UNIVERSITI PUTRA MALAYSIA

# *DECOLOURIZATION OF SELECTED TEXTILE DYES BY WHITE ROT FUNGUS CORIOLOPSIS SP. STRAIN AFF17*

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# DECOLOURIZATION OF SELECTED TEXTILE DYES BY WHITE ROT FUNGUS CORIOLOPSIS SP. STRAIN AFF17

By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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**DECOLOURIZATION OF SELECTED TEXTILE DYES BY WHITE ROT FUNGUS CORIOLOPSIS SP. STRAIN AFF17**

By

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**October 2014**

**Chairman : Professor Mohd. Arif Syed, PhD**
**Faculty : Biotechnology and Biomolecular Sciences**

Synthetic dyes are replacing natural dyes in modern textile industries. However, each year, about 10% of dyestuffs wastes enter to the environment and causes water pollution. As synthetic dyes are designed to resist fading therefore they are recalcitrant in the environment. Since physicochemical treatments have limited efficiency and disadvantages, bioremediation of dyestuffs wastes with microorganisms is gaining scientific interest as an alternative pollution treatment. A previously locally isolated white rot fungus, *Coriolopsis* sp. Strain aff17 (EU863193) from UPM, Serdang was screened for its ability to decolourize azo dyes. It was able to decolourize eleven out of thirteen azo dyes that were chosen. Only seven azo dyes were used in the studies namely Ponceau 2R (C.I. 20470), Amaranth (C.I. 16185), Orange G (C.I. 16230), Naphthol Blue Black (C.I. 20470), Remazol Black B (C.I. 20505), Trypan Blue (C.I. 23850) and Sirius Light Blue BRR (C.I. 34140). Charcaterizations of decolourization ability by *Coriolopsis* sp. Strain aff17 were investigated. Decolourization rates were shown to be higher in shake cultures as compared to static cultures. The best agitating speed for decolourization of dyes was found to be 75 rpm. The best medium for the decolourization of dyes was distilled water. The presence of chromium, arsenic, cadmium and lead did not inhibit the decolourization of by *Coriolopsis* sp. Strain aff17, but slightly increase the decolourization process even at a high concentration of 2.0 mg/L. Copper which is essential for laccase, surprisingly showed lower down the decolourization at the concentration above 1.5 mg/L. Mercury inhibited the decolourization even at a low concentration of 0.5 mg/L. The detection of LMEs was studied in 1L and 2L conical flasks. Larger surface area was shown to have a better decolourization rate when 500 mL of working volume was placed into both 2L flask and 1L flask. Two Litre flask's culture has a larger surface area providing more contact with air therefore providing a better condition for dye decolourization. Laccase and Manganese peroxidase were found in *Coriolopsis* sp. Strain aff17. These Lignin Modifying Enzymes' activities show inverse relationship to the concentration of azo dye. *Coriolopsis* sp. Strain aff17 was grown on agricultural wastes such as sugarcane bagasse, sugarcane peel and paddy straw, to serve as the alternative inoculum in the 2L decolourizing system to replace the utilization of Potato Dextrose Broth.

*Coriolopsis* sp. Strain aff17 that was grown on paddy straw showed the fastest decolourization, which was six days, followed by sugarcane peels (eight days) and sugarcane bagasse (twelve days). *Coriolopsis* sp. Strain aff17 was then tested on raw wastewaters that were collected from rivers near textile factories of Pahang, Malaysia, to further study its ability to decolourize raw wastewater. *Coriolopsis* sp. Strain aff17 was able to decolourize limited raw wastewater without addition of glucose by 12.74 to 24.89%. However with the addition of glucose, the decolourization percentage can reach over 84%. In conclusion, *Coriolopsis* sp. Strain aff17 is proven to be able to decolourize various azo dyes. More studies should be continued to determine if *Coriolopsis* sp. Strain aff17 is able to decolourize dyes in harsher conditions found in raw wastewater. *Coriolopsis* sp. Strain aff17 also should be tested on other pollutants.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGURAIAN PERWARNA TEKSTIL AZO OLEH KULAT REPUT-PUTIH CORIOLOPSIS SP. STRAIN AFF17**

