DECOLORIZATION AND BIODEGRADATION OF MORDANT ORANGE-1 BY NEWLY ISOLATED *TRICHODERMA HARZIANUM* RY 36 AND *ACREMONIUM SPINOSUM* RY 42

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To my beloved mother, father and sister

Thanks for all your support, love and care…

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ABSTRACT

The synthetics dyes are dangerous for human being and aquatic life when it pollute of water resources. Textile processing manufacture is the largest sector and initiator of fluid sewage in the shape of pollutants containing synthetics dyes. Several wastewater treatment technologies are used to treat of these pollutants including conventional and advances treatment such as Physico-chemical, electrochemical, membrane separation, and reverse osmosis. Nevertheless, all these technologies are high cost operating, need of huge space, limited flexibility and generate by-products. The microbial field which is fungal based bioremediation gives promising treatment for decolorize and degrade the synthetic dye in wastewater from textile industry. It was found to be an environmental friendly, low-cost operation and effective compared to conventional and advances treatments. In this present study, the fungal strains from soil and decayed wood isolated from Universiti Teknologi Malaysia (UTM) Campus and some region in Johor Bahru forest were screened and selected for its ability to decolorize the azo dye, Mordant Orange-1 (MO-1). Two isolates RY 36 and RY 42 showed its ability for decolorization of MO-1 dye, among fifty fungal strains collected. The degradation experiments were conducted in both of the solid and liquid medium amended with 50 ppm of MO-1 dye. The efficient degraders, RY 36 and RY 42 were identified using 18S rRNA sequence analysis and morphology characterization. From the results obtained, these fungi belong to the group of *Trichoderma harzianum* RY 36 and *Acremonium spinosum* RY 42, respectively. Further, the effect of various environmental factors parameters such as carbon and nitrogen sources, surfactant (Tween 80), aromatic compounds and pH on the dye decolorization by *Trichoderma harzianum* RY 36 and *Acremonium spinosum* RY 42 in the liquid medium was assessed. *Trichoderma harzianum* RY 36 showed efficient decolorization with addition of glucose (84.16%), ammonium nitrate (79.41%), tween 80 0.1 mL (27.68%), salicylic acid (84.73%) pH 3 (89.42%) and maximum biomass production of 6840 mg/L was achieved in the presence of yeast extract. Meanwhile, *Acremonium spinosum* RY 42 showed efficient decolorization of MO-1 with addition of glucose (86.6%), ammonium nitrate (70.21%), Tween 80 0.1 mL (12.77%), salicylic acid (84.68%) pH 3 (89.6%) and maximum biomass production of 7850 mg/L was achieved in the presence of Tween 80 (1.5 mL). Further, the degradation products of MO-1 by both of the isolates were identified using Thin Layer Chromatography (TLC) and Gas Chromatography Mass Spectrophotometer (GC-MS). The analytical results showed that maleic acid and Isophthalic acid were formed during the degradation of MO-1 by *Trichoderma harzianum* RY 36. In the case of *Acremonium spinosum* RY 42, salicylic acid and benzoic acid were identified as metabolic products during the degradation of MO-1 dye.

