A COMPARITIVE STUDY ON IMMOBILISED MYCELIUM AND SPORES OF *P. CHYRISOSPORIUM* IN PVA-ALGINATE-SULFATE BEADS FOR TEXTILE DYES EFFLUENT TREATMENT.

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Specially dedicated to To my beloved family:

Espindada and Ami

Shah Khalid

Shah Hassan

Naveed-u-llah khan

Bilal ahmad khan

Azan

And fiancée

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ABSTRACT

Effluents discharging from the textile and dye industries to the neighbouring water are causing serious health concern to the environment and are getting attention of water regulatory agencies. Among the recent proposed treatment methods for treatment of textile effluents biological treatment has gain more attention because of its cheap and effective approach. Biotreatment with white rot fungi seems to be a viable option in the existing biological treatment process. This study, investigates the treatment of textile effluents with immobilized spores and mycelium of *Phanerochaete chrysosporium* separately in PVA-alginate-sulfate beads. Screening for the dye discoloration was done by using Design Expert 9.0.3.1. Screening process was conducted by using a two level factorial programmed with three factors namely temperature, number of beads loaded and initial dye concentration. The responses namely colour and COD reduction, enzymatic activities, reusability, storage stability and toxicity were thoroughly investigated to determine the system efficiency. Results revealed that optimum colour reduction and enzyme activity was achieved in immobilized spores compared to immobilize mycelium at 37 ^{o}C, 10gm beads and 300 mg/L dye concentration also the enzyme activity was comparatively high in immobilized spores as compared to immobilized mycelium. The reusability test also revealed that the immobilized fungus could be reused for up to 5 times to treat dye effluents. Toxicity test also proved the ability of immobilized cells in reduction of toxicity level. In conclusion, spores and mycelium were successfully immobilized in PVA-alginate-sulfate beads and they both serve as a potential mean and methods for treating textile dye effluents.

ABSTRAK

Sisa buangan daripada industri tekstil dan pewarna terhadap air persekitaran akan menyebabkan masalah kesihatan yang serius dan ianya telah mendapat perhatian daripada agensi kawal selia air. Antara kaedah rawatan terbaru yang telah dicadangkan ialah melalui rawatan biologi kerana ianya murah dan berkesan. Rawatan menggunakan kulat reput putih merupakan pilihan yang baik bagi proses rawatan secara biologi yang sedia ada. Projek ini mengkaji rawatan sisa buangan tekstil menggunakan spora dan miselium daripada *Phanerochaete chrysosporium* yang telah disekat gerak secara berasingan dalam manik PVA-alginat-sulfat. Saringan bagi perubahan warna sisa buangan pewarna dilakukan dengan menggunakan Design Expert 9.0.3.1. Proses pemeriksaan telah dijalankan dengan menggunakan tahap dua factorial yang diprogramkan bersama tiga faktor iaitu suhu, jumlah manik yang digunakan dan kepekatan pewarna. Tindak balas dikaji melalui warna dan pengurangan COD, aktiviti enzim, penggunaan semula, kestabilan penyimpanan dan ketoksikan. Ianya telah dikaji dengan teliti bagi menentukan kecekapan sistem yang digunakan. Hasil kajian menunjukkan bahawa pengurangan warna dan enzim aktiviti yang optimum telah dicapai menggunakan spora tersekat gerak berbanding miselium tersekat gerak pada keadaan 37 °C, 10 g manik dan kepekatan pewarna pada 300 mg / L dimana aktiviti enzim adalah agak tinggi pada spora tersekat gerak berbanding miselium tersekat gerak. Ujian kebolehgunaan manik juga mendedahkan bahawa kulat tersekat gerak boleh digunakan sehingga 5 kali bagi merawat sisa buangan pewarna. Ujian ketoksikan juga membuktikan keupayaan sel yang tersekat gerak terhadap pengurangan tahap ketoksikan. Kesimpulannya, spora dan miselium telah berjaya disekat gerak pada manik PVA-alginat-sulfat dan ianya berpotensi bagi merawat sisa buangan tekstil dan pewarna.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Textile, leather, paper, and food industries are widely using dyes as a colouring agent (Ali and El-Mohamedy, 2012). The textile industry is a diverse sector in terms of production of raw materials, operating processes, product development, and equipment. Textile industries are well-characterized for consuming large quantities of water, energy, and discharging high volumes of waste in to public sewage treatment plants. Textile industry uses high volumes of water from the beginning of washing of fibbers until washing of finished products. Subsequently, produces enormous volumes of wastewater which also contaminated with variety of chemicals including Azo dyes, formaldehyde, dioxins and heavy metals such as copper and chromium that might harm human and environment (Le Marechal *et al.*, 2012; Solís *et al.*, 2012).So without prior treatment of dyes effluents before discharging to water would effects the sunlight penetration negatively which in turn will slow down the photosynthesis process and will alter the gas solubility in water (Egli, 2007).

