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## Decomposition of Lignin and Holocellulose on *Acacia mangium* Leaves and Twigs by Six Fungal Isolates from Nature

<sup>1</sup>Djarwanto and <sup>2</sup>S. Tachibana

<sup>1</sup>Forest Products Research and Development Center-FORDA, Jl. Gunung Batu 5, Bogor, Indonesia

<sup>2</sup>Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime, 790-8566, Japan

**Abstract:** This research was conducted in the aim of preventing wild fire through reducing potential energy source to become *in situ* fertilizer. To prevent forest fires by reducing wood waste using lignocellulose-degrading fungi, six fungal isolates were tested for lignin and cellulose-degrading activity with *Acacia mangium* leaves and twigs over a period of 1 to 3 months. The fungi degraded 8.9-27.1% of the lignin and 14-31% of the holocellulose. The degradation rate varied depending on the fungal species. An increase in incubation time tended to decrease the amounts of holocellulose and lignin. However, the hot water soluble tended to increase following a longer incubation period. From the results obtained here, more time was needed to degrade lignin rather than other components in the sample.

**Key words:** Plantation forest, *Acacia mangium* leaf and twig, decomposition, fungi

### INTRODUCTION

Harvested wood is used for the manufacture of wood-based products. Logging operations in plantation forests usually generate abundant amounts of waste such as residual wood, branches/twigs, leaves and bark. The waste accounts for more than 60% of the total biomass (Kuhad *et al.*, 1997). Indonesia, now has planted tropical mangium over 1 million hectares which annual increment at operational scale 15-30 m<sup>3</sup>/ha/year (Cossalter and Pye-Smith, 2003).

In Indonesia, the pulp industry only uses wood from trees with a diameter greater than 10 cm. From industrial plantation forests encompassing 335,000 ha, the harvest will be 7.6 million m<sup>3</sup> per year. This generates waste such as wood from residual trees less than 10 cm in diameter and bark, as much as 1.5 million m<sup>3</sup> per year and 0.76-1.14 million m<sup>3</sup> per year. Anshori and Supriyadi (2001) alleged that the first cutting rotation (9 years) at a mangium plantation forest for pulp (193,500 ha) generated 15.18 t ha<sup>-1</sup> of bark, 31.43 t ha<sup>-1</sup> of wood, 4.01 t ha<sup>-1</sup> of leaves and litter and 5.84 t ha<sup>-1</sup> of stumps. Further, Muladi *et al.* (2001) asserted that the total wood biomass of mangium (*Acacia mangium*) stands at 5-7 years of age was about 60.5-95.8 t ha<sup>-1</sup>; while the corresponding figure for eucalypt (*Eucalyptus deglupta*) stands at 5-9 years was 45.7-116.4 t ha<sup>-1</sup>. The natural decomposition of waste is usually a slow process, resulting in the

accumulation of materials, which when become dry, are a potential risk for forest fires (Nakayama and Siebert, 2001).

Lignin and holocellulose in the litter structure are major energy sources available to decomposer organisms, constituting 70-80% of fresh organic material (Swift *et al.*, 1979). Lignin is the more recalcitrant plant polymer and its mineralization by white rot basidiomycetes plays a major role in carbon recycling (Martínez *et al.*, 2005). Abundant lignin in the forest site may take a role as an energy potential for combustion of fire. Lignin content has positive correlation with gross heat of combustion of wood (White, 1987), therefore it has correlation to the forest fire. By degrading the lignin content on woody material left in forest can hopefully reduce the latent combustibility accident to those materials.

White-rot fungi are wood-degrading organisms capable of decomposing all wood polymers; lignin, cellulose and hemicellulose (Hakala, 2007). Holocellulose, polysaccharide containing cellulose and hemicellulose (Pettersen, 1984) as a major component of wood is 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). Compared to other pretreatment

alternatives, fungal treatment takes a long time, but the energy requirement of the process is low and treatment conditions are mild (Sun and Cheng, 2002).