ABSTRAK

Pewarna sintetik adalah berbahaya kepada manusia dan kehidupan akuatik apabila ia mencemari sumber air. Proses pembuatan tekstil merupakan sektor yang besar dan penyumbang utama air sisa kumbahan yang mengandungi pewarna sintetik. Beberapa teknologi rawatan air sisa yang digunakan untuk merawat pencemar tersebut termasuk kaedah konvensional dan rawatan termaju seperti fiziko-kimia, elektrokimia, pemisahan membran dan osmosis berbalik. Namun, semua teknologi tersebut memerlukan kos operasi yang tinggi, kawasan yang luas, tidak fleksibal dan mewujudkan hasil sampingan. Bidang microbial yang mengunakan kulat sebagai asas permulihan-bio memberikan jaminan rawatan untuk penyingkirkan dan penguraikan pewarna sintetik dari airsisa industri tekstil. Ia juga mesra alam sekitar, kos operasi yang rendah dan berkesan berbanding dengan kaedah kovensional dan rawatan termaju. Dalam kajian ini, *strain* kulat yang telah di dapati dari tanah dan kayu lapuk di kampus Universiti Teknologi Malaysia (UTM) dan sebahagiannya dari hutan di Johor Bahru, telah di saring dan di pilih bagi kebolehan untuk menyingkirkan pewarna *Azo*, *Mordant Orange-1* (MO-1). Dua pengasing seperti RY 36 dan RY 42 menunjukkan keberkesanan dalam penyingkiran pewarna MO-1, dikalangan lima puluh strain kulat yang dikumpul. Kajian penguraian telah dijalankan dalam dua bentuk keadaan iaitu pepejal dan cecair yang telah diubah dengan menggunakan 50 ppm pewarna MO-1. Keberkesanan pengurai dan jenis kulat RY 36 dan RY 42 telah dikenalpasti berdasarkan analisis turutan 18S rRNA dan ciri-ciri bentuk permukaan dimana masing-masing adalah dari jenis *Trichoderma harzianum* RY 36 dan *Acremonium spinosum* RY 42. Seterusnya, kesan-kesan kepelbagaian parameter faktor persekitaran seperti karbon, sumber nitrogen, bahan permukaan (Tween 80), sebatian aromatic dan pH terhadap penyingkiran pewarna oleh *Trichoderma harzianum* RY 36 dan *Acremonium spinosum* RY 42 pada keadaan cecair telah diuji. *Trichoderma harzianum* RY 36 menunjukkan keberkesanan penyingkiran warna dengan penambahan glukosa (84.16%), *ammonium nitrate* (79.41%), Tween 80 0.1 mL (27.68%), asid *salicylic* (84.73%), pH 3 (89.42%) dan penghasilan biomas yang maksimum sebanyak 6840 mg/L telah diperolehi dengan kehadiran estrak yis. Manakala, *Acremonium spinosum* RY 42 menunjukkan keberkesanan penguraian MO-1 dengan penambahan glukosa (86.6%), *ammonium nitrate* (70.21%), Tween 80 0.1 mL (12.77%), asid *salicylic* (84.68%) pH 3 (89.6%) dan penghasilan biomas yang maksimum sebanyak 7850 mg/L telah diperolehi dengan kehadiran Tween 80 (1.5 mL). Seterusnya, penguraian produk dari MO-1 oleh kedua-dua pengasing telah ditentukan menggunakan *Thin Layer Chromatography* (TLC) dan *Gas Chromatography Mass Spectrophotometer* (GC-MS). Hasil kajian analitik menunjukkan asid *maleic* dan asid *isophthalic* telah terbentuk semasa penguraian MO-1 oleh *Trichoderma harzianum* RY 36. Manakala, bagi kajian *Acremonium spinosum* RY 42, asid *salicylic* dan asid *benzoic* dikenalpasti sebagai produk metabolik semasa penguraian pewarna MO-1.

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LIST OF SYMBOLS

%	-	Percent
–	-	Path length of radiation *b*; l, d
λ max	-	Lambda Maximum
-N=N-	-	Mono Azo Dye
-C=O	-	Carbonyl
$(NH4)_2SO_4$	-	Ammonium Sulfate
$\frac{\boldsymbol{A}}{\boldsymbol{bc}}$	-	Absorptivity a, extinction coefficient *k*
$\frac{\boldsymbol{A}}{\boldsymbol{bc}}$	-	Molar absorptivity $\boldsymbol{\epsilon}$, Molar extinction coefficient
°C	-	Celsius
°C/min	-	Celsius per Minute
$C_6H_{12}O_6$	-	Sugar
$C_7H_6O_2$	-	Benzoic Acid
$C_6H_4(OH)_2$	-	Catechol
$C_7H_6O_3$	-	Salicylic Acid
C	-	Carbon
CH_3	-	Methyl
C.I.	-	Color Index
Cl	-	Chloride
Cl_2	-	Chlorine
cm	-	Centimeter
COOH	-	Carboxyl
Cu^+	-	Copper Ion
Cu^{2+}	-	Copper (II) Ion
DNA	-	Deoxyribonucleic Acid
E'_0	-	Redox Potential
e^-	-	Electron

