DECOLORIZATION OF ORANGE II DYE USING BACTERIA IMMOBILIZED ONTO MULTI-WALLED CARBON NANOTUBE CEMENT COMPOSITE

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I would like to dedicate this
thesis to my ever-supportive family
and my supervisor Dr. Shafinaz Shahir who
have been my great inspiration in completing this thesis

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ABSTRACT

In this study, decolorization of Orange II dye using *Enterococcus faecalis* immobilized onto multi-walled carbon nanotube (MWCNT) cement composite was assessed. Multi-walled carbon nanotube (MWCNT) cement composites were made by mixing zeolite (40g), cement (12g), water (8ml) and MWCNT (0.6g) for attachment of biofilm. Biofilm was allowed to develop on the biocomposites for 7 days. The efficiency of decolorization of orange II dye was examined in the presence and absence of biofilm. By comparing the system with and without biofilm, the system without biofilm (using free suspended cells) achieved the highest percentage removal of orange II dye (97.69%). Unexpectedly the lowest percentage of dye decolorization was achieved for the system using cells immobilized onto Hardened Cement Paste + MWCNT (72.74%). This was most likely due to the cytotoxic effect of MWCNT on the bacterial cells. As expected maximum decolorization for all the systems occurred in anaerobic condition and after entering the aerobic condition decolorization had reached a steady state because of the competition between oxygen and dye.

ABSTRAK

Dalam kajian ini, penyahwarnaan pewarna *Orange II* menggunakan *Enterococcus faecalis* yang disekat gerak pada tiub nano karbon berdinding ganda (*MWCNT*) simen komposit telah dikaji. *MWCNT* simen komposit telah dibuat dengan mencampurkan zeolit (40 g), simen (12 g), air (8 mL) dan MWCNT (0.6 g). Lapisan biofilem telah dibangunkan pada komposit selama 7 hari. Kecekapan penyahwarnaan pewarna *Orange II* telah dikaji dalam kehadiran dan ketiadaan biofilem. Kajian mendapati bahawa sistem tanpa biofilem (menggunakan sel bebas) mencapai peratusan penyingkiran tertinggi pewarna *Orange II* (97,69%) manakalaperatusan terendah telah dicapai untuk sistem menggunakan sel-sel yang telah disekat gerak dan membentuk biofilem pada *MWCNT* simen komposit (72,74%). Pencapaian peratusan penyahwarnaan yang rendah ini mungkin disebabkan oleh kesan sitotoksik *MWCNT* pada sel-sel bakteria. Sebagaimana yang dijangka proses penyahwarnaan maksimum telah dicapai dalam keadaan anaerobik. Walaubagaimanpun proses ini telah mencapai keadaan mantap dalam keadaan aerobik akibat persaingan antara oksigen dan pewarna.

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INTRODUCTION

1.1 Introduction

Textile wastewater as one of the industrial wastes always draws attention of researchers and scientists. Textile industries are described by high water and chemical consumption as a result of different processes which give rise to noticeable amounts of colored wastewater. Their conventional treatment like the other waste has some steps and is mostly physico-chemical and biological. Textile companies may face a lack of available water sources due to water insufficiency and restrictions of ground water use. In the near future, many textile companies will have to improve wastewater quality to the fresh water standards for reuse purpose.

Tendency to use biological methods has increased in recent years, because of their ability for removing the organic materials and the problems that chemicals cause to nature. Furthermore for using microorganisms which can degrade these waste, some methods have been suggested but most of them are time consuming, expensive and low efficient versus chemicals.

Dyes are the visual part of textile wastewater and because of that take most attention at first glance and the usual methods are not efficient in decolorizing them and it seems that microorganisms are more important in this part. Types of the dyes

according to their application and chemical composition are diverse and due that we need to use different kind of microorganisms.

Because of that, biological methods should be consist either of one species of special microorganisms or of various kinds of different microorganisms that can establish an ecosystem suitable for dye omitting. At the end dyes can be degraded into simpler compounds and are finally mineralized to water and carbon dioxide by a wide variety of aerobic or anaerobic organisms.

Microorganisms need time, stability and physical support to work efficiently on wastes from effluent and for this matter one of the method is immobilizing enzyme or cell to greatly restrict the freedom of movement of them. So immobilization provides a physical support for cells. Therefore the first consideration is to decide on the support material and then the main method of immobilization.

Lately the use of nanomaterials in bio-related research has increased sharply. Nanomaterials due to their dimension have specific characteristics which make them valuable and provide new ideas for novel research.

1.2 Background of Study

No ideal support material or method of immobilization has emerged to provide a standard for each type of immobilization. Selection of support material and method of immobilization is made by measuring the various attributes of the enzyme or cell application against the properties and limitations of the combined immobilization and support. Several practical aspects should be considered before starting laboratory work to ensure that the final immobilized enzyme or cell preparation is suitable for the planned purpose or application and will operate at optimum effectiveness.

Different methods of immobilization can influence the activity and half-life of a cell-based biotransformation. There are five principal methods for immobilization of enzymes or cells: adsorption, covalent binding, entrapment, encapsulation, and cross-linking.

There are many different support materials such as calcium alginate, polyacrylamide, DEAE-cellulose, glutaraldehyde and agar. Moreover in some research solid and porous materials like sponge have been used. Recently by the emerging and development of nanotechnology, the interest of using them in environmental research regarding to their unique properties has increased.

Azo dyes are the most important groups of synthetic dyes. It had been estimated that about 10% of the dye stuff used during the dyeing processes does not bind to the fibers and is released into the effluents. Because all the industrially produced azo dyes are xenobiotic compounds, it is not surprising that they are recalcitrant in conventional treatment methods (Steffan *et al.*, 2005).

1.3 Objectives of the Study

- To immobilize dye degrading bacterial cells onto hardened cement paste with multi-walled carbon nanotubes (MWCNT).

- To compare the ability of freely suspended and immobilized hardened cement paste with MWCNT to degrade Orange II dye.

1.4 Scope of the Study

The study was concentrated on the decolorization of pure dye solution of Orange II. Composite of zeolite, cement and MWCNT were used to immobilize bacterial cells (*Enterococcus faecalis*) which are able to degrade Orange II. Dye decolorization, pH, optical density (OD) and chemical oxygen demand (COD) were determined and analyzed by standard methods.

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