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Perwarna sintetik telah menggantikan perwarna semula jadi dalam industri tekstil moden. Walau bagaimanapun, 10% sisa perwarna masuk ke dalam persekitaran dan menyebabkan pencemaran air. Memandangkan perwarna sintetik adalah dicipta untuk menahan pemudaran warna, maka ia akan berada kekal dalam persekitaran. Rawatan beasaskan kimiafizik merupakan cara yang biasa gunakan tetapi ia mempunyai kelemahan dan kecekapan yang terhad. Jadi, biopemulihan sisa perwarna dengan menggunakan mikroorganisma semakin mendapat perhatian saintifik sebagai kaedah rawatan pencemaran alternatif untuk perwarna sintetik. Keupayaan mengurai pewarna tekstil azo oleh kulat-reput putih tempatan *Coriolopsis* sp. Strain aff17 (EU863193) yang dipencil daripada UPM, Serdang telah kaji tentang keupayaannya mengurai tiga belas perwarna tekstil yang terpilih. Ia boleh menguraikan sebelas perwarna tekstil. Hanya tujuh perwarna tekstil azo, Ponceau 2R (C.I. 20470), Amaranth (C.I. 16185), Orange G (C.I. 16230), Naphthol Blue Black (C.I. 20470), Remazol Black B (C.I. 20505), Trypan Blue (C.I. 23850) dan Sirius Light Blue BRR (C.I. 34140) telah dipilih untuk menjalakan kajian yang seterusnya. Kadar penguraian adalah lebih tinggi dalam kultur goncang apabila dibanding dengan kultur pegun. Kelajuan yang terbaik bagi penguraian perwarna azo adalah 75 rpm. Medium kultur terbaik untuk penguraian perwarna adalah air suling. Kehadiran kromium, arsenik, kadmium dan plumbum tidak mengpengaruhi keupayaan *Coriolopsis* sp. Strain aff17 untuk mengurai perwarna bahkan ia dapat meningkatkan kadar penguraian walaupun kepekatannya adalah setinggi 2.0 mg/L. Kuprum yang diperlui oleh lakase, tidak meningkatkan kadar penguraian tetapi menjejaskan penguraian pada kepekatan 1.5 mg/L. Raksa menjejaskan kadar penguraian walaupun kepekatannya adalah serendah 0.5 mg/L. Kawasan permukaan yang lebih besar menunjukkan kadar penguraian yang lebih cepat apabila isipadu 500 mL kultur yang diletak dalam 2L kalalang kon dan 1L kelalang kon. Dua Liter kelalang kon mempunyai kawasan permukaan yang lebih besar untuk mendapat lebih banyak udara jadi ia menyediakan persekitaran untuk penguraian perwarna. Enzim yang terdapat dalam *Coriolopsis* sp. Strain aff17 adalah lakase dan mangan peroksidase. Aktiviti enzim-enzim ini berkadar songsang dengan kepekatan perwarna azo. *Coriolopsis* sp. Strain aff17 ditumbuhkan ke atas sisa-sisa pertanian seperti

hampas tebu, kulit tebu dan jerami padi supaya mereka boleh menggantikan media Potato Dextrose Broth yang mahal. *Coriolopsis* sp. Strain aff17 yang ditumbuhkan di atas jerami padi mempunyai kadar penguraian yang terpantas, ia itu enam hari, diikuti oleh kulit tebu (lapan hari) dan hampas tebu (dua belas hari). *Coriolopsis* sp. Strain aff17 juga diuji untuk menguraikan air sisa tekstil yang dikutip dari sungai berdekat dengan kilang tekstil di Pahang, Malaysia. *Coriolopsis* sp. Strain aff17 boleh menguraikan perwarna tanpa tambahan glukosa, tetapi pada kadar yang sangat rendah (12.74 ke 24.89%). Walau bagaimanapun dengan tambahan glukosa, peratus penguraian perwarna mencapai 84%. Kesimpulannya, *Coriolopsis* sp. Strain aff17 dibukti boleh menguraikan perwarna-perwarna tekstil azo. Lebih kajian perlu dijalankan supaya *Coriolopsis* sp. Strain aff17 boleh menguraikan perwarna dalam keadaan yang sukar dalam air sisa tekstil. *Coriolopsis* sp. Strain aff17 juga boleh dicuba atas pencemar-pencemar yang lain.