Fe^{3+}	-	Iron (III) Ion
g/L	-	Gram per Liter
g/mol	-	Gram per Mole
GC	-	Gas Chromatography
GC-MS	-	Gas Chromatography-Mass Spectrometry
H_2O_2	-	Hydrogen Peroxide
H_2O	-	Water
H^+	-	Hydrogen Ion
HCL	-	Hydrochloric Acid
kPa	-	Kilopascal
Lac	-	Laccase
LMEs	-	Lignin-Modifying Enzymes
LiP	-	Lignin Peroxides
$\log \frac{\boldsymbol{P_0}}{P}$	-	Absorbance A, Optical density; extinction *E*
M^+	-	Molecular Ion (peak)
M	-	Molar
MAE	-	Malt Extract Agar
Min	-	Minutes
mL	-	Milliliter
mg/L	-	Milligram Per Liter
mM	-	Millimolar
mm	-	Milliliter
MnP	-	Manganese Peroxides
Mn^{2+}	-	Manganese (II) Ion
Mn^{3+}	-	Manganese (III) Ion
MnO_4	-	Permanganate
MO-1	-	Mordant Orange-1
m/z	-	Mass Spectrum (*Mass-to-Charge Ratio)*
N	-	Nitrogen
NaOH	-	Sodium Hydroxide
NH_2	-	Amines
NH_4NO_3	-	Ammonium Nitrate
NO	-	Nitro

NO_2	-	Amino
Nm	-	Nanometer
O_2	-	Oxygen
O_3	-	Ozone
OH	-	Hydroxyl
OH^-	-	Hydroxyl Radical
PAH	-	Polycyclic Aromatic Hydrocarbons
PCR	-	Polymerase Chain Reaction
PDA	-	Potato Dextrose Agar
PDB	-	Potato Dextrose Broth
pH	-	Power Hydrogen
P, P_0	-	Energy of radian (in ergs) impinging on a 1-cm^2 area of detector per second; radiation intensity I, I_0
$\frac{P_0}{P}$	-	Transmittance T
ppm	-	Part per Million
R_f	-	Retention Factor
rpm	-	Rotor per Minute
rRNA	-	Ribosomal Ribonucleic Acid
S	-	Sulfur
Si	-	Silica
SO_3	-	Sulfur Trioxide
TLC	-	Thin Layer Chromatography
TMS	-	Trimethylsilylation
t_R	-	Retention Time
UV	-	Ultraviolet
UV-Vis	-	Ultraviolet-Visible
v/v	-	Volume per Volume
w/v	-	Weight per Volume
µL	-	Microliter
µg	-	Microgram

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The synthetics dyes are dangerous for human being and aquatic life when it pollute of water resources. Developing countries are faced with serious water pollution. The industries discharge 100 tons per days of wastewater effluent into river body (Allen *et al.*, 2004). The industrial processes including the pulp and paper, textile, chemical and petrochemical industries are generally utilizing dyes as colorant (Noroozi1 and Sorial, 2013). In excess of ten thousand variant synthetic dyes have been surpassed 7×10^5 metric tons per annum in worldwide consumption and including manufactures (Deveci *et al.*, 2004). The presence of toxic dyes in water sources has stimulated much attention in recent decades because of their potential to cause the environmental problems. Moreover, they lead to undesirable effects in the color, odor and taste waters (Attia *et al.*, 2003).

Textile processing manufacture is the largest sector and is initiator of fluid sewage in the shape of pollutants. Moreover, up to 1,000 tons per year are released in the form of wastewater industry based on report study (Ozmen *et al.*, 2007). The process it is predicted that 10%–20% of synthetic dyes will be lost in remaining fluids via partial washing and finishing operations (Deveci *et al.*, 2004). Besides, 1 kg of cloth produced generating approximately 40-65 L of textile effluent (Mezohegyi *et al.*, 2007). Dyed treatment for effluent are presently able to reduce just partially of the dyes lost in wastewater streams. The international, national articles and magazines showed the pollution of rivers, agricultural lands and cases due to drinking water contamination by the effluent, coming out of the textile industries. It has been prompted that find a new effective way is needed for the treatment of dye contaminated effluent to protect the ecosystem.

Several wastewater treatments are used including adsorption, physical-chemical treatment including coagulation, flocculation and filtration, advance treatment such as electrochemical, membrane separation, and reverse osmosis (Arulkumar *et al*., 2011; Edward, 2005). Several literatures described that most of problem with physical-chemical treatment lies on the high cost operating with low efficiency, in addition to the need of huge space and limited flexibility. This is in contrast with another wastewater techniques and the treatment of the wastewater generated. Moreover, alternative by using adsorption process for decolorization of dyes is efficient, high competitive and simple (Sathishkumar *et al*., 2011; Zheng *et al*., 2009). Other innovative natural materials used in the production of carbonaceous solids include products from lignocelluloses substances e.g. palm shell, wood, sawdust, and also from vegetable waste substances e.g. fruit stones, nutshells, etc. (Adinata *et al*., 2007; Alam *et al*., 2009; Baccara *et al*., 2009; Foo and Hameed, 2011; Srinivasakannan and Abu Bakar, 2004; Ucar *et al*., 2009; Ould-Idriss *et al*., 2011; Thio *et al*., 2009; Yorgun *et al*., 2009). The results from these lignocelluloses materials and wastes of vegetable origin reveal that their adsorption capacity was not so effective (Sarier, 2007). Furthermore, all technologies have advantages and disadvantages; these would be essential issues in search of any technology that are low-cost, easily obtained and environmentally friendly.

