Decomposition of Lignin and Holocellulose of Acacia dealbata Link (Mimosoideae) Leaves, Twigs and Barks by Fungal Isolates from Virgin Forest Ecosystem of Doddabetta Belt of Nilgiris

M. N. Abubacker* and M. Prince

1Department of Biotechnology, National College, Tiruchirappalli, India.
2Department of Botany, National College, Tiruchirappalli, India.

DOI: http://dx.doi.org/10.13005/bbra/1187

Decomposition of lignin and holocellulose study was conducted in Acacia dealbata Link [Mimosoideae] leaves, twigs and barks from the virgin forest ecosystem of Doddabetta belt of Nilgiris during monsoon period June, July and August 2013. Four lignin and holocellulose degrading fungi which were dominating the A. dealbata forest ecosystem were identified, viz., Creolophus cirhatus, Hirschioporus abietinus, Schizophyllum commune and Stereum rugosum. The spores collected from the fruit bodies / the mycelia from the degrading material were inoculated individually to the experimental leaves, twigs and barks to analyse the decomposition potentials of lignin and holocellulose. The percentage of degradation of lignin, holocellulose and hot water soluble content varied with the samples and as well as the fungal isolates. However the degradation is a long process which certainly adds nutrients to the virgin forest ecosystem.

Key words: Lignin, Holocellulose, decomposition.

Lignocellulose is the predominant component of woody plant and dead plant materials, and the most abundant biomass on earth. Lignin and holocellulose in the biomass structure are major energy sources available to decomposer organisms constituting 70-80% of fresh organic material (Swift et al., 1979). Lignin is a recalcitrant plant polymer and its mineralization by white rot basidiomycetes plays a major role in carbon recycling (Martinez et al., 2005). White rot fungi are wood degrading organisms capable of decomposing all wood polymers, lignin, cellulose and hemicelluloses (Hakala, 2007). Holocellulose, a polysaccharide containing cellulose and hemicellulose (Pettersen, 1984) is a major component of wood suitable for fungal growth. Polysaccharide content generally ranges between 60 and 80% (w/w) in hardwood (Willfor et al., 2005). However, decomposition rate of cellulose is higher than that of lignin (Fioretto et al., 2005). White-rot fungi belong to the basidiomycetes and their activity is usually related to the moisture content of wood (Blanchette, 1995). The decaying fungi belong to saprophyte fungal organisms, since they live on dead or residual vegetation, decomposing them into simpler molecular compounds (Dubeux et al., 2006; Ohkuma et al., 2001). The use of saprophytic fungi can be expected to accelerate the decay of waste. Laboratory screening revealed that four fungi from the natural Acacia forest of Nilgiris which are capable of degrading lignin as well as holocellulose was identified and their lignin and holocellulose decomposition potentials in Acacia dealbata Link leaves, twigs and barks was worked out in this study.

* To whom all correspondence should be addressed.
E-mail: abubacker_nct@yahoo.com
MATERIALS AND METHODS

Leaves, twigs and barks of *Acacia dealbata* (Silver Wattle)

Sample materials in the form of leaves, twigs and barks were collected from the natural virgin forest of Doddabetta belt of Nilgiris, Tamilnadu, India (Fig. 1, a1 and 2).

Source of fungal materials

Four genera of fungi were identified for their association with decomposition of leaves, twigs and barks of *A. dealbata* material. They were *Creolophus cirhatus* (Fig. 2, a), *Hirschioporus abietinus* (Fig. 2, b), *Schizophyllum commune* (Fig. 2, c) and *Stereum rugosum* (Fig. 2, d). The fungal fruit body was collected in sterile plastic bags and brought to the laboratory and inoculated in the malt-extract-agar medium and the grown mycelia was used for further experimental work. They were cultured in lignocelluloses agar (LCA) modified by Miura and Kudo (1970). LCA contains glucose 0.1%, KH$_2$PO$_4$ 0.1%, MgSO$_4$ *7H*$_2$O 0.02%, KCl 0.02%, NaNO$_3$ 0.2%, yeast extract 0.02% and agar 1.3% (w/v). LCA modified by Miura and Kudo (1970) does not contain lignin or other recalcitrant compounds.

