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Evaluation of lignocellulolytic fungus, *Phanerochaete chrysosporium* as a potential agent for decomposition of *Macaranga triloba* logs in peat eco-system

(Penilaian kulat lignoselulolitik *Phanerochaete chrysosporium* sebagai agen berpotensi untuk pereputan kayu *Macaranga triloba* dalam eko-sistem tanah gambut)

M.S. Umi Kalsom*, A.B. Ismail*, A. Mohamad**, M. Suhaimi*, A.M. Sahilah* and M. Abd. Zalal*

Key words: wood decomposition, peat ecosystem, lignin, cellulose, hemicellulose, *Phanerochaete chrysosporium*

Abstract

A field study was conducted at MARDI Peat Research Station, Sessang, Sarawak, to evaluate the potential of using lignocellulolytic fungi, *Phanerochaete chrysosporium*, as a biological agent to enhance the decomposition of *Macaranga triloba* (Mahang) logs. The logs were treated as follows: 1) inoculated wood above ground (IWAG), 2) uninoculated wood above ground (UWAG), 3) inoculated wood below ground (IWBG), and 4) uninoculated wood below ground (UWBG). After 12 months, the total weight loss was 67, 62, 43 and 35% for IWAG, UWAG, IWBG and UWBG, respectively. The respective lignin loss was 9.6, 6.3, 3.0 and 2.1%; cellulose loss was 17.5, 15.7, 13.8 and 12.5%; and ash content was 3.0, 2.6, 2.2 and 1.7%. The relative decay rates after 3 months were higher (3.2, 3.0, 2.9 and 2.6 mg/g/d, respectively) as compared to that of after 12 months (1.8, 1.6, 1.2 and 1.1 mg/g/d, respectively). The C:N ratio after 12 months was reduced by 55, 51, 32 and 17%, respectively. The study indicated that under natural condition, the introduced fungus, *P. chrysosporium* slightly enhanced wood decomposition in peat eco-system.

Introduction

There are 2.6 mil. ha of peat land in Malaysia, accounting for about 8% of the total land area. Of these, 1.66 mil. ha is found in Sarawak, 0.8 mil. ha in Peninsular Malaysia and 86,000 ha in Sabah (Mutalib et al. 1991). More than 360,000 ha of these areas has been developed, mainly for agriculture. With improved knowledge and the development of new technologies, more areas are expected to be cleared for development.

The development of peat land involves land clearing, which is often implemented by the 'slash-and-burn' method. The practice potentially contributes significantly to air pollution, which on a large scale can result in severe haze problems. In addition, burning of peat also releases more than 80 gaseous compounds of which some are

*Strategic Resources Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia

**MARDI Station, Sessang, P.O. Box 59, 95400 Saratok, Sarawak, Malaysia

Authors' full names: Umi Kalsom Md. Shah, Ismail Abu Bakar, Mohammad Abdullah, Suhaimi Masduki, Sahilah Abdul Mutalib and Abdul Zalal Mahat

E-mail: umishah@mardi.my

toxic, such as aliphatic and aromatic hydrocarbons, furfurals and organic acids containing cancer-causing substances (Okazaki et al. 1999). To avoid these potential hazards, zero burning land clearing is an alternative recommended and currently being practised by some operators. This method, however, leaves behind large amounts of woody debris, which occupy the potential cultivable areas.

Studies at MARDI Peat Research Station at Sessang, Sarawak, indicated that land clearing resulted in about 2,277 m^3/ha of forest debris, occupying 13–17% of the cleared area (Mohammad and Ooi 2001). The volume of the woody debris larger than 1.5 cm was about 169 m^3/ha, with an estimated weight of about 123 x 10^3 kg/ha, and more than half with the sizes of less than 15 cm.

