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Degradation of Crude Oil and Beech Wood by *Coriolus versicolor*

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Abstract: Polynuclear-aromatic hydrocarbons are the most important environmental pollutant agents and relatively resistance to degrading. White rot fungi are wood degrading *basidiomycetes* that not only degrade Lignin but also the vast environmental pollutant. In this research capability of *Coriolus versicolor* for crude oil and beech wood degradation in the presence of surfactant and wood preservative evaluated. There were high correlation among the increasing mycelium growth and the decreasing wood mass and degrading crude oil that shows high capacity for degradation.

Key words: Crude oil, synthetic surfactant, white rot fungi, wood preservative

INTRODUCTION

Growth of fungi in several substrates can be considered as taxonomical and biotechnological instrument[1]. These group of fungi, whether parasitic or saprophyte, degrading wood by secreting special enzyme such as hydrolase, phenyl oxidase and so on[2]. All of durable woods have poisonous material against fungi, but sufficient amount that being preservative affect only hardwood. In sapwood due to high food source (carbohydrate than their absorption is easiest than starch and other storage carbohydrate). It is low durable and affected by wood degrading fungi.

Lignin is a recalcitrant hetropolymer of phenyl-propanoid units present in woody plant tissue that confers them rigidity and resistance to microbial attack[3-6]. In order to depolymerize and mineralize lignin, white rot fungi have developed an oxidative and inspecific system including extracellular enzymes, low molecular weight metabolite and activated oxygen species. Due to the lack of specificity of the system involved in the lignin depolymerziation, white rot fungus such as *Coriolus versicolor* was being studied for application on degrading the aromatic hydrocarbon pollutants that cause environmental problems.

Ability of white rot fungi is an interesting subject with aim of development of environmental biotechnology alternative in order to decrease energy expense and decrease environmental impact in pulp and paper industry. In other way their ability in degrading organic pollutant such as oil can be considered.

The use of some white rot fungi have been showed that the decreasing of wood weight by *Coriolus versicolor* was high. The decreasing in beech wood after 14 week incubation have been 23.5%. In this research that used *Coriolus versicolor* for assay natural durability and treated wood durability of beech wood. It is one of five species fungi that used as wood destructive and determine of sensibility of species against fungi (in laboratory condition) but in nature may be attacked deciduous tree.

The use of phenolic compound by this species can be based for other research. Study of degradation pathway of other substrate are used to treat biological pollutants.

Bioremediation is based on the idea that organisms are capable to take in things from the environment and use it to enhance their growth and metabolism[7-9]. This unique characteristic laid the fundamental principle of bioremediation to use microorganism to take contaminated substance from the environmental or convert it to nontoxic form. Fungi, bacteria and mosses are degrading complex molecules and convert them in simple molecules, CO_2 and water. White rot fungi degrades lignin effectively. The objective of this study was to determine whether oil degrading fungi can be considered major wood destructive.

MATERIALS AND METHODS

Systematic and ecologic situation of *Coriolus versicolor*: This species is from class Basidiomycetes. Family Polyporaceae. It is seen on logs and fallen lumber and

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also on excess of cut off wood in Iran forests. It causes decaying of woody species completely. It is one of five important species that are wood destructive.

Isolation of fungi species: Essential samples were collected from fungi sp. and transferred to laboratory in cold conditions. In order to assay bacterial non-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 CMA medium for reproduction. Inoculum's disks in 5 mm diameter were cut from edge of colony and transferred to center of medium.

Two types of assay was performed: study of wood degrading activity and natural durability of wood in treatment and without treatment condition by measuring mass decrease; study of degrading light and heavy crude oil by measuring changes in radial growth.

Study of wood degrading by decreasing in wood mass: The 25 samples of beech wood in 2×2×6 cm dimension were selected and in equilibrium humidity of laboratory were weighed. Their volume were determined by measuring dimension then set samples in oven at 100±5°C in 24 h and their dry weight and volume were determined.