Decomposition test based on holocellulose and lignin content

The leaf, twig and bark samples of *A. dealbata* (Fig. 1, b 3, 4 and 5) was mixed with a 1% malt extract in distilled water to a water content of about 65% and packed in plastic bags (2.5 g for each sample pack with 3 replicates, then total sample were 32 plastic bags). They were then sterilized in autoclave at a temperature of 121°C and pressure of 1.5 atm., for 20 min. After the medium is cooled, it was then inoculated with the test fungi for 90 days to analyse the decomposition (Fig. c 6, 7 and 8). For control, samples were made of same materials with no fungal inoculation or treatment.

Determination of holocellulose content

Holocellulose content was determined with reference to ASTM 1104-56 (1978). 1 g of sample was placed in an Erlenmeyer flask (250 ml) and 150 ml of distilled water was added. While slowly shaking, 1 g of NaClO$_2$ and 0.2 ml of acetic acid were added and the flask was covered with glass and boiled at 70 to 80°C for 60 min. Again 1 g of NaClO$_2$ and 0.2 ml of acetic acid were added and boiled. After cooling, the sample was filtered using a filter flask and washed with hot water until free of acid. Then, the insoluble portion was dried in an order at 105°C for 4 hr, cooled in a desiccator and weighed repeatedly until a constant weight was obtained. Holocellulose content was calculated as follows:

$$\text{Holocellulose content (\%) = \frac{\text{Oven-dried weight of Holocellulose}}{\text{Oven-dried weight of Initial sample}} \times 100}$$

Determination of lignin content

TAPPI Standard method (2009) T 222 OS-74 procedure was followed for the determination of lignin content. 1 g of air-dried sample was weighed and transferred to a 50 ml beaker, then 10 ml of 72% sulphuric acid was added with a pipette and the mixture was stirred with a glass rod. The mixture was moved quantitatively with a wash bottle of 500 ml and diluted with water until the final volume was 300 ml. The solution was then refluxed for 3 h filtered in a glass filtered and dried in an oven at 105°C for 12 h. The bottle was cooled in a desiccator for 15 min and then weighed. The glass filter containing the lignin was reported as percentage by weight of the dried sample. Lignin content was calculated as follows:

$$\text{Lignin content (\%) = \frac{\text{Oven-dried weight of the glass filter containing lignin}}{\text{Oven-dried weight of Initial sample}} \times 100}$$

Determination of hot water soluble content

The soluble of treated *Acacia dealbata* leaves, twigs and barks were examined with reference to ASTMD1110-87 (2007). Two gram sample was oven-dried and placed into a 250 ml Erlenmeyer flask containing 200 ml of distilled water. A reflux condenser was attached to the flask and the apparatus was placed in a gently boiling water bath for three hours with constant shaking. Special attention was given to insure that the level of the solution in the flask remained below that of the boiling water. Samples were then removed from the water bath and filtered by vacuum suction into a glass filter of known weight. The residue was washed with hot water before the glass-filter was oven-dried at 103 ± 2°C. The glass-filter was then cooled in a desiccator and weighed until a constant weight was obtained. The following formula was used to obtain the hot water soluble of the sample:

$$\text{Hot water soluble (\%) = \frac{W_1}{W_1 - W_2} \times 100}$$

where

$W_1 =$ Weight of oven – Dry test sample (g)
RESULTS AND DISCUSSION

Ability of the fungi to degrade lignin

The lignin content of *A. dealbata* leaves, twigs and barks are shown in Table-1. For measuring lignin content, *A. dealbata* leaf, twigs and barks collected from the forest soil and incubated with the four fungi for one to three months (30-90 days). The initial lignin content of the leaves are 19.13%. After 30 days of treatment with fungi, the lignin content decreased to 15.10 to 17.28%, depending on the fungal genera (Table-1). The genera *Schizophyllum commune* was the most effective (15-10) at 30 days. The initial lignin content of the leaves are 18.6%, after 90 days of treatment, the lignin content decreased to 13.7 to 15.0%. However, the genera *Hirschioporus abietinus* was the most effective (13.7) at 90 days of degradation.

