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Biological Method to Quantify Progressive Stages of Decay in Five Commercial Woods by *Coriolus versicolor*

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Abstract: Biologic agar-block method was developed that allowed wood samples to be evaluated and monitored in terms of colonization and development of the decay by *Basidiomycetes* fungi (*Coriolus versicolor*) and to be directly classified based on mean mass loss. In this research, the *in vitro* decay of five commercial woods by *Coriolus versicolor* was studied by the agar-block method. The selected wood samples were *Abies alba*, *Populus alba*, *Fagus orientalis*, *Platanus orientalis* and *Ulmus glabra*. The results demonstrated the strong resistance of *Ulmus glabra* and the lowest resistance in *Fagus orientalis*. The mass losses (%) were 16.8 and 42.4%, respectively. There were also a high correlation between the mass loss and apparent damage. Therefore biological evaluation of wood regarding biodegradation and the selection of wood types for various application respects will be of high priority.

Key words: Biological method, basidiomycetes, commercial woods, wood decay

INTRODUCTION

Detecting of early wood decay is an important part of evaluation of existing wood construction. Because such decay grows and expands continuously, degrading the wood and eventually compromising the strength of the elements (Gierlinger *et al*., 2003).

Wood decay fungi only attack wet wood. Protecting untreated wood from water is therefore critical to long-term durability (Avango, 2006). The degree of biodeterioration (rot damage)in wooden construction is affected by the severing and duration of moisture exposure and temperature. Besides of the sensitivity of the wooden construction to environmental stress, a constant relative humidity (RH) of above 80% (Zanuttini, 2003) is a risk for fungal growth at temperature between 5-50°C. High nutrient content of the surface stimulates the fungal growth.

The natural durability of wood varies with environmental condition, wood characteristics and the decay organisms (Freitag *et al*., 1991). White rot fungi are predominantly from the subdivision *Basidiomycetes*. They invade wood cells and degrade cell wall components. They have the ability to degrade cellulose, hemicellulose with a bleached appearance. An economic loss due to wood decay is incurred through reduction in grade quality or loses of timber and the requirement to replace affected timber in structural use.

Timber is a sustainable, economical, completely renewable, Co_2- natural organic material, which requires less energy to process that other constructional materials, wood is also a strong and very durable material then properly maintained and used. However under certain conditions of exposure or use particularly when it becomes wet, wood may be rapidly decomposed due to organic decay or natural weathering agents.

The deterioration of wood caused by a combination of biological, chemical and physical process (Zabel and Morrell, 1992). Biological damage is often measured using laboratory experiments because the exact analysis of factors in woody structure (buildings) is difficult.

In forest area in northern of Iran, the most of buildings are consist of wood and wood products, there are several species that are potentially useful timber species but over-exploitation of the few traditional timber species has resulted in the depletion of species.

It is therefore very important to study and document the various properties of lesser-utilized species before they can be correctly selected for appropriate use. As the white rot fungi, *Coriolus versicolor* is among the most

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frequent decomposers of angiosperm wood in northern forest we investigated durability of several commercial wood from Dicotyledonous and Coniferous wood against it.

The main objective of this research was to design a methodology suitable for later standardization for relating anatomical features of wood and weight loss to incipient decay *in vitro*. This study presents details of these methods and in fact It is the first step in a biological control program.

MATERIALS AND METHODS

The experiment was carried out in wood anatomy laboratory of Tehran university during a period of Dec 2005-Apr. 2006.

For better understanding of the problem of the commercial wood durability, it is in any case useful to refer to the European standardization content related to the biological durability of woods (European Committee, 1994). The EN-350-1 standards consider that in case of a possible attack by wood decaying microorganisms, EN-350-1 acts as a guideline for the determination and classification of natural durability of solid wood against attack of wood decaying microorganism.

Wood species and wood anatomy:
Dicotyledonous wood:

- *Fagus orientalis* (From *Fagaceae*)
- *Platanus orientalis* (From *Platanaceae*)
- *Ulmus glabra* (From *Ulmaceae*)
- *Populus alba* (From *Salicaceae*)

Coniferous wood:

- *Abies alba* (From *Pinaceae*)

Anatomical characters are taken in Table 1.