This paper discusses a study conducted to find the possibility of enhancing decomposition of woody materials on the field by inoculation with Basidiomycetes fungus, *Phanerochaete chrysosporium.*

Materials and methods

The study was carried out at the MARDI Peat Research Station, Sessang, Sarawak, which covers 387 ha of intensively logged-over forest sitting on deep peat that ranges from 0.5 m to more than 5.0 m thick (MARDI 1996). The area has been partially drained since early 1990s for adjoining oil palm plantation. Plant diversity in the area was fairly high, with 148 species representing 66 families and 91 genera (Salma et al. 2003). The most common top-level tree species identified was *Macaranga* spp. (mahang), reaching heights of 20–25 m. The sub-tree layer, standing 10–20 m in height, consisted of common species such as *Blumeodendron tokbrai* (merbulan), *Eugenia* spp. (kelat), *Diospyros* spp. (kayu arang), *Litsea* spp. (medang), *Pometia pinnata* (kasai) and *Xylopia corrifolia* (jangkang paya). There were also many species of shrubs and herbs.

The wood decomposition study was carried out on *Macaranga triloba*, which is the most common plant species found in the research station. Only logs with about 15 cm diameter were considered and cut at 30 cm long. The fungus used for the study was *Phanerochaete chrysosporium*, which was obtained from the Biomolecular Sciences Laboratories, University of Manchester Institute of Science and Technology, United Kingdom. The fungus was grown on Potato Dextrose agar (PDA), maintained by sub-culturing on PDA and stored at 4 °C. This fungus was chosen because it is well known to be efficient degraders of both cellulose and lignin (Vallim et al. 1998). The expression of α-galactosidase (enzymes hydrolyzing hemicellulose) gene by this fungus grown on mixtures of wood pulp and wheat bran has been reported (Umi Kalsom 2002). *Phanerochaete chrysosporium* has an optimum temperature tolerance of 37 °C which means it can grow on tropical wood piles which attain high ambient temperatures. This fungus is capable of efficient depolymerization and mineralization of lignin.

Field trial was conducted in a cleared area where six logs were stacked in three layers, put in nylon bags and placed on the ground as well as buried 30 cm below the surface. The logs were enriched and inoculated with the fungus by submerging in solution containing mixtures of 0.5% (w/v) K_2HPO_4, 1.0% $(NH_4)_2.SO_4$ and 0.5% yeast extract and 10^8 spores of *P. chrysosporium* per ml of solution. As control, logs without any treatment were similarly placed. As such, the logs were treated as follows: 1) inoculated wood above ground (IWAG), 2) uninoculated wood above ground (UWAG), 3) inoculated wood below ground (IWBG), and 4) uninoculated wood below ground (UWBG). The experiment was laid out in a completely randomized design with four replicates.

The logs were sampled and analysed at three months intervals for a period of one year. The wood logs were taken out from the

nylon bag and soil debris on the wood surface was cleaned. For each interval of sampling, quarter portion of each six logs in every one nylon bag was taken. These wood log portions were then chipped into small sizes and ground to 1 mm size. The following analysis was carried out to determine:

- The weight loss of the wood calculated from the difference in oven dry weight at the beginning and end of the experiment, expressed as percentage to initial weight.
- Relative decay rate (mg/g/d) estimated from the percentage weight loss per unit time.
- The ash content of the wood by heating 10.0 g of wood chips in a muffle furnace at 550–600 °C for 6 h and the solid residue remaining after complete combustion was measured on dry weight basis.
- The lignin content quantitatively measured using the Klason method (Kirk and Obst 1988) and the cellulose content using modified methods as described previously by Krusong et al. (1999). Briefly, lignin and cellulose were determined using a 72% sulfuric acid digestion. Residual tissue from the digest was considered lignin and mass loss during the digestion was considered cellulose.

Results and discussion

The first set of wood sampling was done three months after inoculation. Fungal white mycelia were observed around the wood samples inoculated with the fungus *(Plate 1).* Wood sampling was subsequently done every three months for a period of one year. The time course of wood degradation with and without inoculation with *P. chrysosporium*, placed above and below ground was shown in *Figure 1*.