The initial lignin content of the twigs of *A. dealbata* was 36.10%. After 30 days of treatment with fungi, the lignin content decrease ranged from 27.15 to 33.2% and after 90 days of degradation the lignin content decrease ranged from 22.5 to 27.2% when compared with the initial content 34.22%. As far as the bark is concerned the initial lignin content was 71.24%, after 30 days of treatment with fungi. The decrease in lignin content ranged from 59.0 to 68.4% and after 90 days of degradation the lignin content was decrease ranged from 52.6 to 56.2% as against the initial content 68.9%.

Fungi require a carbon source, macronutrients such as nitrogen, phosphorous and potassium and certain trace elements for their growth. Carbon serves primarily as an energy source for the microorganisms, while a small fraction of the carbon in incorporated into their cells (Tuomela et al., 2000).

Biomass including leaf, twigs, bark and other residual materials of forest ecosystem, naturally undergo degradation due to fungal enzymatic action. This causes increase in CO₂ in the environment. Therefore, it would be better for

\[ W_2 = \text{Weight of oven – Dry sample after extraction with hot water (g)} \]

**Table 1. Lignin content of *Acacia dealbata* leaves, twigs and barks after treatment with selected fungi**

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Lignin content of Leaves (%)</th>
<th>Lignin content of twigs (%)</th>
<th>Lignin content of barks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Degradation period</td>
<td>30 days</td>
<td>90 days</td>
</tr>
<tr>
<td><em>Creolophus cirhatus</em></td>
<td></td>
<td>16.3 ± 0.55</td>
<td>13.8 ± 1.03</td>
</tr>
<tr>
<td><em>Hirschioporus abietinus</em></td>
<td></td>
<td>15.4 ± 0.48</td>
<td>11.7 ± 1.86</td>
</tr>
<tr>
<td><em>Schizophyllum commune</em></td>
<td></td>
<td>15.1 ± 0.42</td>
<td>11.2 ± 1.86</td>
</tr>
<tr>
<td><em>Stereum rugosum</em></td>
<td></td>
<td>17.2 ± 0.42</td>
<td>11.0 ± 1.61</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>19.1 ± 0.48</td>
<td>11.6 ± 1.52</td>
</tr>
</tbody>
</table>

Values are as Mean ± SD of 3 replicates

Control: Refers to No fungal treatment
Table 2. Holocellulose content of *Acacia dealbata* leaves, twigs and barks after treatment with selected fungi

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Holocellulose content of Leaves (%)</th>
<th>Holocellulose content of Leaves (%)</th>
<th>Holocellulose content of Leaves (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Degradation period</td>
<td>Degradation period</td>
<td>Degradation period</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>90 days</td>
<td>30 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Creolophus cirratus</em></td>
<td>16.4 ± 1.30</td>
<td>12.3 ± 0.96</td>
<td>31.5 ± 0.50</td>
</tr>
<tr>
<td><em>Hirschioporus abietinus</em></td>
<td>15.2 ± 0.48</td>
<td>11.8 ± 0.40</td>
<td>30.7 ± 0.53</td>
</tr>
<tr>
<td><em>Schizophyllum commune</em></td>
<td>16.1 ± 0.31</td>
<td>10.7 ± 0.53</td>
<td>30.2 ± 0.42</td>
</tr>
<tr>
<td><em>Stereum rugosum</em></td>
<td>14.8 ± 0.66</td>
<td>09.8 ± 0.42</td>
<td>29.1 ± 0.66</td>
</tr>
<tr>
<td>Control</td>
<td>17.4 ± 0.45</td>
<td>16.2 ± 1.22</td>
<td>33.7 ± 0.70</td>
</tr>
</tbody>
</table>