The introduction of saprophytic fungi may accelerate the breakdown of forest waste. It is necessary to seek particular fungi capable of decomposing waste. Waste from industrial plantation forests containing lignocellulosic substances can provide beneficial nutrients for saprophytic fungi (Alexopoulos *et al.*, 1979). Decaying fungi belong to saprophyte microorganisms, since they live on dead or residual vegetation, decomposing them into simpler molecular compounds (Dubeux *et al.*, 2006; Ohkuma *et al.*, 2001; Aumen *et al.*, 1983). Corresponding to their cosmopolitan-nature, saprophytic fungi can also grow in plantation forests. The use of indigenous saprophytic fungi can be expected to accelerate the decay of waste. Laboratory screening revealed that six fungi from planted forest areas were capable of degrading lignin as well as holocellulose in mangium wood meal, as much as 14-24 and 11-44%, respectively, depending on the fungal species (Djarwanto and Tachibana, 2009). The present study was carried out with the objective of studying saprophytic fungi capable to decompose lignocellulose waste from mangium plantation forests in a semi-laboratory stage as a way of using the fungi in remediate logging forest harvesting and in an effort to convert waste into bio-fertilizer, in the specific objective of studying to decompose lignin and holocellulose of *Acacia mangium* leaves and twigs.

## MATERIALS AND METHODS

This research project was conducted in the Faculty of Agriculture, Ehime University, Japan and the Forest Product Research Center in Indonesia during 2008 in an effort to find fungi to degrade lignocellulose waste from mangium plantation forests.

**Chemicals:** Acetic acid, agar powder, alcohol, benzene, malt-extract, sodium chlorate and sulfuric acid were purchased from Wako Pure Chemical Company Ltd., Japan.

**Leaves and twigs of *Acacia mangium*:** Sample materials in the form of leaves and twigs were collected from log over area of mangium plantation forest in South Sumatera, Indonesia. According to the sampling of harvesting wastes, found an average comparison part between barks, leaves and twigs was about 12 by 5 by 3 (w/w), respectively, in fresh condition.

**Source of fungal isolates:** Six kinds of fungi isolates were kept from the previous research (Djarwanto and

Tachibana, 2009), i.e., isolates 371 (*Polyporus* sp.), 368 (*Lentinus* sp.), 265 (unidentified), 346 (*Picnoporus* sp.), 345 (*Marasmius* sp.) and 338 (*Ganoderma* sp.) in form of mycelia. The fungi were replicated in malt-extract-agar medium for further use.

### **Decomposition-testing based on holocellulose and lignin**

**content:** Test fungi were selected with reference to Friedrich *et al.* (2007), Terron *et al.* (1995) and Tuomela *et al.* (2000). Test samples containing leaves and twigs of mangium were chopped, mixed with a 1% malt extract in distilled water to a water content of about 65% and packed in plastic bags (500 g for each sample pack with 3 replicates, then total sample were 42 plastic bags). They were then sterilized by an autoclave at a temperature of 121°C and pressure of 1.5 atm., for 30 min. After the medium had cooled, it was inoculated with the test fungi as much as 25 grams per bag and incubated for 1 and 3 months. For control samples was made of same materials with no fungal treatment.

**Determination of holocellulose content:** Holocellulose content was determined with reference to ASTM 1104-56 (1978), Reapproved. Initially, 1 g of sample was placed in an Erlenmeyer flask (300 mL) and 150 mL of distilled water was added. While slowly shaking, 1 g of NaClO<sub>2</sub> 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<sub>2</sub> and 0.2 mL of acetic acid were added and boiled 3 times. After cooling, the sample was filtered using a filter flask and washed with hot water until free of acid. Afterward, the insoluble portion was dried in an oven at 105°C for 4 h, cooled in a desiccator and weighed, repeatedly until obtaining a constant weight. Holocellulose content was calculated as follows:

$$\text{Holocellulose content (\%)} = \frac{\text{o.d. weight of holocellulose}}{\text{o.d. weight of initial sample}} \times 100$$