Total weight, lignin and cellulose loss

The percentage of total weight loss of the log was increased with time. The IWAG showed a higher degradation, shown by

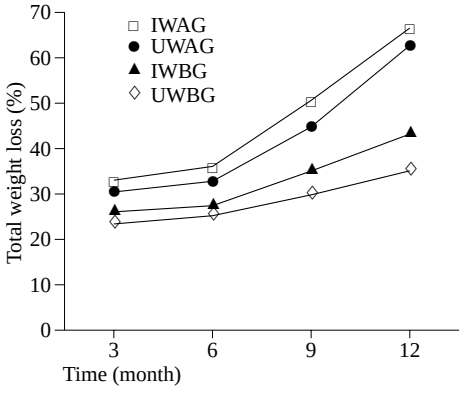

Figure 1. The time course of percentage total weight loss of wood with different types of treatment. All values are calculated from the mean of 4 replicates ± standard error of 2% of measured values

Plate 1. Inoculated wood with white fungal mycelia surrounding the wood logs (left), and uninoculated wood (right)

Table 1. Percentage of total weight, lignin and cellulose loss of wood with different types of treatment after 12 months. All values are calculated from the mean of 4 replicates with ± standard error

Treatment	Weight loss (%)	Lignin loss (%)	Cellulose loss (%)
Inoculated wood above the ground	66.6 ± 1.4	9.6 ± 0.3	17.5 ± 0.5
Uninoculated wood above the ground	62.3 ± 1.3	6.3 ± 0.2	15.7 ± 0.4
Inoculated wood below the ground	43.2 ± 1.2	3.0 ± 0.1	13.8 ± 0.3
Uninoculated wood below the ground	35.1 ± 1.0	2.1 ± 0.05	12.5 ± 0.3

higher percentage of total weight loss, as compared to UWAG, IWBG and UWBG. This indicated that *P. chrysosporium* produced the necessary enzymes that can break lignin and cellulose apart. It was reported that, the initial reactions are mediated by extracellular lignin and manganese peroxidases, produced by the fungus (Kirk and Farrell 1987). The fungus also shown to produce high activity of xylanase that hydrolyze hemicellulose which forms a major part of wood component (Umi Kalsom 2002).

The highest weight loss, lignin loss and cellulose loss occurred in IWAG, followed by UWAG, IWBG and UWBG. After 12 months inoculation, the percentage of wood degraded was 66.6, 62.3, 43.2 and 35.1%, respectively *(Table 1).* The trend was clearly seen in *Figure 2.* Respective lignin loss after 12 months was 9.6, 6.3, 3.0 and 2.1%. A similar trend was also found in the amount of cellulose loss. After 12 months the cellulose loss was 17.5, 15.7, 13.8 and 12.5%, respectively.

Other studies showed that lignin degradation of alder pulp by *P. chrysosporium* increased from 5.2–29.8% with addition of 0.12% nitrogen (dry weight basis) (Yang et al. 1980) whereas, the increase in lignin degradation of hemlock pulp with 0.12% supplemental nitrogen was only 2.2–3.9% and additional nitrogen did not provide further benefit. The differences between plant species are likely to be related to differences in lignin structure, with gymnosperm lignin composed of coniferyl alcohols, angiosperm lignin composed of both coniferyl and sinapyl alcohols, and

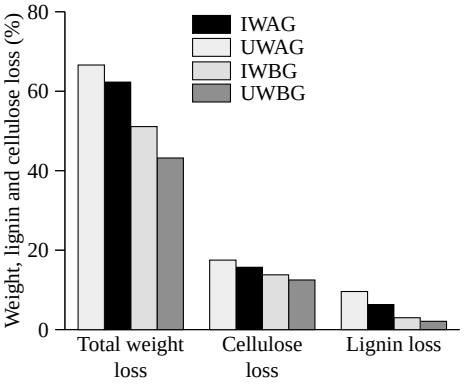

Figure 2. Percentage of total weight, lignin and cellulose loss of wood with different types of treatment after 12 months. All values are calculated from the mean of 4 replicates with ± standard error of 2% of measured values

grass lignin of coniferyl, sinapyl, and p-coumaryl alcohols (Ladisch et al. 1983).