I certify that a Thesis Examination Committee has met on 20 October 2014 to conduct the final examination of Cheng Wei Nee on her thesis entitled "Decolourization of Selected Textile Dyes by White Rot Fungus Coriolopsis sp. Strain aff17" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| 2,6-DMP | 2,6-dimethoxyphenol |
| AMR | Amaranth |
| BJ | Bukit Juntong |
| BS | Biebrich Scarlet |
| CAS | Chemical Abstracts Service |
| C.I. | Colour Index |
| DB71 | Direct Blue 71 (alson known as Sirius Light Blue BRR) |
| EDTA | Ethylenediaminetetraacetic Acid |
| IUPAC | International Union of Pure Applied Chemistry |
| KBM | Kirk Basal Medium |
| KL | Kampung Ladang |
| LiP | Lignin Peroxidase |
| LME | Lignin Modifying Enzyme |
| MnP | Manganese Peroxidase |
| MR | Methyl Red |
| MY | Metanil Yellow |
| NBB | Naphthol Blue Black |
| OG | Orange G |
| O2 | Orange II Sodium Salt |
| PDA | Potato Dextrose Agar |
| PDB | Potato Dextrose Broth |
| PDS | Paddy Straw |
| PS | Ponceau S |
| P2R | Ponceau 2R |
| RBB | Remazol Black B |
| SB | Sugarcane Bagasse |
| SP | Sugarcane Peel |
| TB | Trypan Blue |
| TE | Trace Elements |
| TTZ | Tartrazine |
| WRF | White Rot Fungus |

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# CHAPTER 1

# INTRODUCTION

In recent years, synthetic dyes are mostly replaced natural dyes in a number of industries, such as textile dyeing or paper printing. Each year, India, Eastern Europe, China, South Korea and Taiwan consume approximately 800 thousands tons of dyes, and at least 10-15% of dyestuff wastes are enter to the environment (Kumar *et al*., 2013;  Selvam *et al*., 2012; Xie *et al*., 2012; Sinha *et al*., 2012; Palmieri *et al*., 2005; Levin *et al*., 2004).

Synthetic dyes are designed to resist fading upon exposure to sweat, light water, many chemicals including oxidizing agents, and microbial attack (Selvam *et al*., 2012) and therefore, they are recalcitrant in the environment. Regardless of the advantages of synthetic dyes over natural dyes, synthetic dyes present their own new set of problems. The most noticeable is the aesthetic pollution of waterways caused by the presence of dyes leached from textile factories since they are visible even in minute amounts (Stoyanova *et al.*, 2014; Verma *et al.*, 2012; Banat *et al.*, 1996). The production of potentially carcinogenic aromatic amine compounds from the partial cleavage of synthetic dyes are harmful to human (Zheng *et al*., 2014; Ali, 2010; Yilmaz *et al*., 2010; Pinheiro *et al*., 2004). Therefore, the frequently volumetric rate of dyestuff waste discharges raise concern of stringent legislation, the search for appropriate treatment technologies has become an important priority in textile and paper industries (Rodriguez-Couto, 2012; O'Neill *et al*., 1999).

A number of biotechnological approaches have been suggested by recent researches as of potential interest towards combining this pollution source in a coefficient manner, including the use of bacteria or fungi, often in combination with physicochemical processes (Kumar *et al*., 2013; Yadav *et al*., 2013; Zodi *et al*., 2013; Saratale *et al*., 2011). By far the single class of microorganisms most efficient in breaking down synthetic dyes are white rot fungi (WRF). *Phanerochaete chrysoporium* is among the first to have been shown to have the ability to degrade azo dyes (Kumar *et al*., 2013, Priya and Arulmozhi, 2012; Son *et al*., 2012).

Biodegradation of azo dyes by WRF presents a great potential for large-scale applications after many bioremediation processes being investigated for this purpose. Its biodegradation system, comprising of lignin modifying enzymes are not only efficient but also have a wide substrate range. At this time, most research are focusing on a narrow range of well-known WRF while the rich biodiversity of fungi found in tropical forests such as in Malaysia is ignored most of the time. There are reasons to believe that these undiscovered species might have greater azo dye degrading abilities compared to the ones that are being studied now. Hence, the processes to isolate and screen new white-rot fungi for the biodegradation of azo dyes have to be done intensively.

The objectives of this research were:

I. To characterize the decolourization ability of *Coriolopsis* sp. strain aff17.
II. To determine the ligninolytic enzymes present in *Coriolopsis* sp. strain aff17
III. To evaluate the alternative substrates for *Coriolopsis* sp. strain aff17.

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