

Biodegradation of Woody Substrata by Common Species of Wood Rotting Fungi of Meghalaya

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Abstract—Four commonly occurring wood rotting fungi of Meghalaya were selected to study their efficacy of decomposition of two common woody substrata *Pinus kesiya* and *Michelia champaca* under laboratory conditions. The assessment of the degree of their effectiveness was done on woodblocks over a period of 300 days. It was observed that maximum weight loss was affected by *Trametes versicolor* followed by *Earliella scabrosa*. *Polyporus brumalis* and *Hirschioporus abietinus* shows lesser efficacy in the decomposition of the wood structural components. The assessment of the degree of their effectiveness with respect to lignin degradation, moisture content change and pH change was also done. The study also demonstrated a modified method of studying the efficacy of biodegradation of the wood rotting fungi on natural wood blocks under laboratory conditions.

Keywords: Bio-degradation; bio-deterioration; decay potential; decomposition; wood rotting fungi

I. INTRODUCTION

Fungi are very important agents of bio-deterioration as they are active in the breakdown of plant materials, especially cellulose and lignin [1]. Wood decaying or wood rotting fungi belong mainly to the group of Basidiomycetes. These organisms are of particular interest not only in their own right, but also they are crucial to the creation of habitat for other organisms and for nutrient and carbon cycling [2]. They invade wood cells and degrade cell wall components resulting in detrimental effects on strength and other wood properties [3].

The major structural elements of wood are cellulose, hemicellulose and lignin. Lignin provides strength and rigidity in wood cell walls. Lignin is a water-impermeable aromatic polymer found in all higher plants and serves as a barrier against microbial attack [4]. Wood rotting fungi are so far the only organisms capable of degrading lignin. Lignin is degraded by white rot, brown rot and soft rot fungi. White rot fungi are able to degrade both carbohydrates and lignin, whereas brown rot fungi and soft rot fungi prefer cellulose and hemicellulose as substrates [5].

Decay study of woody substrate by fungi using percentage weight loss has been used. [6]. Weight loss measurement using wood block assay method has been employed to measure the decay potential of wood-decaying fungi by others [7]. A glass Kolle flask is used

according to the European standard EN 113 [8] to determine the toxic values of wood preservatives against wood destroying basidiomycetes cultured on agar medium [9]. The method can be also used to test the natural durability of timber species etc., [10]. The technique of sterilization of wood by autoclaving has been commonly used by several workers [11].

The study was conducted to examine the efficacy of biodegradation of wood of four commonly occurring wood rotting fungi *Trametes versicolor* (L.: Fries) Pilát, *Hirschioporus abietinus* (Dicks. ex Fr.) Donk, *Polyporus brumalis* (Pers.) Ex. Fries., and *Earliella scabrosa* (Pers.) R.L. Gilbertson and Ryv., of Meghalaya. The wood substrata used were woodblocks of *Pinus kesiya* and *Michelia champaca*, one of the most common sources of woody substrata in forests and important sources of timber in Meghalaya.

II. MATERIAL AND METHOD

A. Pure Cultures of Common Wood Rotting Fungi

The pure cultures of the common wood rotting fungi of the region *Trametes versicolor*, *Hirschioporus abietinus*, *Polyporus brumalis* and *Earliella scabrosa* were maintained in the laboratory using 2% malt extract agar (MEA) medium [12]. The pure cultures were then transferred to 250 ml conical flasks under aseptic conditions in a laminar flow chamber and sealed with sterilized cotton wool and aluminum foil. They were then allowed to grow under controlled temperatures of 25±2 °C in a culture room for 5 days or more, until the medium was well covered with the fungal mycelia.

1) Composition of malt extract agar medium (MEA; Difco)

Malt Extract -20 g, Agar - 15 g, Dextrose - 10 g, Peptone - 5 g, Distilled Water - 1000 ml, Streptomycin - 0.5 mg,

B. Wood Block Assay for Estimation of Efficacy of Decomposition

The method is based on that of Cartwright and Findlay [13], Chee *et al.* [7], Fryar *et al.* [11] and the European standard EN 113 [8] and modified as necessary.