**Determination of lignin content:** The procedure was from TAPPI Standard method (2009) T 222 os-74. One gram of air-dried sample was weighed out accurately in a weighing bottle and transferred to a 50 mL beaker, then 10 mL of 72% sulphuric acid was added carefully with a pipette and the mixture was stirred with a small glass rod (which was left in the beaker). The mixture was moved quantitatively with a wash bottle (water) to a 500 mL round-bottle flask and diluted with water until the final volume was 300 mL. The solution was then refluxed for 3 h, filtered in a glass filter and dried in an oven at 105°C for 12 h. The crucible was cooled in a desiccator for 15 min and then weighed accurately. The glass filter containing the lignin was reported as a percentage by weight of the dried sample:

$$\text{Lignin content} = \frac{\text{o.d. weight of lignin}}{\text{o.d. weight of initial sample}} \times 100\%$$

**Determination of hot water soluble content:** The soluble of treated mangium leaves and twigs was examined with reference to ASTM D1110-84 (2007). A 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 fritted glass filter of known weight. The residue was washed with hot tap 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:

W1 = Weight of oven-dry test sample (g)

W2 = Weight of oven-dry sample after extraction with hot water (g)

## RESULTS

**Ability of the fungi to degrade lignin:** The lignin content of mangium leaves and twigs are shown in Table 1. For measuring lignin content, mangium waste was incubated with the six fungi for one to three months. The initial lignin content of the leaves was 38-44%. After one month, the lignin content had reduced to 8-10% (Table 1). Further, after three months, the lignin content of mangium leaves was reduced to 11-16%. As shown in Table 1, isolate 388 was the most powerful degrader of lignin (12%); while, isolate 345 was the weakest degrader (7%). It seems that untreated leaf matter also decreased with the duration of the incubation. In this study, lignin content remained high after 3 months of the testing period.

**Ability of the fungi to degrade holocellulose:** The holocellulose content of mangium leaves and twigs are shown in Table 2. As mentioned above, holocellulose content was measured after incubation of the six fungi for 1 to 3 month. The initial holocellulose content of mangium

leaves was 45.9%. After one month of treatment, the holocellulose content decreased to 35.5-45.1, depending on the fungal isolate (Table 2). Isolate 371 was most effective at degrading holocellulose in mangium leaves (10%), while isolate 265 was least effective (0.8%). Furthermore, after three months, isolate 371 had degraded 11% of holocellulose, while isolate 345 had degraded 1%.

**Hot water soluble content of treated leaves and twigs:** The volume of the soluble content of *Acacia mangium* leaves and twigs in hot water is shown in Table 3. Coincident with the duration of incubation, all the samples increased in solubility in hot water, except for the sample of twigs treated with isolate 388. The value of soluble content varied depending on the isolate i.e., 7-32% in leaves and 8-40% in twigs.

Table 1: Lignin content of mangium leaves and twigs after treatment with the selected fungi

Fungi	Lignin content of leaves (%)		Lignin content of twigs (%)	
	1 month	3 months	1 month	3 months
338	31.8±3.45	26.8±2.49	27.0±2.49	23.7±3.45
368	32.2±3.66	27.0±3.66	27.4±6.92	22.5±6.92
265	32.8±6.45	31.8±3.44	27.0±3.44	23.7±6.45
346	33.8±1.03	32.6±1.03	33.7±1.91	31.5±1.91
345	35.7±0.54	31.9±3.19	35.0±0.54	27.3±3.19
371	33.4±2.01	29.9±2.01	35.2±1.97	33.2±1.97
Control	43.6±5.45	38.1±5.45	44.2±7.55	36.6±7.55

Values are as Mean±SD of 3 replicates. Control means no fungal treatment

Table 2: Holocellulose content of mangium leaves and twigs after treatment with the selected fungi

Fungi	Holocellulose content of leaves (%)		Holocellulose content of twigs (%)	
	1 month	3 month	1 month	3 month
338	43.9±1.30	43.3±0.14	43.5±0.14	42.0±1.30
368	43.6±5.98	41.9±0.52	41.2±0.52	35.2±5.98
265	45.1±0.22	41.5±7.04	44.8±0.22	31.6±7.04
346	43.7±1.21	42.9±1.41	41.7±3.47	31.2(3.47
345	45.0±0.22	44.2±0.15	43.7±0.15	42.9±1.30
371	35.5±1.61	33.4±1.61	36.5±1.61	35.1±2.54
Control	45.9±1.85	44.6±0.43	45.2±0.43	42.9±0.12