The wood samples placed below the ground all showed a lower percentage of total weight, lignin and cellulose loss. A lower percentage of total weight, lignin and cellulose loss in wood placed below the ground were due to the lack of aeration below the ground. This fungus requires nutrient limitation to trigger ligninolytic activity, and it only reaches full degradative potential under oxygen enriched atmosphere (Aust 1990). The process of aerobic respiration uses atmospheric oxygen as a reactant. Many fungi have optimal growth rate at oxygen level between 19–20% of ambient air. Peat soil are very acidic in nature with pH ranging from 3.4–3.9, which again inhibits the fungal growth and hydrolytic enzyme activities as pH optimum for fungal growth and hydrolytic enzyme activities are in the range of pH 4.5–5.5.

Another factor affecting the rate of wood decomposition below the ground is soil moisture. In Sarawak, peat deposits are almost fully saturated with water, with moisture content generally ranging from 85–95% and the water table fluctuates up to 0.2 and 0.3 m above and below the ground respectively (Ong and Yogeswaran 1991). The optimum soil moisture content for wood decomposition to occur is at about 35%. Temperature also affects the many integrated metabolic activities of fungi such as digestion, assimilation, respiration, relocation and synthesis. The metabolic reaction rate increases with increase in temperature until the heat denatures enzymes required for growth. The optimum temperature range for *P. chrysosporium* growth is between 30–37 °C (Umi Kalsom 2002).

Relative decay rate

The time course of the wood relative decay rate is illustrated in *Figure 3*, showing the wood relative decay rate decreased with time. The wood relative decay rate was higher in the IWAG as compared to the UWAG, IWBG and UWBG. The relative decay rate measured after 3 months was higher (3.18, 3.0, 2.9 and 2.6 mg/g/d, respectively) as compared to the relative decay rate measured after 12 months (1.85, 1.60, 1.25 and 1.12 mg/g/d, respectively). At the early stage of wood decomposition, the fungi utilized many types of nitrogen, especially the amino forms and other simpler components such as sugars, protein and carbohydrates found in wood. The fungi affected several strength and mechanical properties of wood before significant weight loss was detected. The slow rate of wood decomposition at the later stage is due to the hemicellulose presence in the wood bind bundles of cellulose fibrils to form microfibrils and they cross-link with lignin, creating a complex web of bonds which provide structural strength, thus challenging degradation by microbes (Ladish et al. 1983). Although the breakdown of chemical compounds is specific to the original chemical composition of the wood, the decay process can be simply followed in the relative proportions of lignin and cellulose.

Yonebayashi et al. (1991) showed that readily decomposable organic matter, namely polysaccharides, tannin, hemicellulose and cellulose in peat soil were decomposed and converted into humic acids in the first year of reclamation whereas the lignin content in peat soil decreased very

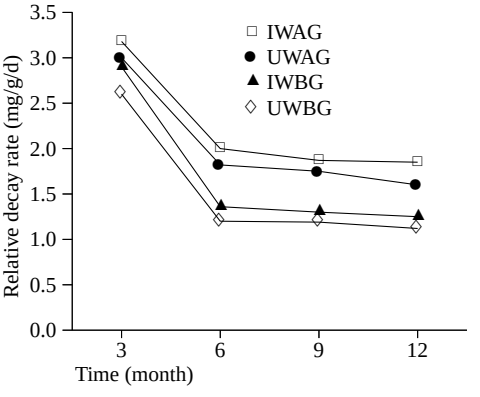

Figure 3. Relative decay rate of wood with different types of treatment after 12 months. All values are calculated from the mean of 4 replicates with ± standard error of 2% of measured values

