resulted in the lowest cellulose content (27.13%). The decrease in cellulose content indicates cellulase activity of the enzyme produced by *P.chrysosporium.* The decrease in cellulose content occurred on day 10, then the cellulose content increased along with length of fermentation time. Therefore, the inoculation during the second fermentation phase (day 15 to 42) was more effective than that during the first fermentation phase (0–14 days) (Zeng et al., 2010). Feng et al. (2011) stated that cellulose were degraded slightly during the initial phase of composting (0–6 days), while rapid decomposition were identified during the thermophilic phase. The decrease of cellulose content followed by decrease in ADF content. The addition of 100 ppm Mn with 10 days of fermentation produces the lowest ADF (52.879%). This in agreement with the statement of Tuomela et al. (2000) that the utilization of readily soluble materials was occurred in the early phase of fermentation. Hemicellulose content of OPF before fermentation is 11.912%, and fluctuated during fermentation time (7.061% - 18.469%). Lopez et al. (2013) found an increase the hemicellulose content of 44,44% corn cobs inoculated with *P. flavido alba* compared with controls (14.0% vs. 25.2%).

NDF and ADF contents. Fermentation and mineral doses significantly (P <0.05) influenced the content of NDF and ADF. Combinations of 100 ppm Mn with 10 days of fermentation resulted in the lowest NDF and ADF content, being 66.457% and 52.879%, respectively. The decrease of NDF content was 18.87% (81.92% vs. 66.457%) and ADF 24.46% (70% vs. 52.879%) compared with the NDF and ADF of OPF before fermentation. These were lower than of Haddadin et al. (2009) who reported a decrease in NDF and ADF contents, being 31.64% and 39.74% respectively on Olive Pomade fermented by *P.chrysosporium* fungus for 10 days. The decrease in NDF and ADF content after fermentation was caused by the breakdown of the cell walls of fungi *P. chrysosporium* which leads to changes in the content of the fiber fraction.

CONCLUSIONS

There were interaction between Mn doses with time of fermentation on dry matter, organic matter, crude fiber, cellulose, hemicellulose, ADF, NDF and lignin contents of OPF and there were no interaction between Mn doses with time fermentation for crude protein and crude fat content of OPF. The time of fermentation 15 days and 100 ppm Mn was the best combination treatment due to the lowest lignin content of OPF.

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