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The Effect Treated of Oil Palm Trunk by Ligninase Thermostable to Improvement Fiber Quality as Energy Sources by Ruminant

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Abstract : The research purposed to find out interaction between oil palm trunk concentrations and ligninase doses to increase the fiber quality of oil palm trunk before using as animal feeding. The oil palm trunk treated by ligninase aimed to hidrolyzed of lignin as cell wall through separation of fiber fraction such as: cellulose, and hemicellulose. The design used in this study was a completely randomized design (CRD) factorial using 2 factors: factor A consists of three levels of ligninase enzyme concenrations A1: 250 units/kg, A2: 500 units/kg,and A3: 750 units/kg. Factor B is the concentration of oil palm trunks B1: 40%; B2: 60% were repeated 3 times. The results showed that there was highly significantly effect of interactions ($P<0.01$) between levels of ligninase enzyme (factor A) with the concentration of oil palm trunks (factor B) to ADF, NDF, cellulose, hemicellulose and lignin contents.The research can be concluded that the optimum concentration of oil palm trukns was 60% (v/w) and 750 U/kg of ligninase that were improved of the fiber fractions quality. The best fiber fraction quality with 60.75; 76.68; 13.02; 52.43 and 5.6% DM for ADF, NDF, cellulosa, hemicellulosa and lgnin respectively.
Keywords : Oil palm trunk, ligninase, NDF,ADF, cellulosa,hemicellulosa, fiber and lignin, enzyamtic hydrolysis, ligninase, ruminant, energy.

Introduction

Most of the developing countries are very rich in agricultural fiber and a large part of agricultural waste is being used as a animal feeding. The agriculture waste contained lignocellulosic materials are composed of cellulose, hemicellulose and lignin which are the most important part of the natural resources that are discharged by the farming and agricultural activities [1, 2].The palm oil production in from 2012 to 2013 was 28.5 million tons in Indonesia and 18.5 million tons in Malaysia, which amounts to approximately 88 % of the worldwide palm oil production[3]. To harvest fresh palm with its high oil productivity, the oil palm tree is recommended to be replanted at intervals of 20–25 years. Consequently, a large amount of oil palm trunk (OPT) is inevitably generated as a byproduct in the process of rebuilding plantation sites. In Indonesia, for example, oil palm trees cultivated in 600,000 ha are annually cut for replanting. In such oil palm plantations, approximately 70 million palm trees are generated annually, affording more than 15 million tons OPT[4]. Some parts of OPT such as its hard outer layer were often used for plywood manufacturing, but most of it tends to be discarded or burnt[5].

Recently, research has focused on the use of OPT as a animal feeding. The OPT is lignocellulosic biomass waste contained the polymer of polysaccharides such as: lignin, cellulose, and hemicellulose which has a great potential for animal feeding. The utilization of OPT is still limited and less noticed by the general public as well as oil palm plantation company itself. Wong[6] added OPT has a complex structure that is difficult degraded by most organisms. Lignin protects cellulose, and hemicelluloses in lignocellulosic linkages, that difficulty of cellulose and hemicellluse can be use as energy resources by animal[7].

The one of the serious problems in the utilization of OPT are excessive hygroscopic properties, although it has been dried to reduce the moisture content, the oil palm trunks can reabsorb water from the air. The limiting factors of the OPT as animal feeding are low palatability, low protein content and the higher of fiber. To increase the quality of OPT as animal feeding its needed the treatment before used such as physical, chemical, biologycal and enzymatics treated. Several biological methods for lignocellulose recycling based on the enzymology of cellulose, hemicelluloses, and lignin degradation have been developed[7]. To date, processes that use lignocellulolytic enzymes or microorganisms could lead to promising, environmentally friendly technologies. Efficient enzymatic degradation of lignocellulosic biomass requires a tight interaction between the enzymes and their substrates, and the cooperation of multiple enzymes to enhance the hydrolysis due to the complex structure.The aim of this study was to determine the effect of treatment with ligninase enzyme dosage and level of the oil palm trunk (OPT) as substrates to improvement of fiber quality that can be use as energy resources by ruminants.

