IMMOBILISATION OF *PHANEROCHAETE CHRYSOSPORIUM* IN PVA-ALGINATE-SULPHATE BEADS FOR DECOLOURIZATION OF TEXTILE EFFLUENTS

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To my beloved family:

Ahmad Nasir Bin Khosri

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ABSTRACT

Textiles industry is one of the main sources that contribute to water pollution due to the release of substances such as wax, surfactant and dyes into water bodies. Until now, many methods for textile effluents treatments were introduced. Unfortunately, most of them are expensive and ineffective. Recently, the ability of microorganisms as an alternative to overcome these problems has attracted attentions. In this study, textile effluents were treated with immobilised the white-rot fungi, *Phanerochaete chrysosporium* cells in PVA-alginate-sulphate beads. Optimisation conducted using Design Expert 6.0.4 software. A two level factorial with three factors programme was used for the optimisation. The efficiency of this system was determined by several tests such as colour and COD reduction, enzymatic activities, reusability, storage stability and toxicity test. Results showed that the optimum colour reduction and enzyme activity was achieved at 37 ºC, 10 g of immobilised cells and 300 mg/L dyes concentration. At the optimum condition, immobilised cells were able to decolourize dyes and reduced COD content up to 50% and 33% higher compared to free cells. Meanwhile, the enzyme activity of immobilised cells was doubled as compared to free cells. The suggested model equation are significant since the predicted and actual value show percentage of error less than 5% for colour, COD reduction and enzymatic activity. Reusability test showed the immobilised cells could be reused up to four times to treat dyes effluents. For the stability test, the ability of immobilised cells to reduce dyes effluents decreased to about 10% after two-month storage as compared to free cells which decreased to about 25%. Toxicity test proved that immobilised cells could reduced the toxicity level up to 4% higher than free cells. In conclusion, *P.chrysosporium* was successfully immobilised in PVA-alginate-sulphate beads and could serve as a potential method for dyes effluents treatment.

ABSTRAK

Industri tekstil adalah satu sumber utama yang menyumbang kepada pencemaran air akibat pelepasan bahan seperti lilin, surfaktan dan pewarna ke dalam air. Sehingga kini, pelbagai cara untuk merawat sisa tekstil telah diperkenalkan. Namun, kebanyakan adalah mahal dan tidak berkesan. Kini, mikroorganisma dilihat sebagai alternatif untuk mengatasi pemasalahan ini. Dalam kajian ini, kulat reput-putih, *Phanerochaete chrysosporium* telah disekat gerak ke dalam manik PVA-alginate-sulfat untuk merawat sisa tekstil. Pengoptimuman rawatan sisa tekstil dijalankan menggunakan perisian Design Expert 6.0.4, program tahap dua faktorial dengan tiga faktor. Kecekapan sistem ini ditentukan dengan ujian nyah warna, COD, aktiviti enzim, guna semula, kestabilan penyimpanan dan ujian ketoksikan. Keputusan menunjukkan nyah warna dan aktiviti enzim yang optimum dicapai pada 37 ºC, 10 g sel sekat gerak dan 300 mg/L kepekatan warna. Pada keadaan optimum, sel sekat gerak dapat menyahkan warna dan mengurangkan kandungan COD sehingga 50% dan 33% lebih tinggi berbanding dengan sel bebas. Sementara itu, aktiviti enzim sel sekat gerak juga dua kali lebih tinggi berbanding dengan sel bebas. Model persamaan yang dicadangkan adalah tepat kerana nilai ramalan dan sebenar menunjukkan peratusan ralat kurang daripada 5% bagi ujian nyah warna, COD dan aktiviti enzim. Ujian guna semula menunjukkan sel sekat gerak boleh digunakan semula sehingga empat kali untuk merawat sisa tekstil. Dalam ujian kestabilan, keupayaan sel sekat gerak merawat sisa tekstil telah menurun sebanyak 10% selepas dua bulan penyimpanan berbanding keupayaan sel bebas telah menurun sehingga 25%. Ujian ketoksikan membuktikan sel sekat gerak boleh mengurangkan ketoksikan sehingga 4% lebih tinggi berbanding sel bebas. Kesimpulannya, *P. chrysosporium* berjaya di sekat gerak ke dalam PVA-alginate-sulfat dan berpotensi untuk merawat sisa tekstil.

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LIST OF ABBREVIATIONS

A_0	Absorbance in a mean time
A_1	Absorbance of initial dyes
A_t	Absorbance at time‘*t*’
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ADMI	American Dyes Manufacturing Index
C_0	Initial concentration of dyes
COD	Chemical Oxygen Demand
g	Gram
m	Dry mass of beads after adsorption
mg	Milligram
mg/L	Milligram/Liter
mL	Milliliter
ºC	degree Celsius
PAHs	Polycyclic aromatic hydrocarbon
PDA	Potato Dextrose Agar
rpm	Revolutions per minute
SEM	Scanning Electron Microscopy
v/v	Volume/ Volume
V_0	Initial volume of culture medium
w/v	Weight/volume

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Dyes are widely used as colouring agent in textile, leather, paper, plastic and food industries (Ali and El-Mohamedy, 2010). Approximately 10,000 different dyes and pigments are produce annually worldwide with a volume of more than 7×10^5 tonnes (Devecci *et al*., 2004). Meanwhile, data shows up to 10% of dyes were found in water bodies as wastes (Devecci *et al*., 2004). Textiles industry is identified as one of the industries that contribute to serious water pollution. This is caused by the releasing of substances such as dyes, wax and surfactant in water bodies (Ali and El-Mohamedy, 2010). These chemicals are used for desizing, scouring, bleaching, dyeing, printing and finishing. Thus, opportunities exist for the release of potentially hazardous compounds into ecosystems at various stages of the operation. Usually, the substances are then released to the nearest water bodies without proper treatment.

