The Effect of Storage Time of Cloud Ear Fungus (*Auricularia polytricha*) Spent Culture Media Made of Three Indonesian Tree Species on Their Saccharification Rate

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The effect of storage time of cloud ear fungus (*Auricularia polytricha*) spent culture media (SCM) made of three kinds of tropical hardwood species (*Falcataria moluccana*, *Tectona grandis*, and *Shorea* sp.) was investigated on their saccharification efficiency as a potential pretreatment of reducing sugar production. When SCM was stored at 25℃ for 2 months (SCM-2), the chemical component analysis showed that lignin content was decreased (26.3 - 49.4%) by storage process compared with unstored SCM. The glucose yield by enzymatic saccharification increased from 7.3 - 9.0% to 10.1 - 12.8%. The highest reducing sugar yield (189.2 mg/g dry spent medium) with the highest hydrolysis weight decrease (24.2%) was obtained in the SCM-2 from *Shorea* sp. Therefore, the optimal storage time is different for each SCM, and storage of SCM is a very useful pretreatment for reducing sugar production.

Key Words

Spent culture media, Storage time, *Auricularia polytricha*, Enzymatic saccharification

1. Introduction

Cloud ear fungus (*Auricularia polytricha*) is one of the edible mushrooms. *A. polytricha* can be cultivated in the wide range of region in the world, such as Indonesia which is one of the tropical countries. Some Indonesian tree species are also suitable for *A. polytricha* cultivation 1). In Indonesia this species has been cultivated by farmers using a simple technology. After several months of cultivation period farmers replace the mushroom media with the new ones, and the spent culture media (SCMs) are usually just dumped away nearby the mushroom farms or burnt out. In fact, these treatments have been giving the bad effects to the environment, mainly air pollution. Thus, SCM after cultivation of *A. polytricha* should be utilized for other applications.

SCM is composed of decayed wood meal that is expected to consist of cellulose, hemicelluloses, and lignin. SCM can be regarded as a lignocellulosic biomass that could be used as a feedstock for enzymic saccharification to produce bioethanol or other bioproducts as an alternative to fossil resources, because lignocellulosic biomass is a renewable resource 2) - 3). In fact, we have succeeded in producing high yield of fermentable sugars from the *A. polytricha* SCM originally made of 5 different Japanese wood species 4). However, the residual lignin content of the SCM from *A. polytricha* cultivation was still rather high. The pretreatment process, therefore, is needed to reduce the residual lignin content in SCM 5).

Storing SCM from *A. polytricha* cultivation could possibly enhance the enzymic saccharification ratio. Enzymic saccharification of Shiitake (*Lentinula edodes*) wood meal media stored at a constant temperature was higher than that of unstored 6). However, there are few studies on SCM from *A. polytricha* cultivation storage.

The objective of this study is to examine the effects of storage of SCM from *A. polytricha* cultivation originally made of Indonesian wood species as a feedstock for enzymic saccharification to produce bioethanol or other biorefinery products. Thus, the contents of wood chemical components in SCM were determined before and after stored for 1...
month (SCM-1) or 2 months (SCM-2). Furthermore, the SCM-1 and SCM-2 were enzymatically hydrolyzed. Based on the obtained results, effects of storage time on the enzymatic saccharification were discussed.

2. Experimental

2.1 Biomass materials and Auricularia polytricha cultivation

Wood meals (9-80 mesh) of three Indonesian wood species (Falcataelia moluccana, Shorea sp., and Tectona grandis) with 8.3-12.1% (w/w) moisture content (MC) were used for cultivation experiment of A. polytricha.

A strain of Auricularia polytricha (Aragekikurage 89, Mori & Company, Ltd., Japan) was used for mushroom cultivation. Commercial rice bran (Satoh Rice, 9-80 mesh size, MC = 11.0%, 12.5% w/w), CaCO₃ (Kanto Chemical Co. Inc., Japan, 6% w/w), and distilled water (to adjust the MC to 70%) were added to wood meal, and 150 g of these mixture was packed in a polypropylene bag (25 x 8 x 4.5 cm) equipped with a porous sterile filter (MilliSeal, 1 cm diameter pore, Millipore, U.S.A.). The medium was sterilized at 121 °C for 20 min. After inoculation of A. polytricha, the media were cultured for 130 days in a culture room as previously described 1.

2.2 Storage condition

The spent culture medium (SCM) (cylinder-shaped, 6 cm diameter, approximate height were 8.5, 5, and 4.5 cm for F. moluccana, Shorea sp., and T. grandis respectively) was stored under the following condition: a total 18 bags of SCM (3 bags for each package) were packed in a bigger polypropylene bag (25 x 15 x 15 cm) equipped with a porous sterile filter (MilliSeal, 2.5 cm diameter pore, Millipore) and stored at 25°C in the dark for 30 days (1 month) or 60 days (2 months). Room humidity was maintained at 60-80% relative humidity. SCMs stored for 1 month or 2 months were designated as SCM-1 or SCM-2, respectively.