The vicinity of fungi with beech wood: For vicinity of wood sample with *Coriolus versicolor* used glassy kolle about 60 cc of MEA medium culture poured in every kolle and tied with cotton and was set in autoclave. After cooling transferred to sterile culture chamfering every kolle inoculated small fragment of *Coriolus versicolor* and in 7 days set in 25-30°C. During this time its mycelium covered all surface of culture and prepare to nearness with beech wood. Treated woods with water soluble salts, Celcure and Basilit separately wounded in aluminum paper and autoclaved. Because woods should not be attacked directly with medium culture, for every kolle prepared two glassy peduncle in 2 mm thickness. Wood samples set on them and transferred to incubator. In order to supply relative humidity water dish set in incubator. During different time, studied development in fungi mycelium. After 14 week performed essential measurements. The decrease in mass achieved according to following formula:

$$\text{Mass decreasing (\%)} = \frac{\text{Primary dry weight - secondary dry weight}}{\text{Primary dry weight}} \times 100$$

In order to determine durability of treatments used Findlay methods[10].

According to these methods from point of natural durability, woods are grouped as follows according to the mass losses:

Very durable	<5%
Durable	5%
Average durable	5-10%
Low durable	10-30%
Non durable	>30%

Wood treatment with water-soluble salts, Celcure and Basilit: Full cell or bethel method was used. This method was for holding maximal preservative materials inside of lumen and membrane of cells. Wood samples were set in saturated cylinder and empty air from lumen, then preservative solution enters in cylinder and by press enter cell structure. In this part two salts, Basilit (1 and 2%) and Celcure (1 and 3%) were used and preservative role of these salts against *Coriolus versicolor* in *Fagus orintalis* was studied.

Study of crude oil degrading process: Inoculums disks in 5 mm diameter were cut from edge of culture colony and transferred to center of petri dish contain agar and 4% (v/v) light or heavy crude oil. About 10 cc (1%) synthetic surfactant (Tween 20) in some of culture medium were added. The crude oil used were export light and heavy crude oil of khark, Iran.

The measurement of radial growth in control and treated groups performed in four fragments of petri dish (Radial fragment) with three repeat daily after day. Average of radial growth in determined time was achieved (during 21 day). That is this action continued for three week and the cure of exponential growth synthetic was drawn up.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA method) was employed to compare average dry weight and two estimates were made:

(i) Variance within every samples $\bar{x}$ (S^2P).
(ii) Variance between samples S^2 . The following formula was used in order to determine the minimum amount of difference that make the difference significant (LSD).

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

$a = 0.05$.

RESULTS AND DISCUSSION

According to Table 1 unsaturated control groups set in series of non durable wood and other saturated groups are set in durable woods series. Radial growth in oily medium is higher than control groups and the rate of growth in culture including surfactant is highest (Table 2).

In this research, durability of control and treated groups were studied by adding water soulble salt, Celcure and Basilit against *Coriolus versicolor* after 14 week inoculation. The decreasing weight in control group and that beech wood which had been in exposure of fungi without wood preservative were high. The lowest value in weight decreasing is in groups that treated with basilit 2% (v/v). That is, wood had remained relatively intact. Then this dose of salt had the most effect of preservation (Table 1) and with regard to Findlay table control group were set in non-durable wood class. Study of control groups showed that average weight decrease was very high. This showed high degradation of wood structure by *Coriolus versicolor*. Then if aim is only against with fungi in economic approach, is adequate wood saturated by 1%(v/v) celcure salt, but if aim beside against is improvement of wood quality, wood should be saturated with soluble salt 3% (v/v) Celcure or 1% (v/v) Basilit. Results from study of crude oil degrading activity by this fungi showed that degrading process in oily medium was high. Radial growth changes in oily medium in comparison with control were meaningful. Comparison of two culture with surfactant and without surfactant in all medium show that growth rate in medium containing surfactant was high and in oily medium with tween (surfactant) degrading process in comparison oily medium without Tween was also fast.