To treat the textiles effluents various techniques had been proposed (Rodríguez Couto, 2009). Biological method gain the attention in textile effluent treatment because of its no deteriorating effect on the environment (Sani *et al.*, 1998). Basidiomycetes fungi is commonly use in biological method to degrade wide range of different synthetic dyes.

The ability of fungi to rapidly adapt their metabolism to varying carbon and nitrogen sources is important for their survival. This metabolic activity is achieved through the production of a large set of intra and extracellular enzymes able to degrade various complex organic pollutants such as polyaromatic hydrocarbons, organic waste, dye effluents and steroid compounds (Pointing, 2001) (Glenn and Gold, 1983) (Cripps *et al.*, 1990). In the past few decades white-rot fungi has been evaluated for their ability to degrade polyaromatic hydrocarbons and discoloration of textile synthetic dyes (Nozaki *et al.*, 2008; Reddy, 1995; Wesenberg *et al.*, 2003).

The dye discolorization rate and effectiveness through microorganisms can be enhanced by cell immobilization (Nicell, 2003). Cell in Immobilizated form gives better operational stability and higher efficiency (Iqbal and Saeed, 2007). The immobilization matrix stimulate ligninolytic enzymes production by providing a surrounding that mimics cell natural habitat, depending on the nature of support matrix (Suhaimi, 2010)

Polyvinyl alcohol (PVA) can be used for cell immobilization because recent researches had showed superior mechanical strength and chemical stability of the PVA- immobilized biomass (Idris *et al.*, 2008; Iqbal and Saeed, 2007) . PVA has these unique properties that it is highly stable, non-toxic to organism and can be produced cheaply at industrial scale (Lozinsky and Plieva, 1998). This study aims to investigate and compare the combine advantages offered by PVA, spores and mycelium of white rot fungi to treat the textile effluents.

1.2 Problem Statement

The textile industries uses dyes which are designed to be recalcitrant and resistant when expose to light, sweat, and water, chemical and microbial attack for a better quality in order to fulfill the needs of humans. Dye color found in effluents would directly give rise to water pollution, and discharged of such highly concentrated contaminant can cause toxic, mutagenic and carcinogenic effects (Chung *et al.*, 1992). In textile industries during processing, about 40% of used dyes are released into the water (Faraco *et al.*, 2009). The dyes which are used in textile might contain grease, wax, heavy metal, surfactants and suspended solid (Ahmad *et al.*, 2012). So without prior treatment of dyes effluents before discharging to water would effects the sunlight penetration negatively which in turn will slow down the photosynthesis process and growth of aquatic biota and will alter the gas solubility in water (Egli, 2007).

Alternative chemical and physical technologies have been reported for cleanup of contaminated textile waste water. But these techniques have certain limitations such as most of them are expensive and produce a huge amount of sludge (Robinson *et al.*, 2001). Thus, to overcome these limitations and problems a quick and cost effective approach is needed.

1.3 Objectives

The specific objectives of this research are as follows:

(a) To immobilize spore and mycelium of P. *chrysosporium* in PVA-alginate-sulfate beads.

(b) To treat textile dyes effluent using immobilized spore and mycelium of *P. chrysosporium* and to compare the degradability ability of both.

(c) To optimize the physical parameter for textile effluents treatment using designs expert method.

1.4 Scope of Research

This research study investigates the *Phanerochaete chrysosporium spores* and mycelium's ability to decolorize textile dye effluents. PVA-alginate- sulfate beads used were a modified version from pervious works (Idris *et al.*, 2008)

To test and compare the decolorizing and degrading ability of immobilized mycelium and spores different test were performed such as American Dyes Manufacturer's Institute (ADMI) reduction, Chemical Oxygen Demand (COD) and enzymatic activities. To optimize the physical parameters for the immobilized spores and mycelium a statistical tool Design Expert 9.0.3 was used. For examing the morphology of immobilized mycelium and spores of *Phanerochaete chrysosporium* Scanning Electron Microscope (SEM) was used. For ensuring the effectiveness of the immobilized matrix we also performed the reusability, toxicity storage stability and cell leakage tests.

1.5 Significant of Research

This study highlights the immobilization of spores and mycelium of *Phanerochaete chrysosporium* in PVA-alginate-sulfate beads in treating textile effluents. Previously researchers have used this immobilization matrix, successfully for immobilizing an enzymes (Idris *et al.*, 2008), and *Phanerochaete chrysosporium* (Idris *et al.*, 2013) and it enhanced the beads' shapes, resulting the best surface area for the cells, reduce cell leakage and cells agglomeration. This study will ascertain between immobilized spores and mycelium of *Phanerochaete chrysosporium* which one will be more able to increase the discolorization rate compared to one another.

The research if successfully accomplished will provide a better understanding of treating the dye effluent of textile waste water and possibly can be add catalogue of the already identified biological treatment methods for dye efflue can serve as seeds for bio-augmentation in biodegradation of textile waste wate..

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