Biodegradation-Of-Lignin

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Biodegradation Of Lignin From Corn Cob By Using A Mixture Of Phanerochaete Chrysosporium, Lentinus Edodes And Pleurotus Ostreatus

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Abstract: Corn cob is agricultural waste containing approximately 76% holocellulose (cellulose and hemicellulose). Cellulose can be converted into bioethanol using a method of SSF. Degradation of lignin from corn cob can be conducted by acid, basic and enzymatic methods. The method applied to the process of enzymatic pretreatment was conducted by using a mixture of white rot fungi (Phanerochaete chrysosporium, Lentinus edodes and Pleurotus ostreatus) to degrade lignin which is environmentally friendly. Results showed that maximum lignin biodegradation, i.e. 96.88%, was achieved after incubation for 30 days by the mixture of white rot fungi

Keyword: biodegradation, lignin, white rot fungi and corn cob

INTRODUCTION

Corn cobs are found as many as 30 b/b% of corn (Koswara, 1991). It is used as an ideal source of lignocellulose which can be found easily. The production of corns in South Sulawesi achieved 424 thousands hectares with the productivity of 4.55 ton per hectare or 1.32 million per year (BPS,2012). The use of corn cobs requires pretreatment (Saha,2003), that can be done by acid, base or enzymatic methods (Isroi, 2011; Alvira et al. 2010; Hendriks and Zeeman 2009; Taherzadeh and Karimi 2008; Hu et al. 2008; Mosier 2005;). An enzymatic pretreatment has several advantages, i.e. enzyme works specifically, therefore sugar produced from hydrolysis will not degraded, the process can be conducted at low temperature and neutral medium, higher product will be obtained, and the maintenance cost will be low because the corrosive materials are not used (Taherzadeh dan Karimi, 2007). Generally, enzymes, which are secondary metabolic from white rot fungi, such as lignin peroxidase (LiP), manganese peroxidase (MnP), laccase (Lac) dan versatile peroxidase (VP). The enzymes play an important role in degradation process of lignin and can oxidize phenolic compounds found in lignocellulose (Van der Merwe, 2002; Higuchi 2004; Wong 2009).

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Therefore, the use white rot fungi can be an alternative solution for degrading lignin. Each species of white rote fungi has specific ability in degrading lignin from corn rob. Degradation of lignin was affected by incubation time of fungi and variation of the mixture of white rot fungi (Zeng et al. 2010; Taccari et al. 2009). P. chrysosporium is a white rot fungus that can produce LiP and MnP, but it cannot produce Lac enzyme (Howard, et al, 2003). Lac enzyme is produced by L. edodes dan VP is obtained from P. ostreatus (Dashtban, et al, 2010). Enzyme produced by P. ostreatus is MnP (Sarkar dkk. 1997). Some researchers have reported degradation of lignin by using just one white rot fungus. This research used three kinds of white rot fungi (P. chrysosporium, L. edodes, and P. ostreatus) for degrading lignin from corn cobs..

MATERIALS AND METHODS

Corn cobs were obtained from waste of corn plants grown by corn farmers from several districts in South Sulawesi Province. Corn cobs were crushed and the corn cob powder obtained were sieved and the powder passed by the 45 meshed filter was collected and dried until the water content of below 10%.

Preparation of delignification media from corn cobs

Corn cob powder (10 g) was put into a 250 mL Erlenmeyer, 0.01 g of glucose and 5 m of 0.004% Tween solution were added into the Erlenmeyer, mixed and then sterilized at 121 ^{o}C for 15 min in an autoclave. A 5 mL medium of fungi inoculums was put into the sterile mixture, covered with cotton and aluminum foil and incubated at room temperature for 30 days was analyzed every 5 days. The same treatment was conducted for cultures of P. chrysosporium, L. edodes dan P. ostreatus.

Analysis of lignin and cellulose

Analysis of lignin and cellulose followed the method of Chesson (Datta, 1981). A mixture containing 1 g of dried sample (a) and 150 mL of aquadest was heated in a water bath at a temperature of 90 - 100 ^{o}C for 1 h. The mixture was filtered and the residue was washed with hot water (300 mL). The residue was dried in the oven until the weight was constant (b). The residue was mixed with 150 mL of 1 N H_2SO_4 and heated in the water bath at 90 - 100 ^{o}C for 1 h. The mixture was filtered and washed with 300 mL of

aquadest and then the residue was dried (c). The dried residue was soaked with 10 mL of 72% H_2SO_4 at room temperature for 4. After that, 150 mL of 1 N H_2SO_4 was added into the mixture and refluxed in the water bath for 1 h. The solid was washed with 400 mL of aquadest, heated in the oven at 105 ^{o}C and weighed until the constant weight (d). Finally the solid was heated until become ash and weighed (e).The percentage of cellulose and lignin was calculated as follows:

$$\% \text{ cellulose} = \frac{c-d}{a} \times 100\% \ldots (1)$$

$$\% \text{ lignin} = \frac{d-e}{a} \times 100\% \ldots\ldots (2)$$

where: a = sample weight (g), c = the residue weight (g) at the third weighing, d = the residue weight (g) at the forth weighing, e = the weight of ash (g).

