APPLICATION OF NANOSTRUCTURED MICROBIAL CATALYSTS FOR BIOREMEDIATION OF SOME ORGANIC WASTES

By

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B.Sc. Agric. Sci. (Soil Science), Fac. Agric., Cairo Univ., 2002
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APPROVAL SHEET

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SUPERVISION SHEET

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ABSTRACT

This study focuses on the isolation, screening of bacterial isolates and azo dye removal by bacteria isolated from dumping site of textile industry wastewater as well as using nanotechnology applications to enhance the bioremediation process. Fourteen isolates were isolated and used for further experiments. One of them was the most efficient in decolourization of Reactive Blue (RB) azo dye. This isolate was identified by morphological, biochemical and 16s rDNA sequences as *Pseudomonas aeruginosa* strain OS4 (NCBI accession number: KC762943). Bioremediation of azo dyes was found to occur efficiently under two sequential phases; starting with anoxic phase and followed by aerobic one.

A prototype bioreactor with dual oxygenation levels (anoxic and aerobic) was designed for the bioremediation of RB dye residues. The bioreactor contained 2 compartments; Upflow fixed-film column (UFC) and continuously stirred aerobic (CSA) container. *Pseudomonas aeruginosa* strain OS4 was applied to both compartments either as free or immobilized bacterial cells. The bioreactor operated at flow rate of 50 ml h^{-1} with HRT of 20 h. The CSA was directly fed in continuous mode with the output of UFC. The sequential anoxic–aerobic treatment of wastewater at dilution rate of 10% of wastewater with 90% water resulted in 99% decolourization. The activities of eight enzymes were assessed under anoxic and aerobic conditions. Azoreductase enzyme gave high activity under anoxic conditions only. The induction of Laccase, Mn Peroxidase and Lignin Peroxidase was observed under both anoxic and aerobic conditions. Four other enzymes; Polyphenol oxidase, Tyrosinase, Pyrochatechase and Ascorbate oxidase were activated under aerobic conditions only. The UV–visible spectrophotometer, TLC and HPLC analyses were used to assess the biotransformation of the dye into non-aromatic intermediate compounds.

The Lignin Peroxidase enzyme was partially purified. Magnetic (Fe_3O_4) nanoparticles were prepared by co-precipitation technique. The size and shape of Fe_3O_4 was examined by TEM and the average particles size was 16- 20 nm. The surface charges of Fe_3O_4 magnetic nanoparticles were modified using Glutaraldehyde as cross-linker. These active groups support the Covalant bioconjucation properties of enzyme immobilization. The modified Fe_3O_4 was used in immobilization and stabilization of LiP enzyme. The immobilized LiP was stable until 100 ˚C and pH 12. The immobilized enzyme-regeneration cycle were performed 20 times

Key words: *Pseudomonas aeruginosa* strain OS4, prototype bioreactor, bioremediation, RB azo dye, 16s DNA sequences, enzyme, immobilization, nanotechnology.

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CONTENTS

INTRODUCTION

Industrialization is vital to a nation's economy because it serves as a vehicle for the community development. The major problem associated with some industries is an introduction of the industrial effluent containing waste products into the environment. Many of these products are problematic because of its persistence, low biodegradability and high toxicity. The textile industry is among the largest industries in Egypt. This industry does not have any efficient effluent treatment technology and produces effluents containing several types of chemicals such as dispersants, acids, alkalis, carriers and various dyes. Due to lack of efficient treatment technology, partially treated textile industrial effluent thrown into the drinking water resources leads to environmental as well as aesthetic problems. The treatment of textile dyes containing wastewaters still presents a technical challenge. As regulations are becoming even more stringent, there is urgent need of technically feasible and cost effective methods. The stability and the xenobiotic nature of the azo dyes make them recalcitrant hence they are not totally degraded by conventional wastewater treatment processes that involve light, chemicals or activated sludge. The remediation of textile industry wastewater before releasing into the surrounding environment is of significance particularly for the safety of the agricultural ecosystem nearby the textile industries. The recalcitrant nature of these chemicals requires special technologies to remove and/or reduce the risks associated with their discharge into the environment without treatment. Therefore, the

bioremediation of these toxic dyes is among the important approaches for remediation of textile industry wastewater.

Compared to classical bioremediation approaches, the enzymes from bio-agents offer vast capabilities in the area of pollutants removal. Their specificity and targeted effectiveness make them much more effective than synthetic catalysts. However, the lack of stability, difficulty of reuse inhibits their ability to provide cost effective options. To solve these problems, enzyme immobilization technology will be used in this study to improve the stability and subsequent persistence of enzymes for bioremediation processes. Since nanoparticles are promising carrier for immobilization, magnetic nanoparticles will be prepared and modified to enhance conjugation between enzymes and nanoparticles. Immobilized enzymes are expected to withstand more extreme conditions, such as high/low pH, high contaminant concentration, high salinity, and high/low temperature. In this study, enzymes bound to nanoparticles for treatments of textile azo dyes causing threat to agricultural ecosystem is one of the goals that are striven for. In this respected, the immobilized enzyme will be applied for dyestuff treatment.

