Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.6, No.4, 2016

Effect of White Rot Fungi to Enzimatic Activity and Lignin on Fermentation Process of Cocoa Pod

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Abstract
This study was conducted to determine the enzyme activity ligninolitik and lignin content in the fermentation process of cocoa pod. The substrate was used the cocoa pod while the fungi used white rot fungi were Phanerochaete chrysosporium, Pleurotus ostreatus and Schizophyllum commune. Preparation of cocoa pod was chopped, finely ground and then dried. Preparation of fungi by growing fungi in liquid medium. Research methodology was the optimization of fermentation was fermentation conducted with different fungus at a temperature of 30^0 C and pH 7 using five treatments and three replications. T0 = fermentation of cocoa pod without fungus, T1 = fermentation of cocoa pod with added Phanerochaete chrysosporium, T2 = fermentation of cocoa pod with added Pleurotus ostreatus, T3 = fermentation of cocoa pod with added Schizophyllum commune, P4 = fermentation of cocoa pod with added Phanerochaete chrysosporium, Pleurotus ostreatus and Schizophyllum commune. Fifty gram cocoa pod in erlenmeyer and added fungus 10% from the weight of substrat. Variables observed Lignin peroxsidase and Mangan peroxsidase and also lignin. This study was designed using research design completely randomized design with a unidirectional pattern analysis of variance (oneway ANOVA). Significant variables followed Duncan's multiple range test (Duncan Multiple Range Test/DMRT). Conclusion from this research was fermentation of cocoa pod with different fungus indicates that fermentation used Phanerochaete chrysosporium has the best results and showed the highest enzyme activity of LiP 0.223±0.00 U/ml and MnP 0.091±0.01 U/ml and also has the lowest lignin content was 26.86 ± 0.12%.
Keywords: Fermentation, Enzyme, Lignin, Cocoa pod, White rot fungi

1. Introduction

Waste food crops and plantations have an important role and potential in the supply of forage for ruminants such as cattle, goats, sheep and buffalo, especially in the dry season. In the dry season forage grasses are stunted, making forage available was less in terms of both quantity and quality. Even in areas of specific fodder grass will dry up and die, causing a crisis forage. In addition, ruminant rearing system was still largely dependent on forage in the form of grasses and other forage with little or no additional feed.

Cocoa pod has an important role and potential in the supply of ruminant feed, especially goats, especially in the dry season. Cocoa pod utilization as animal feed can be given in the form of fresh or in the form of flour after processing. The chemical composition of cocoa pod contains 7.75% protein and energy of 3900 kcal / kg which exceeded the composition of elephant grass of 6.9% and a total energy of 3800 kcal / kg (Puastuti et al., 2009). Cocoa pod was an agro-industrial waste produced cocoa plant (Theobroma cacao L.) cocoa consisting of 74% rind, 2% and 24% seed placenta. Proximate analysis results containing 22% protein and 3-9% fat (Nasrullah and Ella A., 1993).

Phanerochaete chrysosporium was a microorganism that has the ability to selectively degrade lignocellulose (Tuomelo et al. 2000) that degrades the lignin component first, followed by the cellulose component. Cellulose and hemicellulose utilized by fungi as a carbon source. This fungi also has the ability to grow at a relatively high temperature was 36-40^{o}C so suitable for use in fermentation processes that produce a lot of heat (Tuomelo et al. 2002). Lignin degradation of high efficiency and minimal in utilizing cellulose polymers compared to other white rot fungi Phanerochaete chrysosporium make the best choice in the treatment of lignin degradation.

Fungi degradate lignin are most active white-rot fungi, such as Phanerochaete chrysosporium and Coriolus versicolor able to hemicellulose, cellulose and lignin from plant waste into CO_2 and H_2O (Paul, 1992; Limura, 1996). In general, white-rot basidiomisetes synthesize three kinds of enzymes, Lignin-peroxidase (LiP), manganese-peroxidase (MnP) and laccase. The third enzyme plays an important role in the degradation of lignin (Srinivasan et al., 1995).