Table 2. The ratio of bacteria:fungi:actinomycetes, ash content and relative decay rate of wood with different types of treatment after 12 months. All values are calculated from the mean of 4 replicates with ± standard error

Treatment	Bacteria:Fungi: Actinomycetes ($x10^{-5}$)	Ash content (%)	Relative decay rate (mg/g/d)
Inoculated wood above the ground	760:8:38	2.96 ± 0.08	1.9 ± 0.05
Uninoculated wood above the ground	190:15:47	2.63 ± 0.06	1.7 ± 0.05
Inoculated wood below the ground	70:14:1	2.20 ± 0.06	1.3 ± 0.03
Uninoculated wood below the ground	10:5:1	1.69 ± 0.04	1.1 ± 0.04

slowly with reclamation. *Table 2* showed the relative decay rate of wood with different treatments after 12 months. Wood inoculated with *P. chrysosporium* showed a higher relative decay rate. Other researchers also showed an enhanced rate of decomposition in plant biomass with fungal inoculation (Samada et al. 1995).

Since the study was carried out under natural environment, other natural soil microbes contributed to the process of degradation. *Table 2* showed the population ratio of bacteria:fungi:actinomycetes. The highest population of microbial ratio was found in IWAG (760:8:38) and UWAG (190:15:47) as compared to IWBG (70:14:1) and UWBG (10:5:1). Not surprisingly, the difference of total weight loss between IWAG and UWAG was only 4.3% i.e. 66.6% verses 62.3%. The natural soil microbes would also affect the relative decay rate of wood.

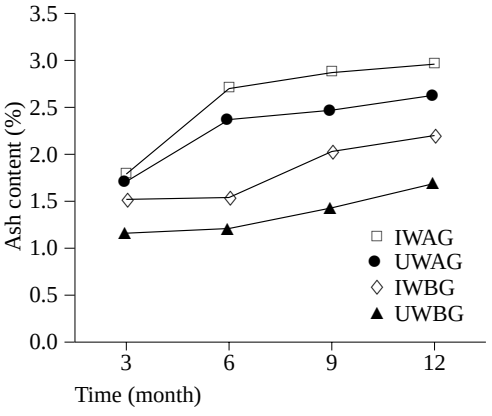

Figure 4. Ash content of wood with different types of treatment after 12 months. All values are calculated from the mean of 4 replicates with ± standard error of 2% of measured values

Ash content

A similar phenomenon was observed in the percentage of the ash content in the wood. The time course of ash content during the process of degradation is shown in *Figure 4*. The ash content was higher in IWAG, followed by UWAG, IWBG and UWBG. The ash content after 12 months was 2.96%, 2.63%, 2.20% and 1.69%, respectively *(Table 2).* The lower ash content in IWBG could be due to the poor aeration below the ground. Other studies also showed that lignin degradation is primarily an aerobic process, and in anaerobic environment lignin can persist for very long period (Van Soest 1994). Other factors which affect the lower ash content in wood placed below the ground were moisture, pH and temperature.

The initial ash content in the wood before incubation was in the range 1.3–1.5%. The higher ash content will indicate more organic matter has been degraded. The increase in ash contents reflected the occurrence of more decomposition. The percentage of weight loss increased with increased percentage of ash content *(Figure 5).* The overall ash content in peat soil is in the range of 2–5% and decomposed peat generally has ash content up to 25% (Esterle et al. 1991).