REVIEW OF LITERATURE

1. Textile wastewater: characteristics and environmental impact

Dye wastewater from textile mills is a serious pollution problem because it is high in organic content (Table 1) (Imtiazuddin *et al.*, 2002). A dye is a coloured substance that can be applied in solution or dispersion to a substrate in textile manufacturing, thus giving a colour appearance to textile materials. Discharging of dyes into water resources even in a small amount can affect the aquatic life and food web (Ahmed *et al.*, 2012).

One of the main problems regarding textile wastewaters is the coloured effluent. The coloured effluent contains visible pollutants. The primary concern about effluent colour is not only its toxicity but also its undesirable aesthetic impact on receiving waters (Imtiazuddin *et al.*, 2002). Non-biodegradable nature of most of the dyes reducing aquatic diversity by blocking the passage of sunlight through the water represents serious ecological problems to the environment. In some cases, dyes in low concentration are harmful to aquatic life. Since many dyes have adverse effect on human beings, the removal of colour from the effluent or process has appeared of importance for ensuring healthy environment (Imtiazuddin *et al.*, 2002). Textile finishing wastewater, especially dyehouse effluents contain different classes of organic dyes, chemicals and auxiliaries (Table 1) thus they are coloured and have extreme pH, COD, BOD and AOX values and they contain different

salts, surfactants, heavy metals, mineral oils and others (Golob *et al.*, 2005).

Table 1. Major pollutant, chemical types and process of origin in textile wastewater.

Pollutants	Chemical types	Process of origin
Organic load	Starches, enzymes, fats, greases, waxes, surfactants and acetic acid	Desizing, Scouring, Washing, Dyeing
Colour	Dyes, scoured wool impurities	Dyeing, Scouring
Nutrients (N, P)	Ammonium salts, urea, phosphate-based buffers and sequestrants	Dyeing
pH and salts	NaOH, mineral/organic acids, sodium chloride, silicate, sulphate, carbonate	Scouring, Desizing, Bleaching, Mercerising, Dyeing, Neutralisation
Sulphur	Sulphate, sulphite and hydrosulphite salts, sulphuric acid	Dyeing
Toxic compounds	Heavy metals, reducing agents (sulphide), oxidising agents (chlorite, peroxide, dichromate, persulphate), biocides, quaternary ammonium salts	Desizing, Bleaching, Dyeing, Finishing
Refractory organics	Surfactants, dyes, resins, synthetic sizes (PVA), chlorinated organic compounds, carrier organic solvents	Scouring, Desizing, Bleaching, Dyeing, Washing, Finishing

Somasiri *et al.* (2006) reported that a composite wastewater from an integrated textile plant consist of following materials: starches, dextrin, gums, glucose, waxes, pectin, alcohol, fatty acids, acetic acid, soap, detergents, sodium hydroxide, carbonates, sulphides, sulphites, chlorides, dyes, pigments, carboxymethyl cellulose, gelatin, peroxides, silicons, flourcarbons and resins. Meanwhile, Karim *et al.* (2006) indicated that the colour of reactive dyes is due to the presence of N=N

azo bonds and chromophoric groups. They added that such dyes are first absorbed on the cellulosic and then fibre and that offer the fixation of these dyes on the fibre about 10-15% of initial loading is present in the dye bath effluent. Reactive dyes in both ordinary and hydrolyzed forms are not easily biodegradable even after treatment and thus colour may be present in the effluent. In this respect, Santhy and Selvapathy (2006) found that the conventional processes such as coagulation, flocculation and biological methods adopted for decolouration of effluent containing reactive dyes were no longer able to achieve adequate colour removal. While, under anaerobic conditions foul smelling compound such as hydrogen sulphides may be produced. This will consequently upset the biological activity in the receiving stream (Ramalho, 2005).

The textile industry requires large volumes of water during the printing and dyeing processes and therefore produces great quantities of wastewater that is often discarded into fresh water sources in developing nations, such as Egypt. These effluents, or waste products, contain dyes such as indigo and azoic as well as large quantities of heavy metals, bleaching agents and acids, which are extremely toxic to all living creatures. The environmental effects of the textile industry have increased in a negative way during the last decade with the introduction of “fast fashion,” in which the media introduce new seasonal trends for each fashion season (Bhimani, 2011).

2. History of dyes

The first use of dye in ancient Egypt can be traced to a red dyed fragment found at the Medium site from the third or fourth dynasty