

PEATLAND FUNGI: IDENTIFICATION, APPLICATION IN DYE
DECOLOURIZATION AND BACTERIAL INACTIVATION IN GREYWATER

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DEDICATION

This thesis is dedicated to:

Soul of my beloved Father: Ali Noman

Whose words of encouragement and push for tenacity ring in my ears

My beloved mother: For her endless love, support and encouragement.

Treasure, My lovely husband: Adel Al-Gheethi

Who has always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve. I am truly thankful for having you in my life.

To my beloved kids

HALA, JULIA, AMIR and ALI

All of you have been my best cheerleaders.

To my beloved younger brothers and sisters



PTTA
PERPUSTAKAAN TUN AMINAH

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ALHAMDULILLAH



PERPUSTAKAAN PUNKU TUN HUSSEIN ONN MALAYSIA

ABSTRACT

Fungi have unique characteristics since they have several applications in the environment and industry due to its ability to produce the different enzyme. This study aims to isolate a new fungal strain from Pontian peatland, Johor, Malaysia to be used for dye decolourizing in the synthetic greywater as a function of laccase (LAC), manganese peroxidase (MnP) and lignin peroxidase (LiP). The bio-synthesized nanoparticles (bimetallic Zn/Cu NPs) in the secondary metabolic products generated during the enzyme production in pumpkin peels medium was evaluated for inactivating *Escherichia coli* and *Staphylococcus aureus* seeded in greywater. The fungal isolates were identified according to phenotypic characteristics and by molecular characteristic at D1/D2 region and ITS (ITS₁- ITS₄) sequences. The decolourization, enzyme production and inactivation process were optimised using response surface methodology (RSM). The mechanism of decolourization and inactivation process was investigated based on Field Emission Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy (FESEM-EDX), Fourier transforms infrared spectroscopy (FTIR), atomic force microscopy (AFM) and Raman Spectroscopy analysis. The results revealed that *Aspergillus iizukae* EAN605, *Aspergillus arenarioides* EAN603, *Penicillium pedernalense* EAN604, *Purpureocillium lilacinum* EAN601, *Paraconiothyrium brasiliense* EAN202 and *Parengyodontium album* EAN602 were new strains and first time recorded in Malaysia. The best dye decolourization (78.34%) was 57.15 mg 100 mL⁻¹ of dye, pH 6 and after 8.5 days (R²=77.9%). The oxidative enzyme production was recorded with 20 g 100 mL⁻¹ of pumpkin peels, 1, 4.6 mL/100 mL⁻¹ of inoculum size, at pH 5.5 and after 10 days, where 6.15, 2.58 and 127.99 U mg⁻¹ of LAC, MnP and LiP was produced, respectively. The inactivation of *E. coli* and *S. aureus* by Zn/Cu NPs was effective with 0.028 mg mL⁻¹ of Zn/Cu NPs, at pH 6 and after 60 mins with 5.6 and 5.2 log reduction respectively. The decolourization mechanism took place due to the action of oxidative enzymes on the inner membrane of fungal mycelium and in the surrounded medium. The inactivation process

acts by the destruction of the chemical composition of the bacterial cell wall and membrane. In conclusion, this study demonstrates that peatland has high fungal diversity to be used in the dye decolourization and synthesis of NPs for inactivating pathogenic bacteria in the greywater.



ABSTRAK

Kulat mempunyai ciri unik kerana mempunyai beberapa aplikasi dalam persekitaran dan industri kerana kemampuannya menghasilkan enzim yang berbeza. Kajian ini bertujuan untuk mengasingkan strain fungi baru dari tanah gambut di kawasan Pontian, Johor, Malaysia untuk digunakan sebagai penyahwarna untuk pewarna dalam air kelabu sintetik sebagai fungsi laccase (LAC), manganese peroxidase (MnP) dan lignin peroxidase (LiP). Nanopartikel bio-sintesis (bnetallic Zn/Cu NPs) dalam produk metabolik sekunder yang dihasilkan semasa penghasilan enzim dalam medium kulit labu dinilai dalam menyahaktifkan *Escherichia coli* dan *Staphylococcus aureus* yang diinokulasikan dalam air kelabu. Pengasingan kulat dikenalpasti mengikut ciri fenotipik dan ciri molekul di kawasan D1/D2 dan jujukan ITS (ITS1- ITS4). Proses pewarnaan, penghasilan enzim dan penyahaktifan dioptimumkan dengan menggunakan metodologi permukaan tindak balas (RSM). Mekanisme proses penyahwarna dan penyahaktifan ditentukan melalui Mikroskopi Elektron Pengimbasan Pelepasan Lapangan dengan Spektroskopi X-Ray Tenaga Dispersif (FESEM-EDX), spektroskopi inframerah transformasi (FTIR), Mikroskop Daya Atom (AFM) dan analisis Spektroskopi Raman. Hasil kajian menunjukkan bahawa *Aspergillus iizukae* EAN605, *Aspergillus arenarioides* EAN603, *Penicillium pedernalense* EAN604, *Purpureocillium lilacinum* EAN601, *Paraconiothyrium brasiliense* EAN202 dan *Parengyodontium album* EAN602 adalah strain baru dan pertama kalinya direkodkan di Malaysia. Penyahwarna pewarna terbaik (78.34%) ialah 57.15 mg 100 mL⁻¹ pewarna, pH 6 dan selepas 8.5 hari ($R^2 = 77.9\%$). Penghasilan enzim oksidatif dicatatkan dengan 20 g 100 mL⁻¹ kulit labu, 4.6 mL / 100 mL⁻¹ ukuran inokulum, pada pH 5.5 dan selepas 10 hari, di mana masing-masing 6.15, 2.58 dan 127.99 U mg⁻¹ LAC, MnP dan LiP dihasilkan. Ketidakaktifan *E. coli* dan *S. aureus* oleh Zn / Cu NPs berkesan dengan 0.028 mg mL⁻¹ Zn / Cu NPs, pada pH 6 dan selepas 60 minit dengan masing-masing dengan penurunan 5.6 dan 5.2 log. Mekanisme penyahwarna berlaku kerana tindakan enzim oksidatif pada membran dalaman