1) Wood sampling and treatment of wood

Experimental woodblocks (1.5 x 1.5 x 1.5 cm) were prepared from the outer heartwood (adjacent to sapwood) of matured and carefully selected 25-30 year old *Pinus kesiya* Royle ex. Gordon., and *Michelia champaca* L. trees. The wood blocks with knots, visible decay, stains, etc., were rejected. Each wood block was then weighed and serially numbered for easy identification. The wood blocks of *P. kesiya* weighed 1.46 g (\pm 0.14) and *M. champaca* weighed 1.74 g (\pm 0.13) respectively. The woodblocks were sterilized carefully to avoid contamination. They were oven dried at 110°C for 24 hrs, each wood block was then weighed, marked and then immediately kept inside carefully sealed conical flasks and sterilized in an autoclave for 2 hrs and kept ready for transfer to the conical flasks containing the cultured test fungi.

2) Inoculation of woodblocks with wood rotting fungi

The sterilized wood blocks were aseptically transferred to the conical flasks containing pure cultures of fungal mycelium of the wood rotting fungi *Trametes versicolor*, *Hirschioporus abietinus*, *Polyporus brumalis* and *Earliella scabrosa*. A control set was also maintained as sterilized woodblocks in MEA medium only. Five woodblocks each was introduced into the Control sets and the conical flasks containing the MEA medium and the test fungi growing on it. The flasks were incubated at 25 \pm 2 °C for 300 days. Sampling was done at 30-day intervals. On sampling, the woodblocks were taken out from the flasks and superficial fungal mycelium on the woodblocks was carefully scraped off without damaging them.

C. Weight Loss

The initial oven-dried weight (W_1) of each woodblock before inoculation was recorded. After inoculation and incubation, the fungus covering each woodblock was carefully removed and the block was again oven dried at 110°C for 24 hrs to get the final weight (W_2).

The percentage weight loss was then calculated as follow [6]:

$$\text{Percentage weight loss} = [(W_1 - W_2) / W_1] \times 100$$

where, W_1 = Initial weight
 W_2 = Final weight

D. Lignin Content

The decayed wood blocks from treated and control sets were then ground to powder in Wiley Mill Grinder for the estimation of the lignin content as outlined by Peach and Tracy [14]. 0.3 g of the powdered sample was taken from each sample into a 250 ml conical flask. 25 ml of 72% concentrated sulphuric acid (H_2SO_4) was added to each replicate and kept in deep freeze for 24 hrs. It was then filtered in a Whatman No. 1 filter paper

and the residue left in the filter paper was washed several times with tap water to remove the last traces of sulphuric acid (H_2SO_4). The residue was then washed finally into a watch glass, oven dried and weighed. The amount obtained was recorded as total lignin.

E. Wood Moisture Content

The proportional wood moisture content u , expressed as a percentage is determined gravimetrically by measuring the wood mass before and after drying a wood sample at 103 \pm 2°C [10]:

$$u (\%) = [(W_1 - W_2) / W_2] \times 100$$

Where, W_1 = Mass of wet wood, W_2 = Mass of dried wood

F. Wood pH

The procedure of pH determination was adopted from TAPPI with slight modification [15]. The decayed wood blocks from treated and control sets were grinded to small pieces in a Wiley Mill Grinder for the measurement of the pH. 1g each of the grinded wood pieces was then transferred into a 100-ml beaker and distilled water (pH~ 6.7) was added until the specimens were wet. Distilled water was added again to bring the total volume to 70-ml. The mixture was stirred well and allowed to soak for 1 hour at room temperature. A battery powered pH meter (Fisher Scientific, Accumet 1003) was used for the measurement.

III. RESULTS AND DISCUSSION

A. Weight Loss

Weight loss was observed in both the woodblocks inoculated with the wood rotting fungi. It was observed that maximum weight loss was affected by *Trametes versicolor* on both the woodblocks where it was 67.24 \pm 0.94% on the woodblocks of *Pinus kesiya* (Fig. 1a) and 34.53 \pm 0.67% on *Michelia champaca* (Fig. 1b) at 300 days. *Earliella scabrosa*, *Polyporus brumalis*, and *Hirschioporus abietinus* also showed weight loss where it was 45.07 \pm 1.67% and 24.35 \pm 0.46%, 28.08 \pm 1.89% and 12.34 \pm 0.25%, and 13.36 \pm 1.79%, 8.57 \pm 0.40% for the woodblocks of *P. kesiya* and *M. champaca* respectively at 300 days. The control replicates also showed minute weight loss with a maximum of 8.72 \pm 2.19 and 8.22 \pm 0.08 on the test woodblocks of *P. kesiya* and *M. champaca* respectively after 300 days of the incubation period.

This is in conformity with those observed where weight loss was recorded from twenty isolates of basidiomycetes [7]. It has also been observed that in a very late stage of attack by wood decaying fungi, a wood mass loss of up to 97% was measured [16]. It has been observed that the ability to cause weight loss in intact wood is a safe indication of the ability of test organisms to degrade lignin [17].