Microbial decolorization and biodegradation is greatly considered as an alternative for removal of textile dye effluent. Biological decolorization of dyes using fungi and bacteria has also been evaluated (Hadibarata *et al*., 2011a; Maas and Chaudhari, 2005; Rodriguez-Couto *et al*., 2003; Pearce *et al*., 2003) to decolorized of azo, heterocyclic, polymeric dyes and triphenylmethane by white-rot fungus, *Polyporus* and *Phanerochaete chrysosporium* (Glen and Gold, 1983; Rodriguez *et al*., 1999; Hadibarata *et al*., 2011b; Pazarlioglu *et al*., 2005). These fungi materials have been known to have good biodegradable capacity (Hadibarata *et al*., 2011).

The microbial field which is fungal based bioremediation gives promising treatment on decolorization and degradation of polluting synthetic dyestuff. The fungi strain that have capability to decolorize azo dye, type of Mordant Orange-1 (MO-1) were screened, isolated and identified. The forest is the best places for collecting fungi. The diversity of forest give fungi different capability to decolorize and degrade Mordant Orange-1 (MO-1). In addition, screening stage is the most important part to search new varieties of fungi strains in order to find the best degrader of Mordant Orange-1 (MO-1). A selected fungal strain was identified based on the 18S rRNA sequencing and microscope method to know the taxonomy and morphology characterization. Study of the effect of environmental factors (carbon, nitrogen sources, surfactant (Tween 80), aromatic compounds and pH) on decolorization was performed in the liquid medium and analyzed by UV-Visible Spectrophotometer. Finally, the metabolite product of MO-1 was analyzed in Gas Chromatography-Mass Spectrometry (GC-MS).

1.2 Problem of Statements

In Malaysia textile industry is mainly essential export sector besides plantation, oil and natural sources. This sector has been the seventh largest contributor to total earnings from manufactured exports, due to its high market either from inside and outside Malaysia. In Malaysia, the textile industry is concentrated mainly in the states of Johor, Perak, Penang and Selangor (Pang and Abdullah, 2013). Based on report from Department of Environment of Malaysia 2010, the textile industry produced approximately 743.99 metric tons per year of wastewater released by into river bodies and one of the substances were azo dyes. These compounds are one of the most difficult to treat. The majority of them are carcinogenic and toxic to living ecosystem. In fact, 1 ppm concentration might less rather than another chemical establish in wastewater, it can be visible instead at low concentrations (Godlewska *et al*., 2009; Medvedev *et al*., 1988).

1.3 Objectives of Study

The present study was carried out to investigate the applicability of the fungi for the removal of mordant orange-1. The objectives of the study are:

1. To screen, isolate and identify the fungal strain isolated from nature for decolorization of an azo dye, Mordant Orange-1 (MO-1).
2. To investigate the effect of several environmental factors on decolorization of Mordant Orange-1 (MO-1).
3. To determine and identify the metabolite pathway of Mordant Orange-1 (MO-1) degradation.

1.4 Scope of Study

The study utilized the filamentous fungi to decolorize Mordant Orange-1 (MO-1) in a solid and liquid medium in 15 and 30 days incubation time. The certain effect of environmental factors in decolorization such as carbon sources, nitrogen sources, effect of surfactant (Tween 80), aromatic compounds and initial pH value were investigated in the liquid medium. Identification of selected fungi was performed by using 18S rRNA sequence and microscope method. Metabolite pathway was determined by identify some products yield in degradation using some instruments such as TLC, UV-Visible Spectrophotometer, and GC-MS.

1.5 Significance of Study

The significance of this research is to provide an alternative method for the decolorization and biodegradation of an azo dye Mordant Orange-1 (MO-1) by utilizing a new promising fungi from nature. Furthermore, the application of fungi for decolorization and degradation of synthetic dye is expected to be more environmental friendly to human life, and contribute an effective method to solve problem in the treatment of wastewater from textile industry.

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