Values are as Mean±SD of 3 replicates. Control means no fungal treatment

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

Fungi	Hot water soluble content of leaves (%)		Hot water soluble content of twigs (%)	
	1 month	3 month	1 month	3 month
338	19.0±0.91	29.1±3.24	26.2±3.67	10.4±3.02
368	19.2±1.08	24.3±2.02	19.7±0.68	28.9±2.81
265	10.7±2.01	26.3±0.96	8.4±1.37	24.3±1.12
346	19.8±1.68	23.4±2.0	18.2±0.80	30.0±3.47
345	19.5±0.90	24.3±3.06	18.6±3.05	32.1±3.18
371	7.9±0.86	32.7±1.86	17.8±3.08	40.4±1.22
Control	9.7±0.85	24.7±2.18	9.2±3.28	26.8±2.10

Values are as Mean±SD of 3 replicates. Control means no fungal treatment

## DISCUSSION

**Decaying ability of selected fungi on lignin:** As shown in Table 1, the lignin content of *Acacia mangium* leaves and twigs was reduced after incubation with the six fungi, respectively, for one and three months. The initial lignin content of the leaves was 38-44%. After one month, the lignin content reduced 8-10% (Table 1). Fackler *et al.* (2006) stated that delignification was significant after 3 days treatment with fungi and the activities of extracellular ligninolytic enzymes (laccase, manganese peroxidase and/or lignin peroxidase) could be detected in fungal cultures even at low levels. Meanwhile, in this study, lignin content was reduced to 8-10% after one month. Those result was much lower than Osono *et al.* (2003) reported that lignin weight loss by basidiomycetes could range from 23.7 to 39.6%, but slight 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. This means that 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). This showed that the extractive content of the mangium leaves has a negative effect on fungal growth. Stecher *et al.* (1968) reported that mangium trees produce acacic acid which functions as an allelopathic agent to protect against competition from other plant including as a fungal retardant. Further, after three months, the lignin content of mangium leaves was reduced to 11-16%. As shown in Table 1, isolate 388 was the most effective at degrading lignin in mangium leaves (12%); while, isolate 345 was least effective (7%). Isolate 388 is most likely more suitable to degrade leaves which are rich in nutrients rather than wood, even though it showed the weakest coloration on guaiacol medium (Djarwanto and Tachibana, 2009).

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 is incorporated into their cells (Tuomela *et al.*, 2000). Biomass including discarded wood materials, branches and roots of shade and forest trees and wood residues, intentionally or not, have been burned or degraded and returned to soil. This causes environmental problems such as increases in CO<sub>2</sub>, concomitantly with temperature increases or the accumulation of deleterious substances, influencing the

health of human beings and wild life. Therefore, it would be better for the woody materials to be recycled by biological degradation or removal of lignin (Watanabe *et al.*, 2003).

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 (Tuomela *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 leaf litter could be indicated by measuring the lignin-degrading properties of fungi (Saparrat *et al.*, 2008).

It seems that untreated leaf matter also decreased with the period of incubation, probably affected by wild decomposers including bacteria and fungi. It was found that the waste degradation rate varied between fungi. Blanchette (1995) reported differences in weight loss depending on the species of fungi used in decay tests and species of wood used as a substrate.

In this study, it was found that lignin content remained high after 3 months of the incubation. Berg and McLaugherty (2008) stated that in the later stages of decomposition, recalcitrant substances become more abundant and in some cases the rate of decomposition approaches zero. Therefore, in some cases, the concentration of certain component like lignin may increase in the residue of litter. One can not distinguish between what is respired as carbon dioxide and what is leached out of the litter or lost due to fragmentation. Moreover, Bernhard-Reversat and Schwartz (1997) found that the lignin content of fresh litter was lower than that of decomposed litter due to the cellulose and the extractive component was degraded faster and therefore lignin accumulated in the residue. In some cases, the decomposition rate of cellulose is higher than that of lignin (Fioretto *et al.*, 2005). During ligninolysis, white rot fungi excrete peroxidases, but there is no correlation in an analytical sense between peroxidase activity and the decrease in lignin content (Fackler *et al.*, 2006).

