Biodegradation of coffee husk substrate during the mycelia growth of *Pleurotus ostreatus* and the effect on in vitro digestibility

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Abstract

The aim of this studies were conducted to evaluate culturing of mushroom P.ostreatus on coffee husk in solid state fermentation as means of improving the nutritive value of coffee husk for ruminant animals. The influence of P.ostreatus on coffee husk biodegradation was investigated. The dry matter and composition changes of coffee husk substrate for P.ostreatus cultivation were analysed on day 0, 30 and 60 after seeding. The profile of cellulose, hemicellulose and lignin were changed when it was used by P.ostreatus. Meanwhile their rate of change varied at different growing day. The increase of protein content and the reduction of lignocellulose content increase dry matter digestibility of coffee husk substrate. This fact could provide an alternative of biofermentation product based on coffee husk substrate which is safe for environment.

Keywords: biodegradation, coffee husk, digestibility, substrate, P. ostreatustion

Introduction

Pleurotus ostreatus is one of the popular cultivated mushroom. It can be cultivated on a wide range of lignoselulosic substrates such as wheat straw, cocoa husk and cotton stalks (Fazaeli *et al.,* 2004; Li *et al*., 2001; Alemawor, 2009). *Pleurotus ostreatus* belongs to white rot fungi which are able to degrade lignin because produce ligninolytic extracellular enzymes, such as laccases, lignin peroxidases and Mn peroxidases (Kerem *et al.,* 1992; Chang and Miles, 2004).

The ability of *P.ostreatus* degrades a wide variety of lignoselulosic substrates, enabling it to play an important role in managing organic wastes whose disposal is problematic. *Pleurotus* species have been used by human for their nutritional value,

medicinal properties, transformation of wastes into animal feed and other beneficial effects (Hadar and Arazi, 1986; Gregori *et al.* 2007; Adamovic *et al*., 1998).

Coffee husk is the major byproducts produced during the operation of coffee cherry to get coffee grain by sun drying (Fan *et al*., 2004). In coffee-producing regions, coffee husk is barely utilized. Therefore, it is considered the most abundant pollutant material. Coffee husk has potency as a source of ruminant feed. The protein content is 9.2-11.3% equally to rice bran protein (±10.4%) and has metabolic energy around 3.356 Kcal/kg (Zainudin and Murtisari, 1995). The content of lignin is 35.0-40.0% (Fan and Soccol, 2005). The digestibility of these materials are limited by the presence of lignin which prevents access of hydrolytic enzymes to cellulose and hemicelluloses.

Application of *Pleurotus ostreatus* is worth considering for improving the nutritive value of coffee husk. This study was carried out to asses the effect of a solid state fermentation involving *Pleurotus ostreatus* on the nutrition composition of coffee husk and to evaluate in vitro digestibility. In addition, fermentation period on the process was evaluated.

Materials and Methods

Coffee husk were obtained from coffee hulling plant at Rejang Lebong Residence Bengkulu Province. Coffee husks were air-dried to moisture content 10-15%. The solid state substrate were prepared with the composition adopted from sawdust standard medium (Herliyana *et al.* 2008). The mushroom substrate may be defined as a kind of lignocellulosic material supports the growth, development and fruiting of mushroom. The substrate were consisted of 82,5% coffee husk, 15% rice bran, 1,5%gips and 1,0% $CaCO_3$. The clean water were added to the substrate as much as 60-65% (v/w). All these components were placed in polypropylene bag in amount 400 gram per bag. Each bag was closed with a small cotton plug inserted in the middle of its opening. The bags were sterilized at 121^{o}C for 30 minutes. After cool, each of bag was seeded with 15 gram (3,75%) of *Pleurotus ostreatus* spawn. All spawned bag were placed in growing room with the temperature was 22-28^{o}C and relative humidity 60-80%. After 30 days, the substrate was fully colonized, and on 60 days primordial started to appeared.

The content of protein was analyzed using Kjeldahl method. The cell wall components (NDF, ADF, Lignin, cellulose and hemicelluloses) were analyze using deterjent analyze method as described by Goering and Van Soest (1970). *In vitro* dry matter digestibility was evaluated according to Tilley and Terry method (1963).

The treatment was the fermentation time consisted of 0 untreated), 30 and 60 days after seeding. The nutrient composition changes were described descriptively. For the dry matter measurement the treatment was arranged in Block Randomised Design (3x4). The rumen inoculum were obtained from four cattles as block.

Significant differences were calculated using Duncan's multiple range test following analysis of variance.

Result and discussion

The celluloses, hemicelluloses and lignin are the main sources of carbon and energy for *P.ostreatus* growth, while protein serves as the nitrogen source. Their degradation and utilization can greatly affect *P.ostreatus* growth and resulting feed value of the substrate. The change of nutrient composition contents during the *P.ostreatus* mycelia growth period are shown in Table 1.

There were increasing of protein content and decreasing of fiber fraction (lignin, NDF and ADF) produced by biofermentation. The decreasing of fiber fraction is the indication that *Pleurotus ostreatus* can degrade the cell wall component of coffee husk.

The decreasing of NDF and ADF from coffee husk suggested that these fungi could utilize the cell wall component as carbon source and energy for growth. The decreasing of NDF and ADF contents of treated coffee byproduct has been reported by Penaloza *et al*., (1985). The decreasing of NDF, ADF and ADL in the first 30 days of mycelia growth were 2.339%, 4.586% and 19.874%, respectively. Meanwhile in 60 days, the decreasing of NDF, ADF and ADL were 16.587%, 15.036% and 31.161%, respectively from the initial value.