miselium kulat dan di medium yang dikelilinginya. Proses penyahaktifan bertindak dengan merusakkan komposisi kimia dinding sel dan membran sel bakteria. Sebagai kesimpulan, kajian ini menunjukkan bahawa tanah gambut mempunyai kepelbagaian kulat yang tinggi untuk digunakan dalam penyahwarnaan pewarna dan sintesis NP untuk menyahaktifkan bakteria patogenik pada air kelabu.



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Internal rate of return

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LIST OF ABBREVIATION

<i>AB</i>	- Acid Blue
<i>ABR</i>	- Azo Black Reactive 5 Dye
<i>ABTS</i>	- 2,2'-Azino-Bis (3-Ethylbenzothiazoline-6-Sulphonic Acid
<i>AFM</i>	- Atomic Force Microscopy
<i>a_w</i>	- Water Activity
<i>BB</i>	- Brilliant Blue
<i>BOD</i>	- Biochemical Oxygen Demand
<i>BPA</i>	- Bisphenol A
<i>BPB</i>	- Bromophenol Blue
<i>BSA</i>	- Bovine Serum Albumin
<i>CBX</i>	- Cationic Blue X-Grl
<i>CCD</i>	- Central Composite Design
<i>CCZ</i>	- Cup-Plate Clearing Zone
<i>CM</i>	- Cell Membrane
<i>COD</i>	- Chemical Oxygen Demand
<i>CR</i>	- Congo Red
<i>CSB</i>	- Chicago Sky Blue
<i>CW</i>	- Cell Wall
<i>CY</i>	- Cationic Yellow 28
<i>CYA</i>	- Czapek Yeast Extract Agar
<i>CZ</i>	- Czapek-Dox Agar
<i>CZR</i>	- Cz Medium With Rose Bengal
<i>DMP</i>	- 2,6-Dimethoxylphenol

<i>DMSO</i>	- Dimethyl Sulphoxide
<i>DR</i>	- Direct Red 28
<i>DW</i>	- Distilled Water
<i>DY</i>	- Direct Yellow 12
<i>EVA</i>	- Pumpkin Medium (Eva)
<i>FA</i>	- Ferulic Acid
<i>FESEM-</i>	- Field Emission Scanning Electron Microscopy With Energy
<i>EDX</i>	Dispersive X-Ray Spectroscopy
<i>FTIR</i>	- Fourier Transform Infrared Spectroscopy
<i>GA</i>	- Gallic Acid
<i>GC-MS</i>	- Gas Chromatography-Mass Spectrometry
<i>HABs</i>	- Harmful Algae Blooms
<i>HBT</i>	- 1-Hydroxy-Benzotriazole
<i>HPLC</i>	- High Performance Liquid Chromatography
<i>IC</i>	- Indigo Carmine
<i>IU</i>	- International Units
<i>LiP</i>	- Lignin Peroxidase
<i>MB</i>	- Methylene Blue
<i>MEA</i>	- Malt Extract Agar
<i>MG</i>	- Malachite Green
<i>MHA</i>	- Müller-Hinton Agar
<i>MnP</i>	- Manganese Peroxidase
<i>MO</i>	- Methyl Orange
<i>NMR</i>	- Nuclear Magnetic Resonance
<i>NP</i>	- Nonylphenol
<i>NPs</i>	- Nanoparticles
<i>PDA</i>	- Potato Dextrose Agar
<i>PDR</i>	- Polymeric Dye R

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