The vicinity of fungi with wood samples: For vicinity of wood samples with *Coriolus versicolor* used glassy kolle. About 60 cc of MEA medium culture poured in every kolle and tied with cotton and set in autoclave. after cooling, transferred to sterile culture cabin (lamin air flow). Every kolle were inoculated by small Fragments of *Coriolus versicolor* and set in 25-35°C for 7-14 days During this time its mycelium covered all surface of culture and prepare to nearness with wood samples. Since woods should not be attacked directly with medium culture for every kolle prepared two glassy peduncle in 2 mm thickness and wood samples set on them and transferred to incubator. In order to supply relative humidify set water dish in incubator during different time studied development in fungal mycelium. After 16 week performed essential measurement. The decrease in mass evaluated according to following formula.

Determination of dry mass loss: The dry mass of the test pieces and the relative moisture (F_i) were determined for the control of each series as follows.

$$F_i = 1 - (\frac{m_o - m_1}{m_o})$$

F_i = Initial moisture factor
m_0 = Conditional mass; m_1 = initial oven dry mass

Having determined the mean F_i for each series the oven dry mass (m_1) of the equivalent set of test specimen was calculated using the following formula

$$F_i \times m_o = m_1$$

The percentage of dry mass loss due to fungal degradation was then calculated

$$\text{\% final loss of dry mass} = (\frac{m_1 - m_3}{m_1}) \times 100$$

Table 1: Anatomic characteristics of wood species

Species	Family	Characteristics
Abies alba	*Pinaceae*	Growth ring boundaries conspicuous. Generally abrupt No resin canals, rarely traumatic resin canals in tangential rows may be present. Rays without tracheides.
Fagus orientalis	*Fagaceae*	Diffuse-Porous or semi-ring porous. Very numerous solitary and clustered pores in early wood. Early wood pores may with gums tyloses. Thick walled fibres simple perforation plates. Fibre-tracheids. uniseriate to multiserate rays (False ray) and very large rays.
Platanus orientalis	*Platanaceae*	Diffuse to semi-ring porous-very numerous pores early wood pores generally in clusters and latewood pores solitary or small irregular groups. Simple and scalariform perforation plates several pits in horizontal, opposite rows Fibers may fibre-tracheids.
Polpulus alba	*Salicaceae*	Diffuse to semi-ring porous-pores solitary or in radial groups. Grow ring boundary more or less distinct with homogenous ray. libriform fibres, large ray-vessel pits.
Ulmus glabra	*Ulmaceae*	Ring porous-Growth ring boundary distinct with hetrogenous rays, big vessel and libiriform fibres.

Where m_3 = Final dry mass

In order to determine durability of species used Findlay methods.

According to these methods from point of natural durability woods ore grouped as follows according to the mass loss.

- Very durable <5%
- Durable 5%
- Moderate durable 5-10%
- Low durable 10-30%
- No durable >30%

Statistical analysis: Analysis of variance (ANOVA method) (Scheffe, 1999) was employed to compare average dry weight. And two estimate were made:

- variance within every sample (S^2P)
- Variance between samples ($S^2\bar{x}$)

The following formula was used in order to determine the minimum amount of difference that makes the difference significant (LSD)

$$LSD = t(a, r(n-1)\sqrt{\frac{2}{n}S^2P}$$

$\alpha = 0.05$

Isolation of fungi species: In order to sure for non-bacterial contaminated, glucose 3% (v/v) was used. Pure culture was prepared in manner of single spore in 7 stages. At last prepared 5 explants in agar medium from pure culture then transferred to MEA medium for reproduction.

Inoculum's disks in 5 mm diameter were cut from edges of colony and transferred to center of medium.

Study of wood degrading by the decreasing in mass: The several samples of 5 commercial woods in 1.5×2×5 cm dimension were selected and in equilibrium humidity of laboratory were weighed. Their volume were determined by measuring dimension then set wood samples in oven at 100±5°C for 24 h and their dry weight and volume were determined.

RESULTS

After 16 weeks of culture under certain condition *Coriolus versicolor* grew completely and became a fibrous whitish mass. In all samples there were apparent damage. mycelium coverage was about 100%. Dry weight decreasing was high. the lowest value weight decreasing was in *Ulmus glabra* and highest value in *Fagus orientalis* and with regard to Findlay grouping set in this orders, respectively from non durable to nearly durable.

DISCUSSION

According to Table 2-4 all groups of studied woods influenced with growing fungi. the decreasing weight in studied samples showed that *Coriolus versicolor* can grows quickly and may rapidly affect the appearance and degraded wood and regard to findlay table dry weight decreasing were high. the lowest value weight decreasing was in *Ulmus glabra* and highest value in *Fagus orientalis*. This is according with the study of crude oil and beech wood by *Coriolus versicolor* (Olfat and Karimi, 2005) and the study of decay woods had been colonized by *C. versicolor* (Heilmann and Boddy, 2005).