The fermentation time was important to improve the nutritive value of straw. The longer fermentation period led greater depletion of carbohydrate source of coffee husk by fungi. This condition could improve the digestibility of coffee husk as result of the changes in non structural carbohydrate to structural carbohydrate ratio. Decreasing of lignin in coffee husk could be a result of lignin degrading enzymes produced by *Pleurotus* (Hong *et al*., 2003). These result are supported by the report from Widiastuti *et al*. (2008) who noted ligninolityc enzyme activities followed the pattern of lignin disappearance from substrate and directly corrected with time of its disappearance. Plat and Hadar (1983) noted that during the mycelia growth period, *P.ostreatus* mycelia were more capable to degrade lignin, and the degradation of lignin played an important role in mycelia development.

The rapid decreasing of hemicellulosic component in 30 days fermentation showed that hemicelluloses were the first substrate utilized by mycelia as the carbon and energy sources at the beginning phase of growth. The decreasing of hemicelluloses was 31,578% from initial value in 30 days fermentation. This suggest that hemicellulose is more easily degraded than cellulose and lignin. *Pleurotus ostreatus* mushroom secreted enzym to demolish the easier used compound. *Pleurotus ostreatus* needs a carbon source which is easier to metabolize (Crawford, 1981). Hemicelluloses were degraded easier than cellulose and lignin (Perez, 2002).

The cellulose content increased 35.574% in 30 days and 27.063% in 60 days.

Biofermentation broke the lignocelluloses bond. Delignification has important role in mycelia growth which cleavage polysaccharide component (cellulose and hemicelluloses) (Agosin and Odier, 1985). This component will be utilized by fungi as substrate for their growth (Hatakka, 2004).

During the mycelia growth, the protein content increased 0.927% in 30 days and 17.220% in 60 day fermentation. Mycelia in 60 days were thicker than 30 days. Fungal cell in mycelia contributed the protein content of subtrate because 60 and 70% of nitrogen present in the fungal cell is protein (Chang and Miles 2004). The higher protein content in 60 days in the substrate were prepared to transferable nitrogen into fruit bodies. The extensive formation of primordia in 60 days indicated the end of the vegetative growth phase of *P.ostreatus*. As coffee husk substrate was degraded and nutrient used by *P.ostreatus*, the total organic matter of substrate decreased (Table 1).

The increasing of protein content and the decreasing of lignocelluloses of coffee husk after fermentation showed that Coffee husk could be used as substrate *P.ostreatus* cultivation. The improving nutrition value after fermentation especially on 60 days indicated that the substrate can be used as a product feed.

In vitro dry matter digestibility tests for ruminant were conducted for the digestibility of untreated and treated coffee husk. Four replication were conducted and the result are shown in figure 1. Average dry matter digestibility (Table 1) increased significantly 4.983% in 60 days fermentation and decreased 14.435% in 30 days fermentation from untreated coffee husk. The possibility of this condition is that in 30 days fermentation the higher level of cellulose made digestibility lower.

Table 1. Changes of nutrient contents and average *in vitro* dry matter digestibility of coffee husk substrate during *Pleurotus ostreatus* mycelia growing (0, 30, and 60 days fermentation) (as % dry matter)

Nutrient contens (%)	0 days (Untreated)	(Treated) 30 days after seeding	(Treated) 60 days after seeding
Organic matter	93.710	92.950	86.599
Crude Protein	10.360	10.456	12.144
NDF	95.177	92.950	79.390
ADF	87.184	83.186	74.075
Hemicelluloses	7.993	5.469	5.3170
Cellulose	19.514	26.456	24.795
Lignin	65.421	52.419	45.035
Dry Matter Digestibility (%)	29.518 ± 1.249^{a}	25.257 ± 0.721^{b}	30.989 ± 1.263^{c}

Different superscript in the same row means significantly different ($P<0.05$)

It suggested that on 30 days, the degradation of lignocellulosic component was not optimal yet. Therefore, it could be acceptable to use the coffee husk substrate after *P.ostreatus* cultivation on 60 days fermentation as ruminant feed.

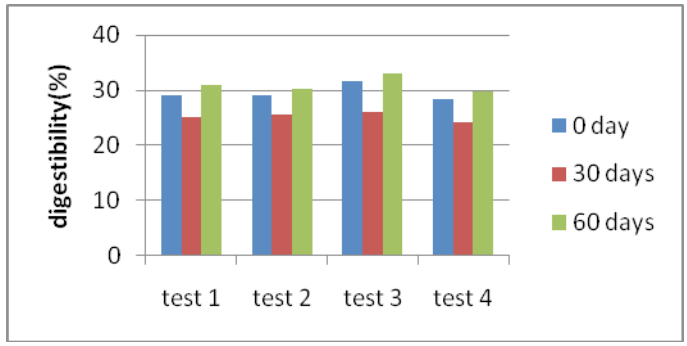

Figure 1. *In vitro* dry matter digestibility of coffee husk during *Pleurotus ostreatus* mycelia growing

Conclusion

It was concluded that protein content and cell wall components in coffee husk substrate changed during *Pleurotus ostreatus* mycelia growing period. In 60 days of fermentation times, cellulose, hemicelluloses and lignin contents in the substrate were decreased and protein content increased as compared with the untreated coffee husk. This could contribute to the increasing in dry matter digestibility of the substrate. It is suggested to use the coffee husk substrate as a ruminant feed especially in 60 days fermentation.

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