Values are as Mean ± SD of 3 replicates

Table 3. Weight loss of the treated samples after extraction with hot water for 3 h

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Hot water soluble content of Leaves (%)</th>
<th>Hot water soluble content of Leaves (%)</th>
<th>Hot water soluble content of Leaves (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Degradation period</td>
<td>Degradation period</td>
<td>Degradation period</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>90 days</td>
<td>30 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Creolophus cirratus</em></td>
<td>32.1 ± 0.42</td>
<td>61.3 ± 0.45</td>
<td>66.3 ± 0.45</td>
</tr>
<tr>
<td><em>Hirschioporus abietinus</em></td>
<td>33.3 ± 0.55</td>
<td>70.7 ± 0.02</td>
<td>61.8 ± 0.31</td>
</tr>
<tr>
<td><em>Schizophyllum commune</em></td>
<td>29.3 ± 0.53</td>
<td>60.5 ± 0.26</td>
<td>67.2 ± 0.48</td>
</tr>
<tr>
<td><em>Stereum rugosum</em></td>
<td>31.8 ± 0.40</td>
<td>71.2 ± 0.41</td>
<td>60.5 ± 0.40</td>
</tr>
<tr>
<td>Control</td>
<td>28.5 ± 0.50</td>
<td>52.0 ± 0.60</td>
<td>53.8 ± 0.40</td>
</tr>
</tbody>
</table>

Values are as Mean ± SD of 3 replicates

Control: Refers to No fungal treatment
the woody materials to be recycled by biological degradation or removal of lignin (Watanable et al., 2003).

In this study, it was found that lignin content of either leaf, twigs or bark degraded effectively by naturally occurring lignin degrading fungi of *A. dealbata* forest ecosystem. Lignin a heterogeneous plant cell wall biopolymer consisting of phenyl-propanoid units and the principal source of aromatic compounds found in nature, is extremely resistant to attack by most microorganisms (Thomela et al., 2000; Dekker et al., 2002). Lignin is a branched polymer of substituted phenylpropane units joined by carbon and ether linkages. Lignin is polymerization pattern and assembly is guided by the orientation of cellulose and the structure of hemicelluloses (Levine et al., 2001). However, the decomposition of woody materials could be indicated by measuring the lignin-degrading properties of fungi (Saparrat et al., 2008).

Fackler et al. (2006) stated that delignification was significant after 3 days of treatment with fungi and the activities of extracellular liginolytic enzymes (laccase, manganese peroxidase and/or lignin peroxidase) could be detected in fungal cultures. The present study leaves that the reduction of lignin content is
2 to 4% after 30 days and 3 to 5% after 90 days. Those results was much lower than that of Osono et al. (2003) who reported that lignin weight loss by Basidiomycetes could range from 23.7 to 39.6%, but slightly higher than lignin content in Quercus litter that only 8% after exposure for 3 years (Fioretto et al., 2005). Vargas-Garcia et al. (2007) reported that lignin degradation by Bacillus licheniformis activity was reached up to 68%. Osono and Takeda (2006) stated that mass loss of lignin in Abies ranged from 4.2 to 36.0% at 20°C and from 0.9 to 13.3% at 10°C and that in Betula ranged from 1.9 to 72.8% at 20°C and from 20.4 to 32.9% at 10°C. These variations due to the fungi used to degrade lignin were not as powerful on wood meal as in the previous study which was capable to degrade 14.6 to 24.9% of lignin content on the sample (Djarwanto and Tachibana, 2009). It is to be noted that the fungi involved in the lignin degradation are also different from those who worked on such degradation.