2.3 Chemical component analysis

Before chemical analysis, the samples were ground by a rotary speed mill (P-14, Fritsch, Germany) and then sieved to collect samples in 40-80 mesh size. After that, the samples were dried in the oven at 45°C. To determine the amount of the organic-solvent extractives, 5 g of sample was extracted with 120 mL mixture of 95% ethanol and toluene (1:2, v/v) using a Soxhlet extractor for 6 h 2. Amounts of holocellulose and Klason lignin were obtained by using the organic-solvent extractives-free sample 3. Then, the amount of α-cellulose was determined using holocellulose sample 4. Acid soluble lignin was determined with using the clear filtrate obtained in the procedure of Klason lignin determination 5. Although the fresh medium (FM) and SCM contained rice bran, CaCO₃, and mycelia, ordinary methods of chemical analysis for wood were applied for determining the amounts of chemical components in these samples. Chemical component analysis was conducted 3 times for each sample.

2.4 Enzymatic saccharification

A commercial enzyme, Meiselase (Meiji Seika, Japan), was used for the saccharification of each sample. Two hundred milligram of oven-dried sample (40-80 mesh size) was saccharified at 40°C for 48 h according to the method previously reported 6. The hydrolysis weight decrease (HWD) and amounts of monosaccharides were determined by using the methods of our previous study 6. Reducing sugar yield was determined by the dinitrosalicylic acid (DNS) method 7. Enzymatic saccharification was conducted 3 times for each sample.

3. Results and Discussion

3.1 Dry weight change in the media

Table 1 shows the dry weight change of media from fresh medium (FM) before cultivation of A. polytricha to the SCM after cultivation of A. polytricha and to the SCM stored for 1 or 2 months. The dry weight change from FM to SCM varied between 17.4-43.7% after A. polytricha cultivation, due to the degradation of wood components which were incorporated into the fruiting bodies and partly emitted into the atmosphere as carbon dioxide through respiration during mushroom growth 8. After the storage for 1 month and 2 months, the dry weight change from SCM to SCM-land SCM-2 varied between 2.4 - 15.5% and 8.7 - 23.9%, respectively. Statistically, significant difference was found in dry weight change of the media from FM to SCM in all tree species and from SCM to SCM-1 and SCM-2 in the medium made of T. grandis. However, no significant difference was found after storage in the media made of F. moluccana and Shorea sp.

3.2 Chemical analysis

Chemical analysis of the sample was conducted to determine the Klason lignin, acid soluble lignin, holocellulose, and α-cellulose (Table 1). After A. polytricha cultivation, the highest decrease ratio (72.2%) of lignin was obtained in F. moluccana and that of α-cellulose (59.5%) was found in F. moluccana. These results indicate that A. polytricha is a non-selective white-rot fungus. Acid soluble lignin increased after A. polytricha cultivation in all tree species. It seems that A. polytricha degraded lignin during its mycelial
Table 1  Dry weight change (%), chemical components and amounts of monosaccharides released by enzymatic hydrolysis at 40°C (g/100 g dry biomass) of wood meal, fresh media, unstored spent culture media, and stored spent culture media

<table>
<thead>
<tr>
<th>Component</th>
<th>WM</th>
<th>FM</th>
<th>SCM</th>
<th>SCM-1</th>
<th>SCM-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight change</td>
<td>-</td>
<td>100</td>
<td>a</td>
<td>56.3 ± 1.6 b</td>
<td>53.9 ± 3.0 b</td>
</tr>
<tr>
<td>Chemical component</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klason lignin</td>
<td>23.3 ± 0.3 a</td>
<td>19.4 ± 0.5 b</td>
<td>53 ± 0.4 c</td>
<td>5.6 ± 0.2 c</td>
<td>39 ± 1.4 d</td>
</tr>
<tr>
<td>Acid soluble lignin</td>
<td>2.8 ± 0.1 c</td>
<td>2.8 ± 0.1 c</td>
<td>46 ± 0.2 a</td>
<td>4.4 ± 0.0 b</td>
<td>4.4 ± 0.0 b</td>
</tr>
<tr>
<td>Holocellulose</td>
<td>80.8 ± 0.6 a</td>
<td>71.9 ± 0.3 b</td>
<td>39.4 ± 1.7 c</td>
<td>397 ± 2.3 c</td>
<td>313 ± 6.9 d</td>
</tr>
<tr>
<td>α-Cellulose</td>
<td>45.3 ± 11 a</td>
<td>40.0 ± 0.2 b</td>
<td>16.2 ± 0.5 c</td>
<td>128 ± 0.4 d</td>
<td>121 ± 3.2 d</td>
</tr>
<tr>
<td>Monosaccharides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>1.4 ± 12 a</td>
<td>3.1 ± 0.0 b</td>
<td>8.3 ± 0.2 c</td>
<td>8.5 ± 0.3 c</td>
<td>9.3 ± 0.4 d</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.4 ± 0.3 a</td>
<td>0.3 ± 0.0 a</td>
<td>1.9 ± 0.5 b</td>
<td>1.9 ± 0.2 b</td>
<td>2.6 ± 0.0 c</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.5 ± 0.5 a</td>
<td>17 ± 0.0 b</td>
<td>12 ± 0.8 a</td>
<td>12 ± 0.8 b</td>
<td>3.5 ± 0.2 c</td>
</tr>
</tbody>
</table>