B. Lignin Content

All the wood rotting fungi showed a decrease in the lignin content in both the wood blocks. The initially lignin content of the woodblocks was $15.24 \pm 0.15\%$ for *P. kesiya* and $12.64 \pm 0.09\%$ for *M. champaca*. A decrease in lignin content of the wood was observed with increase in time (Fig.2a and 2b). Maximum decrease in lignin content after 300 days was observed in *T. versicolor* which showed significant decrease as shown by the remaining lignin content of $6.31 \pm 0.48\%$ for *P. kesiya* (Fig.2a) and $8.21 \pm 0.43\%$ for *M. champaca* (Fig.2b). A significant decrease was also recorded with *E. scabrosa* with mean values of $7.26 \pm 0.23\%$ and $7.49 \pm 0.20\%$ for *P. kesiya* and *M. champaca* respectively. *P. brumalis* shows a decrease of $9.78 \pm 0.24\%$ and $8.51 \pm 0.31\%$ on *P. kesiya* and *M. champaca*, respectively and least decrease was observed in *H. abietinus* with $10.86 \pm 0.26\%$ and $8.51 \pm 0.31\%$ on *P. kesiya* and *M. champaca* respectively. Control samples did not show any significant change.

The lignin degrading property of several wood rotting fungi has been recorded and studied [18]. It was found that the selected species exhibit such similar properties. It has been found that the correlation between cultural studies and field studies with wood decay fungi was generally good [19]. This may be due to the relative bulk, durability and relatively stable micro-environmental conditions of woody resources [3].

C. Moisture Content

The moisture content of the wood showed increasing trend with time (Fig.3a and 3b). The

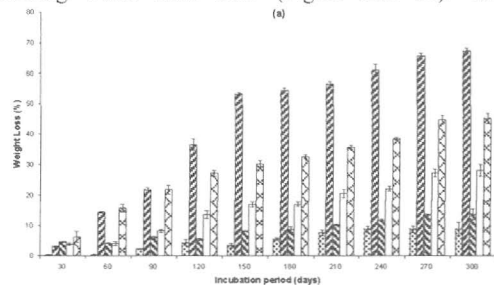


Fig. 1a

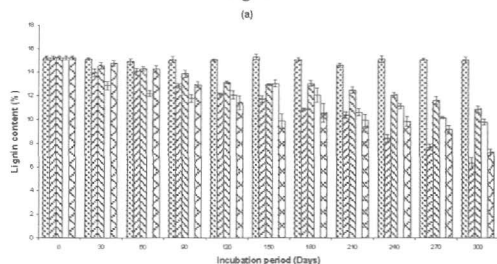


Fig. 2a

moisture content after 300 days was maximum for *T. versicolor* with $82.11 \pm 1.55\%$ for woodblocks of *P. kesiya* (Fig. 3a) and $75.93 \pm 0.66\%$ for woodblocks of *M. champaca* (Fig.3b). *E. scabrosa* was the next species with mean values of $71.08 \pm 0.66\%$ and $66.63 \pm 0.99\%$ followed by *P. brumalis* with $67.27 \pm 0.88\%$ and $48.17 \pm 1.19\%$, *H. abietinus* with $63.81 \pm 0.73\%$ and $53.87 \pm 0.70\%$ for *P. kesiya* and *M. champaca* respectively. The percentage moisture content for control sets was $57.10 \pm 0.56\%$ for *P. kesiya* and $42.68 \pm 1.49\%$ for *M. champaca*.

Wood moisture is the most important factor influencing wood decay by fungi [10]. It has been found that the optimum wood moisture content (u , %) for several wood rotting fungi ranged from 34% - 210% [20]. The increase in moisture content may be influenced to a considerable extent by the test fungi because of changes in the structure of the woodblocks. It has been observed that wood moisture content increased considerably with intensity of wood degradation by white-rot fungi [21].

D. Wood pH

The pH of the wood showed a tendency towards acidity (Figures 4a and 4b). In case of woodblocks treated with *T. versicolor*, the pH at 300 days was 3.70 ± 0.10 for *P. kesiya* and 4.24 ± 0.23 for *M. champaca*. The pH at 300 days was 3.32 ± 0.12 and 3.92 ± 0.20 for *E. scabrosa*, 4.20 ± 0.20 and 4.53 ± 0.17 for *P. brumalis*, 6.14 ± 0.34 and 6.52 ± 0.29 for *H. abietinus* and 5.9 and 6.2 for the control sets with woodblocks of *P. kesiya* (Figure 4a) and *M. champaca* (Figure 4b), respectively.