Various techniques had been proposed in treating textile effluents (Couto, 2009). Attention has shifted towards biological method in textile effluent treatment. It is a result in pursuing the most economical, environmental friendly and efficient technique (Suhaimi, 2010). Basidiomycetes fungi is commonly use in biological method to degrade wide range of different synthetic dyes.

Basidiomycetes fungi is a white rot fungi. This type of fungi has the ability to secrete extracellular lignin-modifying enzymes. These enzymes have low substrate specificity and able to degrade a wide range of xenobiotic compound (Barr and Aust, 1994; Pointing, 2001) including dyes (Glenn and Gold, 1983; Pasti-Grigsby *et al*., 1992; Paszczynski *et al*., 1992; Spadaro *et al*., 1992). Basidiomycetes are considered as the most developed class of fungi because of these specialties (Kunz *et al*., 2001).

The efficiency and effectiveness of dyes decolourization through microorganisms can be enhanced by cell immobilisation (Chen *et al*., 2003). This is due to the protection served by immobilisation matrix towards cells from harsh environment such as high acidity and extreme temperature. The immobilisation matrix would also provide a surrounding that mimics cell natural habitat by stimulating ligninolytic enzymes production, depending on the nature of support matrix (Suhaimi, 2010). Moreover, immobilised cells are able to be reused up to several times thus resulting in decreasing the operational cost.

Basically, immobilised matrix can be divided into synthetic and natural polymer. One of the most natural polymers that widely used is alginate which exists naturally in the cell wall of brown algae. Alginate offers lots of advantages such as availability, non-toxic to microorganisms, cost-effective and eases to handle (Suhaimi, 2010). Unfortunately, alginate is susceptible to their counter ion, such as phosphate that would let them disintegrated resulting the failure of the gelation process.

A synthetic polymer which is Polyvinyl alcohol (PVA) which is a synthetic polymer had been widely used to replace alginate due to its properties. It offers advantageous likes alginates, which are non-toxic to microorganisms, economic and ease to use. The use of PVA as immobilisation matrix seems as an alternative.

Thus, the idea of this study is to combine the advantages offer by PVA and white rot fungi to treat the textile effluents with the improvement of the existing technique.

1.2 Problem Statements

In order to fulfil human demand, dyes are designed to be recalcitrant and resistant when expose to light, sweat, water, chemical and microbial attack. However, certain dyes used are toxic, mutagenic and carcinogenic (Chung *et al*., 1992). During textiles' processing, about 40% of used dyes are release into the water bodies (Faraco *et al*., 2009). Used dyes might contain grease, wax, heavy metal, surfactant and suspended solid (Ahmad *et al*., 2002). Thus, without improper management and treatment, textile effluents can cause serious water pollution to the ecosystems. Dyes released might hinder sunlight to penetrate water bodies, retarding the photosynthesis process and inhibit the growth of aquatic biota and affects gas solubility in water bodies.

Undeniable, the existing chemical and physical methods used to treat textile effluents are effective. However, they also pose drawbacks such as cost consuming, high sludge formation and generate toxic by-products (Couto, 2009). Meanwhile, biological approach by using bacteria would be unfeasible due to the production of colourless dead-aromatic amines by certain bacteria which is more toxic than the parental compounds (Couto, 2009). Thus, a quick, efficient and cost effective approach is necessary to overcome these problems.

1.3 Objectives

The objectives of this study are:

1. To immobilize *Phanerochaete chrysosporium* into PVA-alginate-sulphate beads.
2. To remediate dyes in shake flask using the immobilised cells and compare the degradation with free cells.

3. To optimize the physical parameter for the immobilised cells to treat textile effluents by using Design Expert 6.0.4 software.
4. To study the reusability and stability of the immobilised cells.
5. To examine the toxicity level of treated textile effluents.

1.4 Scope of Research

This study investigates the ability of *Phanerochaete chrysosporium* to decolourize textile effluents collected from Razali Batik, Kelantan. PVA-alginate-sulphate beads used were a modified version from pervious works (Idris *et al*., 2008).

The ability of this immobilised fungus to decolourize and degrade textile dyes was examined in several tests such as American Dyes Manufacturer's Institute (ADMI) reduction, Chemical Oxygen Demand (COD) reduction and enzymatic activities. Design Expert Software 6.0.4 was used as a statistical method to optimize the physical parameter for immobilised *Phanerochaete chrysosporium*. Scanning Electron Microscope (SEM) was used to study the morphology of the matrix and immobilised fungus. In addition, reusability, storage stability and toxicity tests were conducted to ensure the effectiveness of this immobilisation matrix.

1.5 Significant of Research

This study highlights the ability of *Phanerochaete chrysosporium* immobilised in PVA-alginate-sulphate beads in textile effluents treatment. Previously, this immobilisation matrix had successfully immobilised an enzymes, enhanced the beads' shapes, resulting the best surface area for the cells, reduce cell leakage and cells agglomeration (Idris *et al*., 2008). Additionally, immobilised cells are proven to give better results for dye decolourization compared to free cells. Immobilised cells also give better COD reduction, higher enzymatic activities, reusability, more stable in longer period of storage and lower toxicity level compared to free cells (Wang *et al*., 2007).

Thus, the ability of immobilised *Phanerochaete chrysosporium* in treating textile effluents will be beneficial since this method is inexpensive and easy to handle at the industrial scale (Wang *et al*., 2007). The successfulness of this research would be an outstanding knowledge to treat textile effluents in the future.

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