Decomposition is often inhibited during the dry season compared with the wet season in tropical seasonal forests (Swift and Anderson, 1989; Swift *et al.*, 1979), but in this study, a retarding effect was not obvious because at the time of the research there was no extreme limit in the field between dry and wet seasons. Lignin is less readily available to the decomposers than holocellulose and often retards the decomposition of litter (Fogel and Cromack, 1977; Berg *et al.*, 1984) because (1) lignin, an aromatic compound made up of phenylpropane-based monomers

linked via a variety of bonds, is highly refractory and persistent and the delignification depends on the availability of non-lignified carbon energy sources (Kirk *et al.*, 1976); (2) lignin forms a resistant shield around holocellulose to form lignocellulose in plant cell walls (Cooke and Whipps, 1993) and, as a consequence, most of the holocellulose in litter must be delignified for carbohydrate assimilation; and (3) lignin decomposition products may form stable nitrogenous compounds making nitrogen less readily available to decomposer organisms (Berg 1988). The lignocellulose index (LCI), reflecting the relative availability of holocellulose in the lignocellulose matrix, is thus suggested as a useful index of the availability of carbon energy sources to decomposer organisms (Berg *et al.*, 1984; Melilo *et al.*, 1989; Aber *et al.*, 1990).

**Ability of selected fungi to degrade holocellulose:** The holocellulose content of both leaves and twigs of *Acacia mangium* also decreased depending on the fungal isolate (Table 2). Besides lignin, the main components of organic matter are holocellulose, proteins and lipids. Lignocelluloses contain around 40% cellulose, 20% hemicellulose and 20-30% lignin (Tuomela *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). Cellulose is the main polymeric component of the plant cell wall and is the most abundant polysaccharide on earth (O'Sullivan, 1997), it is therefore a prime energy source for fungi.

Basidiomycetes are the most potent degraders of this polymer because many species grow on dead wood or litter, in environments rich in cellulose (Baldrian and Valaskova, 2008). Holocellulose is the main polymeric component of the plant cell wall, the most abundant polysaccharide and an important renewable resource. Cellulose contains both highly crystalline regions where individual chains are linked to each other and less-ordered amorphous regions. Although chemically simple, the intermolecular bonding pattern can result in a very complex morphology. As mentioned above, holocellulose content was measured after incubation of the six selected fungi for one to months (Table 2). The initial holocellulose content of mangium leaves was 45.9%. After one month of treatment, the holocellulose content decreased to 35.5-45.12%, depending on the fungi. Isolate 371 was the most effective at degrading holocellulose in mangium leaves (10%), while isolate 265 was the least effective (0.8%). After three months, isolate 371 had degraded 11% of the holocellulose; while isolate 345 had only degraded 1%. The fungal capability in degrading holocellulose of mangium leaves and twigs was lower than that of wood meal which reached up to 38% by isolate 371 (Djarwanto and Tachibana, 2009). Vargas-Garcia *et al.* (2007) reported

that *Brevibacillus parabrevis* able to reduce the concentration of cellulose 51%. Osono and Takeda (2006) stated that mass loss 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.

It seems that untreated leaf matter also reduced with the period of incubation (Table 3), probably affected by wild decomposers including bacteria and fungi from the environment. It was found that the waste degradation rate varied between fungi. Blanchette (1995), reported differences in weight loss depending on the species of fungi used in decay tests and species of wood used as a substrate.