Materials and Methods

Production of Ligninase Thermostable

The ligninase enzyme produced by bacteria was performed in 5000 ml (5 L) Erlenmeyer flask containing 50 g of OPT. The medium was prepared with the following composition (g/l) 10.0g; urea, 0.3; peptone, 0.75; yeast extract, 0.25; (NH4)2SO4, 1.4; KH2PO4, 2.0; CaCl2, 0.3; MgSO4.7H2O, 0.3 and trace elements (mg/l): FeSO4.7H2O, 5; MnSO4. 4H2O, 1.6; ZnSO4.7H2O, 1.4 and CoCl2.6H2O, 20. The medium and the trace elements were autoclaved separately. The flask was cooled down at room temperature and a known amount of sterilized trace elements was added. The flasks were then inoculated with (50 ml) (1×10^6 spores/ml) of the bacteria and incubated for 60 hour at the 70^0 C[7].

Preparation of Oil Palm Trunk

Oil palm trunk (OPT) collected from field used as substrate for enzyme production, it was ground and sieved to 18-20 mesh by shredding machine. The shredded oil palm trunk was dried at 60^0C in an oven for 12 h. The dried oil palm trunk (OPT) was kept ready for the further use. The Shredding machine and OPT after shredded can be seen in Figure 1 and Figure 2.

Figure 1. Shredding machine

Figure 2. Oil palm trunk after shredded

Enzymatic Hydrolysis of Oil Palm Trunk (OPT)

Hydrolysis of oil palm trunk was carried out in reaction mixture containing differents concentration of ligninase and different concentration of oil palm trunk . The reaction mixtures were incubated on a water bath rotary shaker adjusted to 70^0C and 75 rpm for 2 hrs[7] The fiber fractions of treated ligninase of oil palm trunk was measured by Van Soest method[8]

Research Methods

This study used a completely randomized design (CRD) factorial using 2 factors: factor A is composed of cellulose enzyme units: A1 = 250 units/ kg; A2 = 500 units / kg; A3 = 750 units / kg. Factor B is a palm trunk level, namely: B1: 40%; B2: 60%, was repeated 3 times.

Data Analysis

All data were analyzed using analysis of variance (ANOVA) completely randomized design (CRD) factorial and differences treatments were tested by Duncans Multiple Range Test (DMRT) according to Steel and Torrie[9]

Results and Discussion

Effect of Treatment to ADF Content

Based of analysis of variance shows that there was a significantly influenced ($P <0.01$) against factor A (ligninase dosage), factor B (level oil palm trunks) and the interaction between the enzyme dosage and level of oil palm trunk. Further trials using Duncan Multiple RangeTest (DMRT) showed that the best content of ADF was interaction between ligninase enzyme dose and levels of oil palm trunks were on A3B1 treatment (750 units/kg enzyme ligninase and 40% OPT) that produces the lowest ADF content (60.75%). From the Table 1 showed, if OPT concentration increased to 60% could not be the decrease of ADF, it caused that all active sites of enzyme has full to substrates, so increased of the substrate concentration, no substrates can reacted with active sites the enzyme or enzyme could be saturated by substrate, no product can be produced. A groups of researcher has been reported that by Shleev *et al*[10]; Yetti *et al*[11]; Yetti *et al*[7]. The least digestible plant components, including cellulose and lignin, but ADF values are inversely related to digestibility, so forages with low ADF concentrations are usually higher in energy. In this research the ADF content of OPT was higher than oil palm front (OPF) were 60.75% DM compared to 51.7% DM[12] for OPT hydrolysis by ligninase and silage of OPF respectively.

Table 1. Average ADF products hydrolysis of oil palm trunks (%)DM

A. Ligninase (units/kg)	B. Oil Palm Trunk Concentration (%)		
	B1	**B2**	Average
A1	64.00[a]	64.16[a]	64.08
A2	62.31[b]	64.44[a]	63.38
A3	60.75[c]	61.54[bc]	61.15
Average	62.35	63.38	

Note: Different superscript lowercase letters indicate highly significant effect (P <0.01).