RESULTS AND DISCUSSION

The reduced of cellulose and lignin content as a function of the incubation time using a mixture of 3 species of the white rot fungi was given in Figure 1. The maximum result of corn cobs delignification was obtained with the small amount of cellulose reduced because in the presence of the white rot fungi, different enzymes were produced. The smallest lignin content (0.5 %) for the mixture containing 3 species of fungi was obtained at the incubation time of 30 min at the day-30. At the same condition, the cellulose content was 32.92 %. The maximum lignin content reduced was 96.88 %. Therefore at the day-30, the percentage of lignin reduced was 96,88 % whereas the percentage of cellulose reduced was 17,70%. This occurs because with the presence of the white rot fungi, enzymes of oxalate oxidase and oxalate dehydrogenase were produced. The enzymes oxidized oxalic acid and therefore, the cellulose will be degraded. The mixture of 3 species of fungi can produce enzymes optimally that worked together to degrade lignin in corn cobs maximally with the smallest percentage of cellulose reduced. This result showed the maximum initial treatment because lignin cannot be removed completely (100%) due to the presence of bonds between lignin and cellulose that has not been known completely (Steffen, 2003). According to Achmadi (1990) , more than 2/3 part of phenyl propane in lignin was related via eter bonds and the rest was related through carbon-carbon bonds.

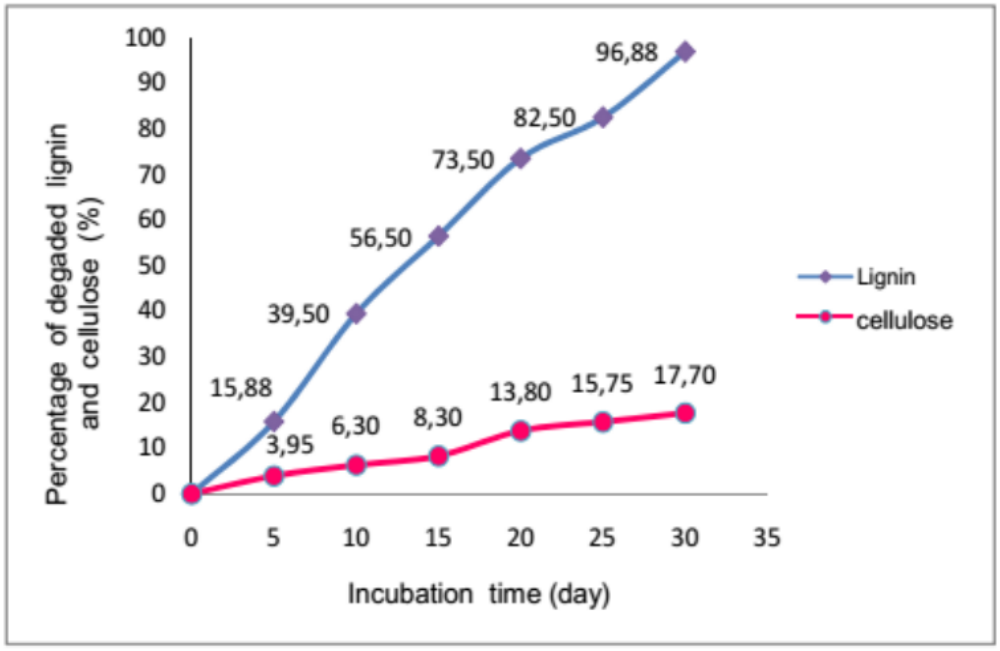

Figure 1.

The reduced percentage of cellulose and lignin as a function of the incubation time using a mixture of 3 species of the white rot fungi (P. chrysosporium, L. edodes and P. ostreatus)

At the delignification process using inocculum consisting 3 species of white rot fungi, several enzymes can be produced that worked together to degrade lignin. The enzymes working in the ligninolysis of corn cobs was LIP acting as the main catalyst for degrading non-phenolic units, MnP oxidizing Mn^{2+} to Mn^{3+} to break the phenolic unit of lignin. The other enzyme work in the process was Lac (oxidizing enzyme) working through a dimethylation process changing metoxy groups to methanol. The radical compounds spontaneously or step by step will remove the intermolecular bonds and some of them will destroy the aromatic rings. Therefore, the bond between lignin and cellulose degraded. Figure 2 shows the presence of Lac produced by L. edodes that can oxidize phenolic groups to quinon (Arora and Sandhu, 1985) and produce brown or black colour at corn cobs after incubation with the white riot fungi at the day-30.

a). before delignification b). After delignification

Figure 2.

The product of corn cob delignification using mixed inocculum of the white rot fungi (P. chrysosporium, L. edodes and P. ostreatus)

CONCLUSION

Degradation of lignin using white rot fungi (P. chrysosporium, L. edodes and P. ostreatus) achieved 96.88% and the degradation present age of cellulose was 17,70%.

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