Biodelignification of sugarcane shoots: agricultural waste management strategy as an alternative feed for ruminants

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Abstract. This research aimed to reduce the lignin content and increase the nutrients content of sugarcane shoots as forages for a ruminant. This research used an experimental method using a completely randomized design (CRD) in factorial patterns, whereas A factor was a type of fungi (Pleurotus ostreatus and Aspergillus oryzae), and B factor was biodelignification time (14, 21 and 28 days). The variables observed in the experiment were laccase enzyme activity, Crude Protein (CP) content, and the percentage of lignin decreased. The research results showed that biodelignification using Pleurotus ostreatus fungi for 28 days resulted: 1.62 U/ml of laccase enzyme activity, 9.23% crude protein content, and 12.83% of lignin decreased. From this research, the best treatment for bio-delignification of sugarcane shoots was with Pleurotus ostreatus fungi for 28 days, producing the best sugarcane shoots with 9.23% of crude protein and 12.83% of lignin decreased.

Keywords: Sugarcane shoots, Bio-delignification, laccase, Pleurotus ostreatus, Aspergillus oryzae

1. Introduction

Ruminant livestock development in tropical countries especially Indonesia generally emphasizes livestock systems that do not cause land use and food needs competition. However, the obstacle faced is the minimal forage availability, so alternative feed ingredients are needed to substitute forage. One potential alternative is plantation waste such as sugarcane shoots. Sugarcane shoots contain 27.29% dry matter; 7.59% Crude Protein; Crude Fiber 40.39%; and BETN 40.67% and lignin 14% [1] [2].

Utilization of sugarcane shoots as feed requires a touch of technology to reduce crude fibre and lignin, thereby enhancing digestibility. The low digestibility is due to lignin, which hinders breaking down polysaccharides in the cell walls by microbes in the rumen. The bound lignin and hemicellulose layers are essential to separating from sugarcane shoots so that cellulose can be hydrolyzed. Also [3] states that the separation of lignin and the breakdown of hemicellulose bonds can be carried out by delignification processes using chemicals, enzymes, and the utilization of microbes. Delignification is carried out as a preliminary process to facilitate the release of cellulose and remove lignin contained in the sugarcane shoots. Hydrolysis is carried out to break the remaining lignin and hemicellulose bonds.
around cellulose and break down cellulose into glucose. One alternative that can be used is to utilize microbes that are expected to be beneficial compared to using chemicals or enzymes directly.

\textit{Pleurotus ostreatus} mold is a white rot suitable for degrading lignin and breaks down hemicellulose and cellulose polymer bonds. White rot mold in degrading lignin can be considered because of its environmentally friendly process. Besides, [4] states that treatment using \textit{Pleurotus ostreatus} indicates a decreased negative impact on the environment because it reduces the use of chemicals in the process. Degradation using fungi is thought to be able to save energy in the process of converting wood or biomass into lignocellulose-based chemicals. The material is then hydrolyzed using \textit{Aspergillus oryzae} mold.

According to [5] \textit{Aspergillus oryzae} is known as the mold that produces the most enzymes. The resulting enzymes such as cellulase, amylase, pectinase, protease and lipase can break down substances that cannot be digested by livestock such as cellulose, hemicellulose, starch, lipids, and their polymers into simple sugars so that the fermented material has good digestibility which is higher than the original material [6]. According to [7], using \textit{Aspergillus oryzae} inoculum as much as 5% can increase the best amount of biomass and enzyme activity. Furthermore, based on the research from [8], Aspergillus oryzae shows high enzyme activity at fermentation time of 2 to 4 days on coconut dregs substrate.

The study aimed to reduce the lignin content and increase the protein content of sugarcane shoots. Biodelignification using \textit{Pleurotus ostreatus} and \textit{Aspergillus oryzae} molds will reduce the lignin content of sugarcane shoots and improve the protein content.

2. Material and methods

2.1. Materials

\textit{Pleurotus ostreatus}, \textit{Aspergillus oryzae}, sugarcane top, rice brand, H2SO4, 0.1 N NaOH, aquades, and PDA medium.

2.2. Research design

The study design used a 2 x 3 factorial Completely Randomized Design (CRD) with three replications, namely: Factor A (1. \textit{Pleurotus ostreatus}, 2. \textit{Aspergillus oryzae}), while Factor B is fermentation duration (14 days, 21 days, and 28 days). The variables measured were the activity of the enzyme laccase (Leonowicz & Grzywnowicz, 1981), protein content (Kjedhal method), and lignin (Van Soest, 1963).

A total of 100 grams pulverized sugarcane shoots are put into plastic and distilled water until the water content reaches 60%. After the medium cooled, it was inoculated with the fungi \textit{Pleurotus ostreatus} and \textit{Aspergillus oryzae} with each treatment of 1 testube (5 x 10^6 cfu/ml) grown then incubated for 14, 21, and 28 days. After reaching the day according to the treatment, the medium is ready to be harvested and weighed in fresh weight, then dried in the oven at 60 oC. The sample is prepared to be tested.