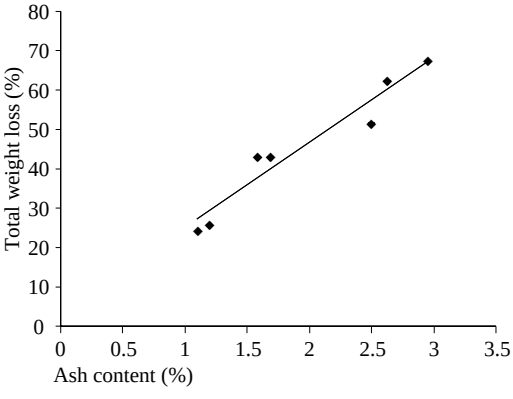

Figure 5. The relation between percentage of ash content and total weight loss. The percentage of weight loss increased with increased percentage of ash content

Carbon and nitrogen content

Table 3 showed the percentage of carbon, nitrogen and C:N ratio of degraded wood after 12 months. The lowest C:N ratio was recorded in IWAG (83:1), followed by UWAG (90:1), IWBG (125:1) and UWBG (152:1). The original C:N ratio of the wood before the experiment started was 183:1. As

Table 3. The percentage of carbon, nitrogen and C:N ratio of wood with different types of treatment after 12 months. All values are calculated from the mean of 4 replicates with ± standard error

Treatment	Carbon (%)	Nitrogen (%)	C:N ratio
Inoculated wood above the ground	45.43 ± 0.6	0.55 ± 0.1	82.6:1
Uninoculated wood above the ground	46.67 ± 0.5	0.52 ± 0.1	89.8:1
Inoculated wood below the ground	47.46 ± 0.9	0.38 ± 0.1	124.9:1
Uninoculated wood below the ground	48.74 ± 0.4	0.32 ± 0.2	152.3:1

such, the C:N ratio after 12 months was reduced by 55, 51, 32 and 17%, respectively.

Conclusion

The study indicated that the introduced fungus, i.e. *Phanerochaete chrysosporium*, slightly enhanced wood decomposition in peat eco-system. There were minimal differences in all the decomposition parameters analysed between the inoculated and uninoculated logs, both on and below the surface. For practical and economic reasons, therefore, this does not warrant it to be practised under natural condition in the open field. The effect is expected to be more pronounced under controlled environment, whereby substrate water potential, temperature, pH, quality and quantity of substrate and additional carbon source can be manipulated.

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Abstrak

Satu kajian telah dijalankan di Stesen Penyelidikan Tanah Gambut MARDI, Sessang, Sarawak, untuk menilai potensi menggunakan kulat *Phanerochaete chrysosporium* sebagai agen biologi untuk mempercepat proses pereputan kayu mahang *(Macaranga triloba).* Keratan batang kayu diberi perlakuan yang berikut: 1) disuntik dengan kulat diletak di atas tanah (IWAG), 2) tanpa suntikan diletak di atas tanah (UWAG), 3) disuntik dengan kulat diletak di bawah tanah (IWBG), dan 4) tanpa suntikan diletak di bawah tanah (UWBG). Selepas 12 bulan, peratus pengurangan jumlah berat masing-masing ialah 67, 62, 43 dan 35% untuk IWAG, UWAG, IWBG dan UWBG. Peratus pengurangan lignin masing-masing ialah 9.6, 6.3, 3.0 dan 2.1%; pengurangan selulosa masing-masing 17.5, 15.7, 13.8 dan 12.5%; dan kandungan abu masing-masing 3.0, 2.6, 2.2 dan 1.7%. Kadar pereputan bandingan yang diukur selepas 3 bulan adalah lebih tinggi (masing-masing 3.2, 3.0, 2.9 dan 2.6 mg/g/d) berbanding dengan kadar selepas 12 bulan (masing-masing 1.8, 1.6, 1.2 dan 1.1 mg/g/d). Nisbah C:N selepas 12 bulan pula masing-masing telah berkurangan sebanyak 55, 51, 32 dan 17%. Kajian ini menunjukkan pada persekitaran semula jadi, kulat yang diperkenalkan *P. chrysosporium* dapat meningkatkan sedikit pereputan kayu dalam eko-sistem tanah gambut.

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