**Hot water soluble content of treated leaves and twigs:** In this study, the hot-water solubility of treated samples increased significantly with incubation time which meaning that some lignocellulose content was degraded, presumably supported by the monosaccharides in mangium like xylose, mannose and glucose (Pinto *et al.*, 2005), which are soluble in water, beside the degradation of cellulose-containing polymers and polysaccharides into simpler components like monomers through fungal activity (Blanchette *et al.*, 1994). After one month of incubation, the hot water soluble content of all treated samples was increased, except for the sample treated with isolate 265 which was similar to that of the control sample due to slow growth of the fungi (Table 3). However, after three months, the hot water soluble content was increased significantly especially in leaves and twigs treated with isolate 371 (Table 3). The hot water soluble content of mangium twigs treated with isolate 338 was decreased after three months. The soluble matter was likely consumed for energy by the fungi, since the lignin and holocellulose content were less decreased. Isolate 371 seemed most capable of degrading leaves and twigs.

## CONCLUSION

The rate of degradation varied depending on the fungal species. An increase in incubation time tended reducing both lignin and holocellulose content. However, the reduction rate was not significant; therefore, more time may be needed to degrade lignin rather than other component in the sample.

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## REFERENCES

- Aber, J.D., J.M. Melillo and C.A. McLaugherty, 1990. Predicting long-term patterns of mass loss, nitrogen dynamics and soil organic matter formation from initial fine litter chemistry in temperate forest ecosystems. *Can. J. Bot.*, 68: 2201-2208.
- Alexopoulos, C.J., C.W. Mims and M. Blackwell, 1979. *Introductory Mycology*. 3rd Edn., John Wiley and Sons, New York, pp: 632.
- Anshori, S. and B. Supriyadi, 2001. Potency and management of logging residue of first rotation *Acacia mangium* in Musi Hutan Persada Ltd. Company. Proceedings of seminar Environment Conservation Through Efficiency Utilization of Forest Biomass, 2001, DEBUT Press. Jogjakarta, pp: 155-160.
- ASTM D1104-56, 1978. Method of test for holocellulose in wood. <http://www.astm.org/Standards/D1104.htm>.
- ASTM D1110-84, 2007. Standard Test Methods for Water Solubility of Wood. American Standard Test Methods. ASTM International, West Conshohocken, PA.
- Aumen, N.G., P.J. Bottomley, G.M. Ward and S.V. Gregory, 1983. Microbial decomposition of wood in streams: Distribution of microflora and factors affecting lignocellulose mineralization. *Applied Environ. Microbiol.*, 46: 1409-1416.
- Baldrian, P. and V. Valaskova, 2008. Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiol. Rev.*, 32: 501-521.
- Berg, B. and C. McLaugherty, 2008. *Plant Litter Decomposition, Humus Formation, Carbon Sequestration*. 2nd Edn., Springer-Verlag, Berlin Heidelberg, New York, ISBN: 978-3-540-74923-3, pp: 341.