2. Material and Method

2.1 The fermentation process

The fermentation process was done on a laboratory scale sterile space. Cocoa pod fermented in a erlenmeyer. Fifty gram cocoa pod used for each erlenmeyer, then the fungus inoculated used as many as 10% of the weight of the substrate. Erlenmeyer put in an shaker incubator at a speed of 150 rpm and 30° C. After fermentation was complete, cocoa pod removed and dried for 6 hours. Before and after the fermentation weighing the weight of the cocoa pod and then dried in an oven at a temperature of 50° C for 4 days. Cocoa pod was dry milled using a Thomas-Wiley Mill type 4 with a diameter of 1 mm. Cocoa pod has been dry milled using a Thomas-Wiley (type 4) with a diameter of 1 mm.

2.2 Experimental Procedures.

The substrate was used the cocoa pod while the fungi used white rot fungi were Phanerochaete chrysosporium, Pleurotus ostreatus and Schizophyllum commune. Preparation of cocoa pod were chopped, finely ground and then dried. Preparation of fungi by growing fungi in liquid medium. Fifty gram cocoa pod put in erlenmeyer and added fungus 10% from the weight of substrat. Research methodology was the optimization of fermentation was fermentation conducted with different fungus at a temperature of 30^0 C and pH 7 using five treatments and three replications. T0 = fermentation of cocoa pod without fungus, T1 = fermentation of cocoa pod with added Phanerochaete chrysosporium, T2 = fermentation of cocoa pod with added Pleurotus ostreatus, T3 = fermentation of cocoa pod with added Schizophyllum commune, P4 = fermentation of cocoa pod with added Phanerochaete chrysosporium, Pleurotus ostreatus and Schizophyllum commune.

2.3 Data Analysis

Data collected was designed using research design completely randomized design with a unidirectional pattern analysis of variance (oneway ANOVA). Significant variables followed Duncan's multiple range test (Duncan Multiple Range Test/DMRT).

3. Results and Discussion

Results of the average of enzyme activity are listed in Table 1.

Table 1. Datasheet enzyme activity (U/ml)

Variable	Treatment				
	T0	T1	T2	T3	T4
LiP	0.026^{a}±0.00	0.223^{d}±0.01	0.133^{b}±0.00	0.166^{c}±0.00	0.134^{b}±0.00
MnP	0.020^{a}±0.00	0.091^{d}±0.00	0.055^{c}±0.00	0.059^{c}±0.00	0.040^{b}±0.00

Results mean enzyme activity Lignin peroxidase (LiP) are listed in Table 1. The mean of five consecutive treatments that T0 = 0.026 ± 0.00, T1 = 0.223± 0.01, T2 = 0.133 ± 0.00, T3 = 0.166 ± 0.00, and P4 = 0.134 ± 0.00 Uml showed highly significant results (P <0.01).

Fermentation with Phanerochaete chrysosporium has highest enzyme activity was indicated in the treatment of T1 = 0.223 ± 0.03 U/ml. Results of this study was similar to Puspita study (2007) showed that the fungi Pleurotus sp has the highest LiP enzyme activity on the 6th day of incubation in the amount of 0.430 U/ml. LiP was a major catalyst in the process ligninolitik by fungus because it can break down the non-phenolic units which make up 90 percent of the structure of lignin (Srebotnik et al., 1998). LiP catalyze an oxidation of aromatic compounds to form non-phenolic lignin aryl radical cation. In addition. because LiP was a strong oxidant that this enzyme also has the ability to oxidize phenolic compounds, amines, ethers aromatic and polycyclic aromatic compounds. Lignin substructure oxidation catalyzed by LiP begins with the separation of the aromatic ring electron donor substrate and produce aryl cation radicals which then under various reactions post enzymatic.

Phanerochaete chrysosporium was one of the white rot fungi in the wood. These fungi produce extracellular enzymes LiP, MnP, and laccase. LiP was instrumental in weathering timber, degrading waste, and lignin. The high activity of LiP issued by Phanerochaete chrysosporium fungi because the cocoa pod was an organic waste lignocellulosic enzyme LiP act as inducers. Cocoa pod was also rich in sugars that are naturally easy to be metabolized by Phanerochaete chrysosporium.

