MCM-41 AS SUPPORT FOR IMMOBILIZATION OF NAR-1 BACTERIAL CONSORTIUM IN THE BIODEGRADATION OF REMAZOL BLACK B

KOGULABALAN A/L ILAN SOLLAN

A thesis submitted in fulfillment of the requirements for the award of the degree of Master of Science (Bioscience)

Faculty of Biosciences and Bioengineering
Universiti Teknologi Malaysia

FEBRUARY 2013
“Specially dedicated to my father who had passed on. This is a special gift to my mother for her endless love and care”.
ACKNOWLEDGMENTS

First of all, I wish to express my warmest thanks and appreciation to my supervisor, Professor Noor Aini Abdul Rashid for her high level inspiration, her extensive editing of the thesis, constructive criticism and the driving force to ensure the completion of this thesis. Also thank you to my co-supervisor, Professor Alias Mohd Yusof who assisted with the chemistry aspect of the research.

My special appreciation to my good friend Shankar who had shared some brilliant ideas and also for the pushing factor which inspired me go on thriving; without which it would have been very difficult. Not forgetting my friends; Aswati and Zarini, who had been sharing ideas and brought smiles to my face during the lowest points in my pursuit for knowledge. Thank you very much for your company and for always being there for me.

I would like to thank the Molecular Biology and Microbiology lab assistant, Puan Fatimah Harun and those who had given their assistance and support in any way. I would like to express my gratitude to the Faculty of Biosciences and Bioengineering for the research facilities, MOSTI for the research funding from vote 79147, PSZ for the endless supply of research materials and UTM for offering me a chance to pursue my education here.

Last but not least, my love and utmost appreciation to my family especially my mom and my late dad for their untiring love, ceaseless support and encouragement. Hope this success will be a gift for her effort in supporting for my education, moral and financial support. There are no words to describe my mother’s sacrifices and contributions!
ABSTRACT

A novel bacterial consortium, NAR-1 consisting of Enterococcus sp. C1 and Citrobacter sp. L17 was immobilised onto an inorganic support material and investigated for its biodegradation ability of Remazol Black B (RBB), micro-aerophilically under both batch and fed-batch continuous systems. To study its efficiency under immobilised condition, the NAR-1 bacterial consortium was adsorbed onto a silica mesoporous material, MCM-41 before conducting decolourisation experiments. Successful synthesis of MCM-41 was verified by FTIR, XRD and FESEM. The MCM-41 powder generated was granulated into spheres of 2-4mm in diameter before immobilisation. Preliminary immobilisation of NAR-1 was done conventionally by observing three parameters namely: agitation speed, contact time and operating temperature in 0.85% (w/v) saline. The highest adsorption of bacteria onto MCM-41 granules was $7.8 \times 10^5$ cfu/ml at 100 rpm, 2 hours contact time and $37^\circ C$. This pre-optimised condition was transferred to RSM for a more precise prediction. RSM predicted a $7.8 \times 10^5$ cfu/ml using an optimised condition of 1.9 h contact time at $34^\circ C$ and 116 rpm but actual lab experiment using the above parameters successfully produced a higher immobilised cell count of $9.0 \times 10^5$ cfu/ml. To compare the reusability of free and immobilised cells with both cell counts fixed at $9 \times 10^5$ cfu/ml, repeated-batch operation was conducted with constant addition of 100 ppm RBB into Modified P5 medium pH7.0, following each decolourisation cycle. Free cells initially took 240 min in the first cycle, declined to 180 min but yet again elevated to 270 min in the third cycle. It took a staggering 420 min to complete the 4th cycle of decolourisation. Improving decolourisation trend was observed from 180 min to 120, down to half the time at 60 min with immobilised cells. Eleven cycles were completed for immobilised cells as compared to 4 for free cells within a time-frame of 19 h. For continuous fed-batch system in an upflow packed bed reactor, the decolourisation rate progressively escalated from 50% within the first 3 hours to 80 % by the 8th hour. Interestingly, beyond that, stabilised decolourisation at almost 90 % was observed, spanning 28 h covering 9.49 cycles. A prominent decline was noticed after the 36th hour and the efficiency plummeted to 0% by the 56th hour. This sharp decline was conceivably due to several factors including bacterial leach out, bacterial cell death due to toxic accumulation and detachment of biofilm. However, MCM-41, an inorganic material remains as a potential support for bacterial immobilisation and can be applied repeatedly in a continuous system due to its rigidity.
ABSTRAK