- Berg, B., 1988. Dynamics of nitrogen (<sup>15</sup>N) in decomposing Scots pine (*Pinus sylvestris*) needle litter: Long-term decomposition in a Scots pine forest. VI. *Can. J. Bot.*, 66: 1539-1546.
- Berg, B., G. Ekbohm and C. McLaugherty, 1984. Lignin and holocellulose relations during long term decomposition of some forest litters: Long-term decomposition in a Scots-pine forest. IV. *Can. J. Bot.*, 62: 2540-2550.
- Bernhard-Reversat, F. and D. Schwartz, 1997. Change lignin content during litter decomposition in tropical forest soil (Congo): Comparison of exotic plantation and native stands. *Earth Planetary Sci.*, 325: 427-432.
- Blanchette, R.A., J.R. Obst and T.E. Timell, 1994. Biodegradation of compression wood and tension wood by white and brown rot fungi. *Holzforschung*, 48: 34-42.
- Blanchette, R.A., 1995. Degradation of the lignocellulose complex in wood. *Can. J. Bot.*, 73: 999-1010.
- Cooke, R.C. and J.M. Whipps, 1993. *Ecophysiology of Fungi*. Blackwell Scientific Publications, Oxford, UK, ISBN-10: 0632021683, pp: 337.
- Cossalter, C. and C. Pye-Smith, 2003. *Fast-Wood Forestry: Myths and Realities*. Center for International Forestry Research (CIFOR), Jakarta, Indonesia, ISBN 979-3361-09-3.
- Dekker, R.F.H., A.M. Barbosa and K. Sargent, 2002. The effect of lignin-related compounds on the growth and production of laccases by the ascomycete, *Botryosphaeria* sp. *Enz. Microbial. Technol.*, 30: 374-380.
- Djarwanto and S. Tachibana, 2009. Screening of fungi capable of degrading lignocellulose from plantation forests. *Pak. J. Biol. Sci.*, 12: 669-675.
- Dubeux, Jr. J.C.B., L.E. Sollenberger, S.M. Interrante, J.M.B. Vendramini and R.L. Jr. Stewart, 2006. Litter decomposition and mineralization in bahiagrass pastures managed at different intensities. *Crop Sci.*, 46: 1305-1310.
- Fackler, K., C. Grading, B. Hinterstoisser, K. Messner and M. Schwanninger, 2006. Lignin degradation by white rot fungi on spruce wood shavings during short-time solid-state fermentations monitored by near infrared spectroscopy. *Enzyme Microbial Technol.*, 39: 1476-1483.
- Fioretto, A., C. Di Nardo, S. Papa and A. Fuggi, 2005. Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. *Soil Biol. Biochem.*, 37: 1083-1091.
- Fogel, R. and K. Jr. Cromack, 1977. The effect of habitat and substrate quality on Douglas-fir litter decomposition in western Oregon. *Can. J. Bot.*, 55: 1632-1640.
- Friedrich, J., P. Zalar, M. Mohorcic, U. Klun and A. Krzan, 2007. Ability of fungi to degrade synthetic polymer nylon-6. *Chemosphere*, 67: 2089-2095.
- Hakala, T.K., 2007. Characterization of the Lignin-modifying Enzymes of the Selective White-rot Fungus *Physisporinus rivulosus*. Helsinki University Printing House, Helsinki, Finland, pp: 60.
- Kirk, T.K., W.J. Connors and G. Zeikus, 1976. Requirement for a growth substrate during lignin decomposition by two wood-rotting fungi. *Applied Environ. Microbiol.*, 32: 192-194.
- Kuhad, R.C., A. Singh and K.E.C. Eriksson, 1997. Microorganisms and Enzymes Involved in the Degradation of Plant Fiber Cell Walls. In: *Advances in Biochemical Engineering Biotechnology*, Eriksson, K.E.L. (Ed.). Vol. 57, Springerverlag, Germany, pp: 46-125.