3. Result and discussion

3.1 Laccase enzyme activity

The research results on the activity of the enzyme Laccase fermented sugarcane shoots are presented in Table 1. Based on the results of further tests, it was found that the treatment had a significant effect (P <0.05) on the activity of the Laccase enzyme. Duncan's further examination showed that the enzyme activity in A1B3 treatment, namely sugarcane shoots-fermented with \textit{Pleurotus ostreatus} fungi for 28 days, was significantly different (2.68) compared to treatment A1B2 (1.62), A2B3 (1.32), A2B2 (1.18), A1B1 (1.16), and A2B1 (1.00).
Table 1. Average laccase enzyme activity (U/mL).

| Type of fungi | Fermentation time | B1         | B2         | B3         |
|---------------|------------------|------------|------------|------------|
|               |                  | 1.16±0.55b | 1.62±0.13b | 2.68±0.83a |
| A1            |                  | 1.00±0.01b | 1.18±0.05b | 1.32±0.08b |
| A2            |                  | 0.25       |            |            |

Note: a, b superscript different means significantly different in a row (p<0.05).

Laccase of sugarcane top-fermented with *Pleurotus ostreatus* for 28 days had the highest activity of 2.68 U/mL. This is because the carbon and nitrogen sources in the sugarcane shoots are still relatively high, so the laccase enzyme activity is high. Similarly, [9] stated that the laccase enzyme activity is influenced by nutritional factors, namely the availability of carbon and nitrogen sources, the nature and concentration of the inducer.

The lowest activity of the laccase enzyme was in sugarcane shoots fermented with *Pleurotus ostreatus* for 28 days. This is because the period of mold growth has begun to end, so that the laccase enzyme activity has begun to stagnate and even decrease. Likewise, the study from [10], who added the laccase enzyme to sterile peat for 25 days. It was found that on days 20-25, the laccase enzyme activity was almost the same and even decreased, namely ± 0.7-0.9 U/mL. Also, [11] highlighted that laccase enzyme activity was high in the mycelium and primordia phases and dropped in the fruiting body phase. The growth of the fungal fruit body causes the laccase enzyme activity to decrease because the mold uses the nutrients in the cane shoots for the development of the fruiting body. Furthermore, [12] stated that the laccase enzyme has the ability to oxidize polyamines, ammoniphenols, lignins, aryl diamines, some inorganic ions and reduce the toxicity of some Polycyclic Aromatic Hydrocarbons (PAH).

3.2 Crude protein content

The results of the variance of lignin reduction showed no significant difference (P>0.05) between the interaction of mold types (A) and fermentation time (B). The longer the fermentation time, the higher amount of sugarcane shoot protein. This increase occurs during the biodelignification process. There is an increase in the amount of microbial biomass and the secretion of several extracellular enzymes and single-cell proteins so that the protein content of the material gains [13].

Table 2. Crude protein content sugarcane top biodelignification (%).

| Type of fungi | Fermentation time | B1 | B2 | B3 | Average |
|---------------|------------------|----|----|----|---------|
| A1            |                  | 8.56 | 9.23 | 8.69 | 8.33    |
| A2            |                  | 8.45 | 8.57 | 9.22 | 8.75    |
| Average       |                  | 8.51 | 8.90 | 8.95 |         |

3.3 Lignin degradation

Sugarcane shoots-fermented with the fungus *Pleurotus ostreatus* was better at reducing the lignin content by 19.01%. Sugarcane shoots fermented for 28 days had a significant effect (P <0.05) higher than 14 days and 21 days fermentation time. The increasing the fermentation time, the higher the lignin that is lowered. According to the statement of [14] and [15], the loss of lignin components tends to increase the length of incubation time.

The amount of percentage reduction in lignin content in sugarcane shoots fermented with *Pleurotus ostreatus* mold shows that there has been a decrease in lignin content from 15.46% to 12.52% (19.01%
decrease). Also, based on the study of [16] biodelignification with *Pleurotus ostreatus* mold can reduce lignin and hemicellulose without damaging cellulose.

Table 3. Average lignin content degradation (%).

| Type of fungi | Fermentation time | Rataan          |
|---------------|-------------------|-----------------|
|               | B1    | B2      | B3      |                  |
| A1            | 13.36 | 18.35   | 25.32   | 19.01±4.91a      |
| A2            | 6.67  | 11.61   | 10.88   | 9.72±2.18b       |
| Rataan        | 10.02±3.34b      | 14.98±3.37ab    | 18.10±7.2a    | 14.37            |

Note: a, b superscript different means significantly different in a row (p<0.05).

Compared to other treatments, the low reduction in A2 was seen in the lower drop in sugarcane shoots lignin after fermentation. The low decrease in lignin is due to the inadequate growth of mold mycelium. Insufficient growth is not triggered by enzyme activity. The resulting enzyme is less and makes the resulting enzyme activity unable to work optimally. The lignocellulose and lignohemisellulose content are still high difficult to digest by rumen microbes.

4. Conclusion

From this research, the best treatment for biodelignification of sugarcane shoots was with *Pleurotus ostreatus* fungi for 28 days that produce the best sugarcane shoots with 9.23 % of crude protein 12.83% of lignin decreased.

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