Since fungi are chemotrophic hetrotrophe, they use organic material as carbon and energy source. Chemical structure of the most carbon source such as wood or crude oil is complicated. In order to use these compounds, there should be changes in fungal physiology. It is necessary to make new enzyme or receive in active stage to change chemical structure of cell. In order to uptake of substrate it is necessary to establish physical contact between degrader and substrate. Use of synthetic surfactant increases this contact and degrading process and induction of enzyme and activation of fungi. Results from degradation of oil in two medium, control and treated with oil show that growth rate in treated medium is high. Because fungi have necessary for carbon source and these source are in oil (chemical structure of oil). Degradation of hydrocarbonic compounds such as oil is enzymatic actions that ordinary are achieved by extracellular enzyme. Then formation of simpler compound and take up it by fungi and intercellular enzyme convert them to CO_2 and water and other necessary metabolite for growth of fungi.

Table 1: Average of decreasing in mass of sapwood after 14 week vicinity of fungi

Treatments	Number	Average decrease in weight (%)	SD	Coefficient of changes%
Control	25	31.22	8.09	26
Saturated by Basilit 1%	25	0.49	0.49	100
Saturated by Basilit 2%	25	0.12	0.16	140
Saturated by Celcure 1%	25	1.06	0.72	68
Saturated by Celcure 3%	25	0.44	0.39	89

The fungi that are degrading wood[11] and oil have similar mechanism to degrade of carbonic compounds and specific aromatic compounds. Of course in the case of oil there is also poisonousness problems. Hydrocarbons of high concentration are poisonous for cytoplasm membrane, but in low concentration are be degraded by phenyl oxidase, These fungi due to have hydrolytic oxidative enzyme are lignin and cellulose degrading.

Lignin is an aromatic hetropolymer of phenyl-propanoid units[5] present in woody plant tissue, that confers their rigidity and resistance to microbial attack. In order to depolymerize and mineralize lignin, white rot fungi have developed an oxidative and in specific system including extra cellular enzyme. Ability of rots fungi in degrading abroad class of organic pollutant such as crude oil is backing in part to activity of this nonspecific system.

The main parts of hydrocarbonic compounds that use by fungi are changed even before and after entering to cell. In outer of fungi cells these changes in broadly in pathway to simple of compounds. In inter of fungi cells, some of metabolic materials oxidize to CO_2 and water and intermediate compounds. In this process fungi achieved necessary energy from degrading wood and oil. Main parts of constructive structure of wood and leaf is carbon and in case of oily hydrocarbon there are about 80-85% carbon, therefore can be considered that studied fungi group use oil as substance of wood structure.

Study of synthetic growth of fungi in control and treated with light and heavy crude oil along with Tween-20 shows that growth is relatively different and there is a delay time in medium without surfactant, but by

Table 2: Comparison of average radial growth in control and treated groups

	Days										
Groups	1st	3rd	5th	7th	9th	11th	13th	15th	17th	19th	21st
Control (Agar)	5	6	8	9.5	10	13	17.5	22	22.5	25	25
Control+Tween	5	6	9	10	11	14	18	24	25	28.5	30
Light crude oil	5	6	8	10	15	25	32.5	38	40	42.5	45
Light crude oil +Tween	5	5.5	8	13	19	30	38	43	45	45	45
Heavy crude oil	5	6	7.5	10	14	23	29	35	40	43	45
Heavy crude oil +Tween	5	6	8	11	18	26	35	40	43	44	45

adding surfactant in medium due to increasing physical contact, degrading rate and the resulted growth rate is high. Results from two type study, i.e., use of preservative salts, Celcure and Basilit 1% (v/v) on wood and use of synthetic surfactant in degrading of oil show that the use of surfactant in induction of activity and uses of preservation in suppression of activity is sufficient.

Interesting in the field of use white rot fungi in biodegrading dangerous wastes are developed. Further research will show the use of white rot fungi in degrading most of environmental pollutants.

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