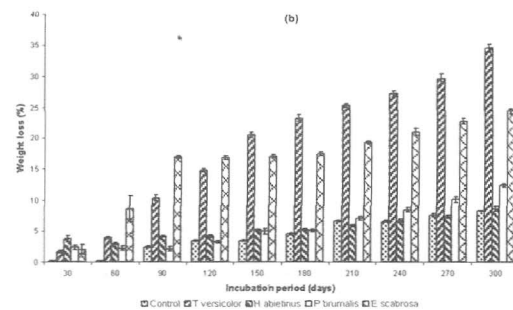


Fig. 1b

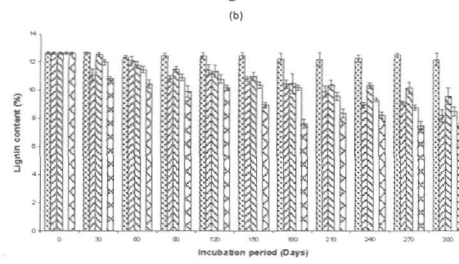


Fig. 2b

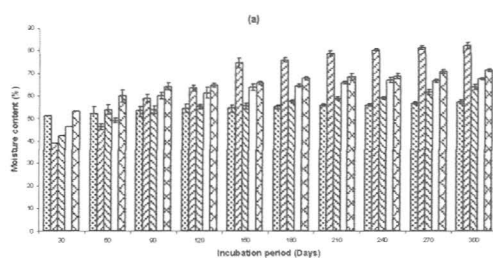


Fig. 3a

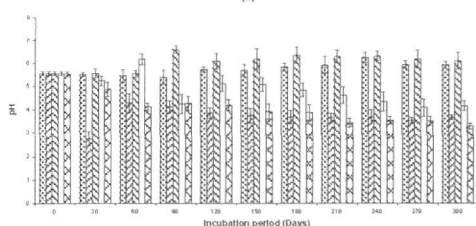


Fig. 4a

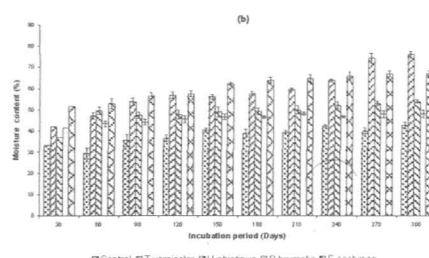


Fig. 3b

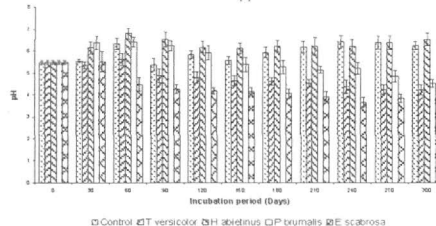


Fig. 4b

Fig. 1a-4b: Change in Weight loss (1a, 1b), Lignin Content (2a, 2b), Percentage Moisture Content (3a, 3b) and Wood pH (4a, 4b) Affected by the Wood Rotting Fungi *Trametes versicolor*, *Hirschioporus abietinus*, *Polyporus brumalis* and *Earliella scabrosa* on Woodblocks of *Pinus kesiya* (1a, 2a, 3a, 4a) and *Melichia champaca* (1b, 2b, 3b, 4b).

In general, the pH of hardwoods varies between 2.8 - 6.8, while it varies between pH 2.7- 8.8 in softwoods [22]. The pH of wood depends to a considerable degree on the presence of volatile acids, the most important of which are acetic and formic acids whose content in living trees may exceed 0.4% per unit of dry weight [23]. Because of the presence of free acids, the pH of fresh wood is often quite low. Fungi have been known to alter their microenvironment to meet their requirements. It has also been revealed that a reduction in the pH of their microenvironment by the ligninolytic fungi is thought to favour the activity of non-enzymatic systems and cellulolytic enzyme activity [24]. The deviation in pH in both the inoculated woodblocks of *P. kesiya* and *M. champaca* from those of the control samples may be attributed to such reasons.

IV. CONCLUSION

In conclusion, it can be asserted that the modified method of the wood block assay for efficacy of decomposition of woody substrata gives a positive result. The different test fungi showed their potential as biodegraders of woody substrata with *Trametes versicolor* as the species showing the highest activity among the four test fungi.

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