It is a fact that among the above studied species the wood of *Ulmus glabra* can be used because of nearly natural durability and tree dimension, height and diameter it has the good anatomical features (Table 1) from the point of view of big vessel, it can be very easy to impregnate chemical..

As shown in Table 2 the most of studied samples the denser woods necessarily were not more durable than the lighter woods, on the other hand were not correlation between mass losing and density because although *Abies alba* is lighter wood than *Fagus orientalis* it is more durable than it this results disagreement with findings of Bultman and Southwell in 1976.

Lignin is an aromatic hetropolymer of phenyl-propanoid units (Britton, 1989; Kenneth, 1995) present in woody plant tissue, that confers their rigidity and

Table 2: Comparison mass loss and denisty

	Fagus orientalis	*Abies alba*	*Platanus orientalis*	*Polpulus alba*	*Ulmus glabra*
Mass loss (%)	42.20	22	37.67	39.26	16.80
Denisty(g cm^{-3})	0.67	0.4	0.55	0.45	0.68

Table 3: Wood groups with regard to Findlay tables

Species	Groups
Fagus orientalis	No durable
Abies alba	Low durable
Platanus orientalis	No durable
Polpulus alba	No durable
Ulmus glabra	Low durable

Table 4: Study of changes in mass during 16 week culture

Species	M1 (mg)	M3 (mg)	M1-M3 (mg)	Loss mass (%)
Fagus orientalis	13.6960	7.9536	5.7424	42.20
Abies alba	07.0226	5.4775	1.5451	22.00
Platanus orientalis	10.4566	6.5177	3.9389	37.67
Polpulus alba	07.5988	4.6150	2.9838	39.26
Ulmus glabra	10.4297	8.6735	1.7562	16.80

resistance to microbial attack (Shah and Nerud, 2002). In order to polymerize and mineralize lignin, white rot fungi have developed an oxidative and in specific system including extra cellular enzyme (Britton, 1989) the ability of rots fungi in degrading abroad clan of organic pollutant (Pointing, 2001) such as crude oil (Olfat, 2005) and crude oil and Beech wood by *Coriolus versicolor* (Olfat and Karimi, 2005) of this nonspecific system.

The main parts of hydrocarbonic (wood) compounds that fungi use are changed even before and after entering to cell. In outer these changes in broadly in pathway to simple of metabolic materials oxidize to CO_2 and water and intermediate compounds In this process fungi achieved necessary energy from degrading woods.

Wood is an important renewable and biodegradable natural resource with a multitude of uses (Olfat, 2005). Wood is used extensively as a structural material for buildings, wharves, telephone poles and furniture due to its high strength per unit weight, its versatilityand its variety.

Wood also serves as the industrial raw material for the manufacture of paper and paper products, wood composites (Mankowski and Morrell, 2000) and other products made from cellulose, such as textiles and cellophane. In many parts of the world wood is used as a fuel for heating and cooking. The primary biotic decomposers of wood are *Basidiomycete* decay fungi, which can attack and degrade both wood in the forest (Buttcher and Drysdale, 1997) and wood in service. In the forest ecosystem wood decay fungi play an important role in carbon and nitrogen cycling and help to convert organic debris into the humus layer of the soil. Some fungi attack living trees; others invade downed timber and slash on the forest floor, lumber and wood in service. Wood decay *Basidiomycetes* colonize and degrade wood using enzymatic and non enzymatic processes (Britton, 1989) and can progressively utilize all major cell wall components, including both the carbohydrates and the lignin. As decay progresses the wood becomes discolored and loses strength, weight and density. Decay and discoloration caused by fungi are major sources of loss in both timber production and wood use, with losses of 15 to 25% marketable wood volume in standing timber and of 10 to 15% in wood products during storage and conversion (Makensen, 2003).then if wood is subject to a moist environment, it should be replaced with pressure treated wood.

CONCLUSIONS

Knowledge on wood natural durability is required in order to be able to decided if we can use a certain type of wood outdoors also is being useful as taxonomic instruments. Moreover it could be useful to integrate the new methods and also mechanical testing. In fact wood decay determine by biological attack and reduced mechanical properties of the product. Finally it would be interesting if this method could supply indication about the service. If the product regarded to in terms of biological durability, it means period which the physical and mechanical would remain adequate for its intended use.

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