Satu konsortium bakteria yang novel, NAR-1 terdiri dari pada Enterococcus sp. C1 dan Citrobacter sp. L17 dijerap pada bahan tak-organik dikaji dalam biodegradasi pewarna azo Remazol Black B secara mikroaerofilik dalam keadaan kultur sesekelompok dan suapan-balik. Keberkesanan keadaan tersekat-gerak dikaji dengan NAR-1 dijerap pada permukaan bahan silika mesoporous MCM-41 sebelum penyahwarnaan. MCM-41 disintesis dan disahkan sifatnya melalui FTIR, XRD dan FESEM. MCM-41 serbuk digranulkan menjadi sfera sebesar 2-4 mm diameter sebelum penjerapan. Eksperimen awal tersekat-gerak secara konvensional dengan NAR-1 melibatkan beberapa parameter seperti kadar agitasi, masa sentuhan dan suhu dalam saline 0.85% (b/i). Penjerapan sel tertinggi direkod pada kadar 7.8 x 10^5 cfu/ml dengan agitasi 100 rpm, masa sentuhan 2 jam dan suhu 37°C. Parameter optimum secara konvensional telah dimasukkan ke dalam program RSM. RSM meramalkan penjerapan bakteria sebanyak 7.8 x 10^5 cfu/ml dengan mengaplikasi parameter optimum iaitu 1.9 jam masa sentuhan, suhu 34 °C dan kadar agitasi 116 rpm tetapi dengan eksperimen maksimal sebenar, ia memberi peningkatan bilangan sel yang disekat-gerak sebanyak 9.0 x 10^5 cfu/ml. Perbandingan penggunaan semula bakteria bebas dan yang tersekat-gerak dengan bilangan bakteria yang ditetapkan pada 9 x 10^5 cfu/ml, eksperimen sesekelompok telah dijalankan dengan penambah sebanyak pewarna RBB dalam gisang tetapi dengan 100 ppm ke dalam medium P5 terubahsuai, pH 7.0. Ujikaji dengan bakteria bebas mengambil masa 240 minit pada kitaran pertama, menurun kepada 180 minit dan menaik kepada 270 minit pada kitaran ketiga. Pada kitaran keempat, ia mengambil 420 minit untuk menyahwarnakan RBB. Seterusnya, bakteria yang terjerap di atas permukaan MCM-41 mengambil masa 180 minit pada kitaran pertama dan berkurang ke 120 minit turun kepada 60 minit. Sebelum kitaran penyahwarnaan RBB dicapai dalam masa 19 jam dengan bakteria yang tersekat-gerak berbanding dengan 4 kitaran untuk bakteria bebas. Untuk eksperimen kultur suapan-balik berterusan yang dijalankan dalam reaktor dasar padat aliran menaik, kadar penyahwarnaan RBB meningkat dari 50 % dalam masa 3 jam ke 80 % pada jam yang ke-8. Kadar penyahwarnaan menjadi stabil pada kadar 90 % dalam 28 jam bersamaan 9.49 kitaran. Penurunan yang mendadak pada kadar penyahwarnaan RBB direkodkan selepas jam ke-36 dan seterusnya menjadi sifar pada jam ke-56. Penurunan yang mendadak disebabkan oleh beberapa faktor seperti larut lepas bakteria dari granul, penjualan sel berikutkan kandungan toksik meningkat dalam medium dan penanggalan biofilm. Walau bagaimanapun, MCM-41 merupakan bahan inorganik yang mempunyai potensi dalam penjerapan bakteria dan boleh diguna dalam sistem selanjar berulangkali kerana ketahanan yang tinggi.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>DEDICATION</td>
<td>iii</td>
</tr>
<tr>
<td></td>
<td>ACKNOWLEDGMENTS</td>
<td>iv</td>
</tr>
<tr>
<td></td>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td></td>
<td>TABLE OF CONTENTS</td>
<td>vii</td>
</tr>
<tr>
<td></td>
<td>LIST OF TABLES</td>
<td>xii</td>
</tr>
<tr>
<td></td>
<td>LIST OF FIGURES</td>
<td>xiii</td>
</tr>
<tr>
<td></td>
<td>LIST OF ABBREVIATIONS</td>
<td>xix</td>
</tr>
<tr>
<td></td>
<td>LIST OF SYMBOLS</td>
<td>xvi</td>
</tr>
</tbody>
</table>

## 1 INTRODUCTION

1.1 Introduction 1
1.2 Problem Statement 4
1.3 Objectives of Study 4
1.4 Scope of study 5

## 2 LITERATURE REVIEW

2.1 Azo Dye 6
2.2 RBB 7
2.3 Decolourization of Azo dye by bacteria 8
   2.3.1 Decolourisation and Degradation of Azo Dyes Using Bacterial Strains 8
2.3.2 The Application of Pure Bacterial Culture and Mixed Cultures or Co-Cultures in Decolourisation.