Results of the average activity of the enzyme manganese peroxidase (MnP) are listed in Table 2. The mean of five treatments that T0 = 0.020±0.00, T1 = 0.091±0.00, T2 = 0.055±0.00, T3 = 0.059±0.00, and T4 = 0.040±0.01 U/ml showed highly significant results (P <0.01).

Fermentation with Phanerochaete chrysosporium has highest enzyme activity was indicated in the treatment of T1 = 0.091 ± 0.00 U/ml. MnP enzyme oxidizes Mn^{2+} to Mn^{3+} and H_2O_2 as a catalyst to generate clusters peroxide (Camarero et al., 1996). Mn^{3+} produced diffuses into the substrate and activate the oxidation process. That was supported also by the activity of the radical cation of veratril alcohol and enzyme-producing H_2O_2. This process will end with the merger of O_2 into the lignin structure (de Jong et al., 1994).

According Pelczar and Chan (2005) the main function of an enzyme was to reduce the activation energy barrier in a chemical reaction. Enzymes known there are two types of extracellular and intracellular enzymes. The main function of extracellular enzymes was to carry out changes in the surrounding nutrient allowing the nutrients to enter cells. Intracellular enzymes synthesize cellular material and also outlines nutrients to provide energy needed by the cell.

Results of the average of lignin are listed in Table 2.

Table 2. Datasheet of Lignin (%)

Replication	Treatment				
	T0	T1	T2	T3	T4
1	36.05	26.78	28.33	29.77	28.65
2	35.79	26.81	28.32	30.75	27.49
3	36.18	27.01	28.72	29.69	28.20
Mean	$36.00^{b} \pm 0.19$	$26.86^{a} \pm 0.12$	$28.45^{c} \pm 0.22$	$30.07^{d} \pm 0.59$	$28.11^{e} \pm 0.58$

The mean content of lignin are listed in table 2. The mean of five treatments that T0 = 36.00±0.19, T1 = 26.86±0.12, T2 = 28.45±0.22, T3 = 30.07±0.59, and P4 = 28.11±0.58% showed highly significant results ($P < 0.01$).

Fermentation with Phanerochaete chrysosporium has lowest lignin content in the treatment of T1 = 26.86 ± 0.12%. Lignin was a component of plant cell walls that had been developed after the plant was undergoing a process of maturation. Cocoa pod as the old plant waste has lignified advanced stages. The magnitude of the lignin content was strongly influenced by fermentation. Changes in lignin content of the substrate occurs due to recast the structure of lignin into simpler components.

Lignin content in T1 was 26.86 ± 0.12% showed the lowest among other treatments. This suggests that the drop of lignin because high enzyme activity in T1 were use Phanerochaete crysosporium to fermentation of cacaco pod. Lignin content in the fermentation time associated with the production of the enzyme ligninase. Jager et al., (1985) reported that the highest enzyme production ligninase occurred on the sixth day after inoculation. The lignin degradation will pave the way for an overhaul of cellulose and hemicellulose.

The decrease in lignin content of the cocoa pod to the treatment of fermentation with Phanerochaete chrysosporium reaches lignolitik phase and immediately degrade lignin, fungi Phanerochaete chrysosporium can degrade lignin effectively by producing enzymes peroxsidase extracellular form of Lignin peroxidase (LiP) and Manganese peroxidase (MnP).

Fermentation of cocoa pod by using Phanerochaete chrysosporium provide the opportunity for fungi to growth well so that production of the enzyme produced was also high that affect to the degradation of lignin in the fermentation process of cocoa pod.

The lignin content in the fermentation process relates to the production of the enzyme ligninase. The lignin degradation will pave the way for an overhaul of cellulose and hemicellulose. White rot fungi Phanerochaete chrysosporium can degrade lignin so well that it becomes lower lignin content. Decreased levels of lignin by the fungi showed that white rot fungi able to degrade lignin, according Kaal et al., (1995), that white rot fungi has ability depolimerizated lignin .

4. Conclusion

Fermentation of cocoa pod with different fungus indicates fermentation used Phanerochaete chrysosporium has the best results and showed the highest enzyme activity of LiP 0.223±0.00 U/ml and MnP 0.091±0.01 U/ml and also has the lowest lignin content was 26.86 ± 0.12%.

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