Effect of Treatment to NDF Content

In the research showed that the hydrolysis product of OPT with various levels of enzymes ligninase an effort to increase the potential of OPT as one of fodder. The results NDF content which can be seen in Table 2. Based on the results of analysis of variance showed that there was a significantly influenced (P <0.01) against factor A (ligninase enzyme dose), factor B (level palm trunks) and the interaction between the enzyme dosage and levels palm trunks. Further trials using Duncan Multiple RangeTest (DMRT) showed that the best NDF content was on A3B1 treatment (750 units /kg enzyme and 40% palm trunks) that produces the lowest NDF content (76.68%). The same reason also happen with NDF like ADF, the saturated enzyme by the substrate of OPT happen, the icreased of substrate concentration can not inreased the hydrolysis product. Varga and Hoover[13] stated that, NDF levels were negatively correlated with the process of hydrolysis. NDF tend to be more susceptible to hydrolysis than ADF because they contain soluble fraction is hemicellulose[14]. NDF is the amount of protective substances obtained from residue after boiling a fodder sample in neutral detergent solution. NDF residue, actually contains very little pectin substances, but may contain negligible amounts of products like starch, nitrogenous substances and tannins[15]

Table 2. Average total NDF products hydrolysis of oil palm trunks (%)DM

A. Ligninase (units/kg)	**B. Oil Palm Trunk Concentration (%)**		
	B1	**B2**	**Average**
A1	80.97[b]	77.18[c]	79.07
A2	81.58[a]	81.80[a]	81.69
A3	76.68[d]	76.76[a]	76.72
Average	79.74	78.58	

Note: Different superscript lowercase letters indicate highly significant effect (P <0.01).

Effect of Treatment to Hemicellulose

The hemicellulose product hydrolysis oil palm trunks can be seen in Table 3. In Table 3 showed that the interaction between enzyme concentration and OPT concentratian to optimal hemicellulose content was at treatment of A1B2 (250 units/kg enzyme and 60% palm trunks) that produced the lowest levels of hemicellulose (13.02%). This was due to the loss of hemicellulose and other easily hydrolyzed materials during the enymatic hydrolysis, the hemicellulose was found on liquid formed. After the majority of the hemicelluloses in OPT were removed during enzymatic hydrolysis, the remaining solid (mainly cellulose and lignin) was more porous and could be more easily hydrolyzed by cellulases into glucose[16] . In this research, the optimum enzymatic reaction need only small enzyme and large of substrate OPT, it might be caused that the conversion of hemicellulose to glucose is two to three times faster than hydrolysis of cellulose to soluble sugars. The most native hemicelluloses are quite water-soluble because, in part, of the substituents attached to the main chain. These side chains disrupt the water structure and help to solubilize the hemicellulose. The debranching enzymes, which remove these substituents, may generally decrease substrate solubility when acting alone and, in turn, lower the polysaccharide's susceptibility to endo-acting hydrolases[17]. The same results also reported by a group of researcher[18,19,20,21]

Table 3. Average hemicellulose products hydrolysis of oil palm trunks (%)DM

A. Ligninase (units/kg)	B. Oil Palm Trunk Concentration (%)		
	B1	B2	Average
A1	16.98^{b}	13.02^{d}	15.00
A2	19.27^{a}	17.36^{b}	18.32
A3	15.93^{c}	15.21^{c}	15.57
Average	17.39	15.20	

Note: Different superscript lowercase letters indicate highly significant effect (P <0.01).

Effect of Treatment to Cellulose

The cellulose contents of OPT after treated by enzymatic hydrolysis using different concentrations of ligninase can be seen in Table 4. In Table 4 showed that the interaction beetwen the treatments was a significantly effect (P <0.01). The best of cellulose content was on the treatment of A3B2 (750 units/kg of enzyme and 60% palm trunks) that produced the lowest cellulose content (52.43%). The enzymatic reaction of OPT to produce cellulose needed more enzyme and substrate, it caused that cellulose more rigid and difficult to degradation compared to other moleculs such as hemicellulose. Cellulose, are main polymeric component of the plant cell wall and the most abundant polysaccharide on earth, has a simple chemical composition: the polysaccharide consists of D-glucose residues linked by β- 1,4-glycosidic bonds forming linear polymeric chains of over 10000 glucose residues. The cellulose molecule is formed by a crystalline part and an amorphous part. The cellulose crystallinity is an important factor that can enhance the results obtained from the enzymatic hydrolysis[21,17,23]