**Ability of the fungi to degrade holocellulose**

The holocellulose content of *A. dealbata* leaves, twigs and bark are shown in Table-2. As mentioned in lignin degradation, holocellulose content was measured after incubation of four fungi for 30 to 90 days. The initial holocellulose content of *A. dealbata* leaves was 17.43%. After 30 days of treatment, the holocellulose content decreased to 14.8 to 16.4%, depending on the fungal genera (Table-2). The genera Stereum rugosum was most effective (14.8) at 30 and 90 days. The initial holocellulose content of the leaves are 16.20%. After 90 days of treatment with fungi, the lignin content decreased to 9.8 to 12.3% of degradation.

The initial holocellulose content of the twigs was 33.7%. After 30 days of treatment with fungi, the holocellulose content decreased ranged from 29.1 to 31.5% and the genera S. rugosum was most effective (29.1) in degradation at 30 and 90 days. The initial holocellulose content of twigs was 30.2%. After 90 days of treatment with fungi, the decrease in holocellulose content ranged from 22.8% to 28.7%. The initial holocellulose content of bark was 53.8%. After 30 days of treatment, the decrease in holocellulose content ranged from 47.8 to 51.2% and the genera S. rugosum was most effective (47.8) in degradation. After 90 days of treatment the holocellulose content decrease ranged from 38.7 to 44.7% when compared with initial level of 49.7% and the genera *S. commune* was more effective (38.7%).

The holocellulose content of leaves, twigs and barks of *A. dealbata* also decreased depending on the fungal genera inoculated with the materials (Table-2). Besides lignin, the main components of organic matter are holocellulose, protein and lipids. Lignocellulose contain around 40% cellulose, 20% hemicellulose and 20-30% lignin (Tommel et al., 2000). Bark accounts for 10-20% of woody plants and is composed of a variety of biopolymers including polysaccharides, lignin, suberin and tannins (Vane et al., 2006). Holocellulose is the main polymeric component of the plant cell wall, the most abundant polysaccharide and an important renewable resource. In the present study the holocellulose content of leaves, twigs and barks of *A. dealbata* decreased due to the degradation of fungi inoculated, depending on the fungi the degradation percentage varied as reported by Btanchette (1995). The fungal capability in degrading holocellulose of *A. mangium* leaves and twigs 0.8% to 11.0% (Djarwanto and Tachibana, 2009). Osono and Takeda (2006) stated that mass loss of holocellulose or often called as total carbohydrate in *Abies* ranged from 2.5 to 16.6% at 20°C and from 0.0 to 13.2% at 10°C and that in *Betula* ranged from 2.3 to 66.8% at 20°C and from 6.4 to 48.1% at 10°C.

**Hot water soluble content of treated leaves, twigs and barks**

The volume of the soluble content of *A. dealbata* leaves, twigs and barks in hot water is shown in Table-3. It was found that the duration of inoculation in all the samples tested in this study increased in solubility in hot water. The value of soluble content varied depending on the fungi inoculated. For leaves, the value of soluble content was 29.35 to 33.38% as against the control sample 28.5% in 30 days. At 90 days, the value of soluble content was 60.5 to 71.24% when compared with control 52.0%.

In case of twigs, the percentage was between 60.5 to 67.2 as against 53.8 for control in 30 days. In 90 days the results are between 74.3 to 81.5% when compared with control 70.78%. For bark samples the percentage of soluble content was 62.30 to 66.70% (Control 62.9%) in 30 days. At 90 days the value of soluble content was 78.05 to
81.3% as against the control 76.20%.

In this study the hot-water solubility of treated samples increased significantly with incubation time meaning that some amount of lignocellulose content was degraded, presumably supported by the monosaccharides in A. dealbata sample like xylose, mannose and glucose (Pinto et al., 2005) which are soluble in water, besides the degradation of cellulose containing polymers and polysaccharides into simpler components like monomers through fungal activity (Blanchette et al., 1994). In both 30 days and 90 days of incubation, the hot water soluble content of all treated samples increased. Perhaps the soluble matter was consumed for energy by the fungi, since lignin and holocellulose content were less decreased.

REFERENCES