*Note: WM, Wood meal; FM, fresh medium, SCM, unstored spent media; SCM-1, stored spent medium for 1 month; SCM-2, stored spent medium for 2 month. Fresh medium and spent culture medium contained rice bran, CaCO₃, and mycelium. Value measures mean of three repetitions ± SD. The same letter shows no significant differences between columns by Tukey-Kramer test at the 5% level.*

growth, leading to the increase of the acid soluble lignin content. The organic-solvent extract content decreased after *A. polytricha* cultivation in all tree species.

Among the chemical components of wood in SCM, the most degraded component after 1 or 2 months of storage was Klason lignin, followed by α-cellulose, holocellulose, organic-solvent extracts, and acid soluble lignin (Table 2). The decrease ratio of Klason lignin, α-cellulose, and holocellulose increased with increase in the storage time. In the SCM made of *T. grandis*, Klason lignin content of SCM was significantly different from that of SCM-1 or SCM-2, while in the SCMs made of *F. moluccana* and *Shorea* sp. not significantly different between SCM and SCM-1 (Table 1). The results show that storage treatment is very effective for the SCM made of *T. grandis*.

In the SCM made of *T. grandis*, the decrease ratio of α-cellulose from SCM to SCM-1 was 22.8%, whereas only 11% from SCM-1 to SCM-2 (Table 2). The increase ratio of...
holocellulose was similar to that of $\alpha$-cellulose. Therefore, the tendency was different from that in the decrease ratio of Klason lignin. These results indicate that the chemical components of SCM were non-selectively degraded by the fungus during the first 1 month, followed by selective degradation of lignin during the next 1 months of storage. Removal of lignin leads to effective saccharification of the stored SCM by enzymes.

The acid soluble lignin content relatively increased after 1 or 2 months of storage. It seems that $A. \textit{polytricha}$ degraded lignin during storage, leading to the increase of the acid soluble lignin content. $A. \textit{polytricha}$ prefers consuming glucans derived from rice bran to those from wood during cultivation. By the time the fruit body was harvested all the rice bran glucan was consumed by the fungus. The consumption of rice bran renders the SCM poor in nutrients, which possibly facilitates the fungal attack of the chemical components of wood during storage.

### 3.3 Enzymatic saccharification

![Fig. 1 Reducing sugar yield and hydrolysis weight decrease in the wood meal, fresh medium, spent culture medium, and stored spent culture medium](image)

Fig. 1 shows hydrolysis weight decrease and reducing sugar yield after enzymatic saccharification. Hydrolysis weight decrease and reducing sugar yield were higher in SCM than in FM, indicating that SCM is a potential material for ethanol production. The hydrolysis weight decrease and reducing sugar yield were not significantly different from those of SCM after 1 month storage, but significant differences were found between SCM and SCM-2 after 2 months storage although the effect depended on wood species. It is considered that cellulases could easily hydrolize cellulose, because lignin encrusting cellulose in SCM was greatly decreased by the mycelia of $A. \textit{polytricha}$ during storage.

After storage treatment of SCM, SCM-1 and SCM-2 of $T. \textit{grandis}$ showed the highest increase ratios of hydrolysis weight decrease and reducing sugar yield (Fig. 1). As shown in Table 2, the highest decrease ratios of Klason lignin from SCM to SCM-1 and SCM-2 were observed in $T. \textit{grandis}$. On the other hand, the $\alpha$-cellulose content remaining in SCM-1 and SCM-2 of $T. \textit{grandis}$ was still high. Thus, it is suggested that rather higher amount of remaining cellulose was easily hydrolyzed by cellulases, resulting in the higher increase ratio of hydrolysis weight decrease and reducing sugar yield in SCM-1 and SCM-2 of $T. \textit{grandis}$.

The mycelial growth during storage is effective in disrupting the chemical bonds between the various components so as to produce the substrates with enhanced reactivity to hydrolytic enzyme. Glucose was
the most abundant monosaccharide among the produced monosaccharides (Table 1). Glucose and galactose yields were higher in SCM-2 compared to SCM. It is known that the larger amount of glucose is favorable to ethanol fermentation\textsuperscript{13). Therefore, storage treatment is considered to be effective for increasing the ethanol yield from SCM.

4. Conclusion

Storage of SCM of \textit{A. polytricha} is a very useful pretreatment for enzymatic saccharification to obtain large amounts of monosaccharides which are fermented to bioethanol in the SCMs made of \textit{Shorea} sp. and \textit{T. grandis}. In addition, SCM-2 gave the higher amounts of monosaccharides from enzymatic saccharification.

References

7) The Japan Wood Research Society, Manual for wood research experiment (In Japanese), Buneido, Tokyo, 2000