Table 4. Average cellulose products hydrolysis of oil palm trunks (%)DM

A. Ligninase (units/kg)	B. Oil Palm Trunk Concentration (%)		
	B1	B2	Average
A1	56.41^{b}	55.38^{bc}	64.08
A2	54.13^{c}	58.07^{a}	63.38
A3	54.06^{c}	52.43^{d}	61.15
Average	54.87	55.29	

Note: Different superscript lowercase letters indicate highly significant effect (P <0.01)

Effect of Treatment to Lignin

The results of lignin content of oil palm trunk after treated with ligninase can be seen in Table 5. Table 5 showed that the best lignin content was treatment of A2B2 (500 units/kg of enzyme and 60% palm trunks), which was produced low lignin content (5.60% DM) from the other treatment. The lignin content of OPT before treated with ligninase was 19.6% DM. According to Hendriks and Zeeman[24] repoted that, lignin removal was sufficient in low heating pretreatment processes for low lignin biomass compounds, but not for high-lignin contained biomass. Since OPT biomass was the trunk of oil palm trees, the encrusted lignocellulosic polymer contained high-lignin and thus might require more harsher conditions such as temperature (>160 °C) in order to achieve higher lignin removal[25]. Jimmy[26] said that the optimum conditions which produce the largest lignin removal (79%) of sugargane leaves was obtained at 11% sodium hydrogen sulfite and 170^{0}C. Characteristics of high content cellulosic products from this stage is a cellulose powder with content of 80% cellulose, 3% hemicellulose and 10% lignin

Table 5. Average lignin products hydrolysis of oil palm trunks (%) DM

A. Ligninase (units/kg)	B. Oil Palm Trunk Concentration (%)		
	B1	**B2**	Average
A1	7.31[b]	7.23[bc]	7.27
A2	7.09[c]	5.60[e]	6.35
A3	6.07[d]	7.67[a]	6.87
Average	6.82	6.83	

Note: Different superscript lowercase letters indicate highly significant effect (P <0.01).

Ligninase enzymes are the main enzyme in lignin degradation process because it can oxidize non-phenolic lignin units. Oxidation of lignin substructure ligninase catalyzed by the enzyme begins with the separation of the aromatic ring electron donor substrate and produces radical cations, which then undergo various post enzymatic reactions[27]. Lignin is a very complex molecule constructed of phenylpropane units linked in a large three-dimensional structure.Three phenyl propionic alcohols exist as monomers of lignin: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Lignin is closely bound to cellulose and hemicellulose and its function is to provide rigidity and cohesion to the material cell wall, to confer water impermeability to xylem vessels, and to form a physic–chemical barrier against enzymes attack[28]. Due to its molecular configuration, lignins are extremely resistant to chemical and enzymatic degradation[7]. Lai *et al*[29] and Dewi *et al*[30] added the lignin component in biomass is responsible to impart strength to plant cell walls so as to protect cellulose and hemicelluose from enzymatic degradation. Lai and Idris[31] also stated that higher lignin content in oil palm trunk biomass was undesirable and has several disadvantages such as lignin would limit the biodegradability of lignocellulosic biomass in the subsequent enzymatic hydrolysis process. Bakkiyaraj *et al*[32] and Moningkey *et al*[33] added various lignocelluloses and the structural architectures of different lignocelluloses influence the lignin hydrolysis.

Conclusions

Lignocellulosic materials including forestry, agricultural and agro-industrial wastes contain several high value substances such as, starch, sugars, lignin, fiber, fraction minerals and protein. The oil palm truk (OPT) are wastes to the soil or landfill causes serious environmental problems, besides to constitute loss of these value added substances. Therefore, the development of processes for reuse of these wastes is of great interest such as: treatment with ligninase enzyme before using as animal feeding. The treated with ligninase can be degraded the lignocellulose and hemicellulose linkage and make the availability of cellulose and hemisellulose as energy sources by ruminantia such as cow, buffalo, sheep and goat. The optimum concentration of OPT was 60% (v/w) and 750 units/kg of ligninase thermostable that improved of the fiber fractions quality to easily digested by animal.

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