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

MOHAMMAD VOJDANI

A dissertation submitted in partial fulfillment of the requirements for the award of the degree of
Master of Science (Biotechnology)

Faculty of Biosciences and Bioengineering
Universiti Teknologi Malaysia

JULY 2012
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.
ACKNOWLEDGEMENT

First and foremost, I want to thank my dear supervisor Dr. Shafinaz Shahir and dear co-supervisor Dr. Zaiton Abdul Majid for their kindness and guidance throughout my entire research. Their encouragement, advices and semi daily observations on my work gave me the inspiration to keep on the right direction during my research project. Without them I could never accomplish my project smoothly. I would also like to thank all those who were involved directly or indirectly in the completion of this project. Last but not least, I would like to express my utmost appreciation for my lovely Mom and Dad who have given me all that I have and my lovely and kind siblings for their love and support of my decision and the path I choose. Indeed they are the best people in my life.
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 biofilm telah dibangunkan pada komposit selama 7 hari. Kecepatan penyahwarnaan pewarna Orange II telah dikaji dalam kehadiran dan ketiadaan biofilm. Kajian mendapati bahawa sistem tanpa biofilm (menggunakan sel bebas) mencapai peratusan penyingkiran tertinggi pewarna Orange II (97,69%) manakala peratusan terendah telah dicapai untuk sistem menggunakan sel-sel yang telah disekat gerak dan membentuk biofilm 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. Walaubagaimanapun proses ini telah mencapai keadaan mantap dalam keadaan aerobik akibat persaingan antara oksigen dan pewarna.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td></td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td></td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td></td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td></td>
<td>v</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td></td>
<td>vi</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td></td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td></td>
<td>xi</td>
</tr>
</tbody>
</table>

## 1 INTRODUCTION

1.1 Introduction 1
1.2 Background of Study 2
1.3 Objectives of the Study 3
1.4 Scope of the Study 4

## 2 LITERATURE REVIEW

2.1 Textile wastewater 5
2.2 Dyes 6
  2.2.1 Dye classification 7
2.3 Azodyes 8
2.4 Methods of color removal 9
  2.4.1 Physico-chemical methods 9
  2.4.2 Physical methods 10
  2.4.3 Chemical methods 10
  2.4.4 Biological methods 12
2.5 Decolorization by Fungi 13
2.6 Mechanism of microbial color removal 14
2.6.1 Aerobic decolorization
2.6.2 Anaerobic decolorization
2.7 Mixed and single(pure) bacterial cultures
2.8 Aerobic fate of aromatic amines
2.9 Biodegradation of Azo Dyes
2.10 Orange II
2.11 Biodegradation of Orange II Dye
2.12 Immobilized microorganisms
2.13 Carbon Nanotube

3 MATERIALS AND METHODS
3.1 Experimental Design
3.2 Microorganism
3.3 Preparation of Stock Solutions
3.4 Medium preparation
3.5 Preparation of starter culture
3.6 Multi-walled carbon nanotube and cement
3.7 Hardened cement paste (HCP) preparation
3.8 Immobilization and Development of Biofilm
3.9 Analysis of Dye Decolorization
3.10 COD Determination
3.11 pH Measurement
3.12 FESEM

4 RESULTS AND DISCUSSION
4.1 Introduction
4.2 Development of bioparticles
4.3 Effect of pH on dye decolorization
4.4 Analysis of Dye Decolorization
4.5 Analysis of Chemical Oxygen Demand (COD)
4.6 Microscopy Examination of Biofilm (FESEM)

5 CONCLUSION
5.1 Conclusion
5.2 Future works

REFERENCES
## LIST of TABLES

<table>
<thead>
<tr>
<th>TABLE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>properties of dyes classified based on their usage</td>
<td>8</td>
</tr>
<tr>
<td>2.2</td>
<td>Advantages and disadvantages of some of the current non-biological methods of dye removal</td>
<td>12</td>
</tr>
<tr>
<td>2.3</td>
<td>Some of the advantages and disadvantages of anaerobic treatment</td>
<td>16</td>
</tr>
<tr>
<td>4.1</td>
<td>Different ratio and combination of materials to develop bioparticles</td>
<td>33</td>
</tr>
<tr>
<td>4.2</td>
<td>Highest decolorization in different systems</td>
<td>35</td>
</tr>
<tr>
<td>4.3</td>
<td>Highest COD removal rate among treatment systems</td>
<td>42</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>General view of the fate of azo dyes and aromatic amines in anaerobic-aerobic systems</td>
<td>19</td>
</tr>
<tr>
<td>2.2</td>
<td>Proposed mechanism for reduction of azo dyes by whole bacterial cells</td>
<td>22</td>
</tr>
<tr>
<td>2.3</td>
<td>structure of Orange II</td>
<td>23</td>
</tr>
<tr>
<td>4.1</td>
<td>Bioparticles which used in treatment</td>
<td>33</td>
</tr>
<tr>
<td>4.2</td>
<td>Changes in pH for free suspended cells grown in Orange II dye medium at anaerobic condition (37°C) and aerobic condition (25°C)</td>
<td>34</td>
</tr>
<tr>
<td>4.3</td>
<td>Changes in pH for HCP, IHCP, HCP + MWCNT and IHCP + MWCNT grown in Orange II dye medium at anaerobic condition (37°C) and aerobic condition (25°C)</td>
<td>35</td>
</tr>
<tr>
<td>4.4</td>
<td>Comparison between pH and decolorization of the free suspended cell system</td>
<td>36</td>
</tr>
<tr>
<td>4.5</td>
<td>Comparison between pH and decolorization of the HCP system</td>
<td>37</td>
</tr>
<tr>
<td>4.6</td>
<td>Comparison between pH and decolorization of the IHCP system</td>
<td>37</td>
</tr>
<tr>
<td>4.7</td>
<td>Comparison between pH and decolorization of the HCP + MWCNT system</td>
<td>38</td>
</tr>
<tr>
<td>4.8</td>
<td>Comparison between pH and decolorization of the IHCP + MWCNT system</td>
<td>38</td>
</tr>
<tr>
<td>4.9</td>
<td>Decolorization of Orange II dye in free suspended cells and immobilized hardened cement pastes with MWCNT in an anaerobic-aerobic system</td>
<td>40</td>
</tr>
<tr>
<td>4.10</td>
<td>Decolorization of Orange II dye in hardened cement pastes and immobilized hardened cement pastes in an anaerobic-aerobic system</td>
<td>41</td>
</tr>
<tr>
<td>4.11</td>
<td>Decolorization of Orange II dye in hardened cement pastes with MWCNT and immobilized hardened cement pastes with MWCNT in an anaerobic-aerobic system</td>
<td>41</td>
</tr>
</tbody>
</table>
4.12 Comparison of COD removal percentage between hardened cement pastes and immobilized hardened cement pastes

4.13 Comparison of COD removal percentage between hardened cement pastes with MWCNT and immobilized hardened cement pastes with MWCNT

4.14 Comparison of COD removal percentage between immobilized hardened cement pastes and immobilized hardened cement pastes with MWCNT

4.15 FESEM image of HCP with 5K magnification

4.16 FESEM image of HCP + MWCNT with 10K magnification

4.17 FESEM image of IHCP with biofilm after 7 days of treatment with 5K magnification

4.18 FESEM image of IHCP + MWCNT and biofilm after 7 days of treatment with 10k magnification
CHAPTER 1

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.
REFERENCES