- Levine, L.H., A.G. Heyenga, H.G. Levine, J.W. Choi, L.B. Davin, A.D. Krihorian and N.G. Lewis, 2001. Cell wall architecture and lignin composition of wheat developed in microgravity environment. *Physiochemistry*, 57: 835-846.
- Martinez, A.T., M. Speranza, F.J. Ruiz-Duenas, P. Ferreira and S. Camarero *et al.*, 2005. Biodegradation of lignocellulosics: Microbial, chemical and enzymatic aspects of fungal attack to lignin. *Int. Microbiol.*, 8: 195-204.
- Melilo, J.M., J.D. Aber, A.E. Linkins, A. Ricca, B. Fry and K.J. Nadelhoffer, 1989. Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. *Plant Soil*, 115: 189-198.
- Muladi, S., R. Amirta, E.T. Arung and Z. Arifin, 2001. Chemical component analysis of wood bark compost on waste of medium density fiberboard industry. *Proceedings of Seminar Environment Conservation Through Efficiency Utilization of Forest Biomass*, 2001, DEBUT Press. Jogjakarta, pp: 124-137.
- Nakayama, M. and F. Siegert, 2001. Comparative study on C and L Band SAR for fire scar monitoring. *Proceedings of 22nd Asian Conference on Remote Sensing*, Nov. 5-9, National University of Singapore, pp: 1-4.
- O'Sullivan, A.C., 1997. Cellulose: The structure slowly unravels. *Cellulose*, 4: 173-207.
- Ohkuma, M., Y. Maeda, T. Johjima and T. Kudo, 2001. Lignin degradation and roles of white rot fungi: Study on an efficient symbiotic system in fungus-growing termites and its application to bioremediation. *RIKEN Rev. Foc. Econ. Sci. Res.*, 42: 39-42.
- Osono, T., Y. Fukasawa and H. Takeda, 2003. Roles of diverse fungi in larch needle-litter decomposition. *Mycologia*, 95: 820-826.
- Osono, T. and H. Takeda, 2006. Fungal decomposition of *Abies* needle and *Betula* leaf litter. *Mycologia*, 98: 172-179.
- Petterson, R.C., 1984. The Chemical Composition of Wood. In: *The Chemistry of Solid Wood*, Rowell, R.M. (Ed.). Am. Chem. Soc., Washington, DC., pp: 57-126.
- Pinto, P.C., D.V. Evtuguin and C.P. Neto, 2005. Structure of hardwood gucuroxylans: Modifications and impact on pulp retention during wood kraft pulping. *Carbohydr. Polym.*, 60: 489-497.
- Saparrat, M.C.N., M. Rocca, M. Aulicino, A.M. Arambarri and P.A. Balatti, 2008. *Celtis tala* and *Scutia buxifolia* leaf litter decomposition by selected fungi in relation to their physical and chemical properties and lignocellulolytic enzyme activity. *Eur. J. Soil Biol.*, 44: 400-407.
- Stecher, P.G., M. Windholz and D.S. Leahy, 1968. An Encyclopedia of Chemicals and Drugs. 8th Edn., Merck and Co, Inc., Whitehouse Station, NJ USA.
- Sun, Y. and J. Cheng, 2002. Hydrolysis of lignocellulosic material from ethanol production: A review. *Biores. Technol.*, 83: 1-11.
- Swift, M.J. and J.M. Anderson, 1989. Decomposition. In: *Ecosystems of the World. 14B: Tropical Rainforest Ecosystems*, Leith, H. and M.S.A. Werger (Eds.). Elsevier, Amsterdam, The Netherlands, pp: 547-569.
- Swift, M.J., O.M. Heal and J.M. Anderson, 1979. Decomposition in terrestrial ecosystems. *J. Ecol.*, 34: 45-50.
- TAPPI Standard Methods, 2009. Fibrous Materials and Pulp Testing. Technical Association of Pulp and Paper Industry, Atlanta.
- Terron, M.C., L. Maria, Fidalgo, G.C. Galletti and A.E. Gonzalez, 1995. Pyrolysis-gas chromatography/mass spectrometry of milled wood lignin of two Chilean woods naturally decayed by *Ganoderma australe*, *Phlebia chrysocrea* and a brown-rot fungus. *J. Anal. Applied Pyrolysis*, 33: 61-75.
- Tuomela, M., M. Vikman, A. Hatakka and M. Itavaara, 2000. Biodegradation of lignin in a compost environment: A review. *Bioresour. Technol.*, 72: 169-183.
- Vane, C.H., T.C. Drage and C.E. Snape, 2006. Bark decay by the white-rot fungus *Lentinula edodes*: Polysaccharide loss, lignin resistance and the unmasking of suberin. *Int. Biodeter. Biodegradat.*, 57: 14-23.
- Vargas-Garcia, M.C., F. Suarez-Estrella, M.J. Lopez and J. Moreno, 2007. *In vitro* Studies on lignocellulose degradation by microbial strains isolated from composting processes. *Int. Biodeter. Biodegradat.*, 59: 322-328.
- Watanabe, T., Y. Watanabe and K. Nakamura, 2003. Biodegradation of wood in dual cultures of selected two fungi determined by chopstick method. *J. Biosci. Boeng.*, 95: 623-626.
- White, R.H., 1987. Effect of lignin content and extractives on the higher heating value of wood. *Wood Fiber Sci.*, 19: 446-452.
- Willfor, S., A. Sundberg, A. Pranovich and B. Holmbom, 2005. Polysaccharides in some industrially important hardwood species. *Wood Sci. Technol.*, 39: 601-617.