2.3.3 Azo Dye Decolourisation Experiments Using Bacterial Consortium NAR-1

2.4 Decolourisation and Degradation of Azo Dyes by Yeast

2.5 Decolourisation and Degradation of Azo Dyes by Fungi

2.6 Decolourisation and Degradation of Azo Dyes by Microalgae

2.7 Decolourisation and Degradation of Azo Dyes by Plants (Phytoremediation)

2.8 Application of Support Materials for Cell Adsorption

2.9 Support Material for Decolourisation

2.9.1 MCM-41 as Support Material

2.10 Response Surface Methodology (RSM)

2.11 Biofilm Reactor for Enhanced Reaction Rate

2.11.1 Biofilm Formation

3 GENERAL MATERIALS AND METHODS

3.1 Microorganism and Storage

3.2 Culture Medium

3.2.1 Nutrient Agar (NA)

3.2.2 P5 Medium

3.3 Preparation of Stock Solutions

3.4 Preparation of Starter Culture

3.5 Optimised Parameters for Decolourisation of Black B by Bacterial Consortium

3.6 Analysis on Decolourisation of Black B
3.7 Drop Plate Method 34

4 SYNTHESIS, CHARACTERISATION AND GRANULATION OF MCM-41

4.1 Introduction 36

4.1.1 Fourier Transform Infrared Spectroscopy (FTIR) 36

4.1.2 X-Ray Diffraction (XRD) 37
to characterise MCM-41

4.1.3 Field Emission Scanning Electron Microscopy (FESEM) to characterise MCM-41 38

4.2 Material and Methods 38

4.2.1 Synthesis of MCM-41 38

4.2.2 FTIR to characterise MCM-41 41

4.2.3 XRD 42

4.2.4 FESEM 42

4.2.5 Granulation of MCM-41 42

4.3 Results and Discussion 45

4.3.1 Fourier Transform Infrared (FTIR) Spectroscopic Analysis of MCM-41 45

4.3.2 X-Ray diffraction (XRD) analysis 47

4.3.3 Field Emission Scanning Electron Microscopy (FESEM) Analysis 47

4.3.4 Granulation of powdered MCM-41 48

5 OPTIMISATION OF BACTERIAL ADSORPTION ONTO MCM-41 GRANULES IN BATCH REACTION USING CONVENTIONAL AND RESPONSE SURFACE METHODOLOGY (RSM) METHOD
5.1 Introduction 50
5.2 Material and methods 51
  5.2.1 Microorganisms 51
  5.2.2 Immobilisation of the bacterial consortium onto MCM-41 granules 51
  5.2.3 Cell count 53
  5.2.4 Central Composite Design 54
5.3 Results and Discussion 56
  5.3.1 Agitation Rate 56
  5.3.2 Contact time 59
  5.3.3 Operating Temperature 61
  5.3.4 Optimisation of temperature, agitation and contact time for the enhancement of bacterial adsorption on MCM-41 granules using Experimental Design 63
  5.3.5 Analysis of Variance (ANOVA) 66
  5.3.6 Graphical Interpretation of the Model for the Cell Adsorption 72
  5.3.7 Application of Optimised Condition on cell adsorption 77

6 DECOLOURISATION OF REMAZOL BLACK B BATCH AND FED BATCH CONTINUOUS CULTURE USING NAR-1 IMMOBILISED GRANULES
6.1 Introduction 78
6.2. Material and methods 78
  6.2.1 Microorganism 78
  6.2.2 Batch immobilisation and decolourisation Process 79
6.2.3 Continuous Decolourisation using a Packed Bed Reactor
6.2.4 UV-Visible Analysis of decolourised samples
6.2.5 Sample Preparation and FESEM (Field Emission Scanning Electron Microscopy) Analysis

6.3 Results and Discussion
6.3.1 Repetitive dye supplementation for free and immobilised cells in Modified P5 Medium pH 7.0
6.3.2 Continuous Decolourisation Process Using Packed Bed Reactor
6.3.3 UV-Visible Spectrophotometry Analysis of decolourised effluent
6.3.4 Analysis of cells in free and immobilised form
6.3.4.1 FESEM analysis of cells
6.3.4.2 FESEM analysis of support matrix with cells
6.3.4.3 Bacterial attachment onto the MCM-41
6.3.4.4 The choice of MCM-41 as support material in the continuous decolourisation of RBB

7 CONCLUSIONS
7.1 Conclusions
7.2 Future Work

REFERENCES
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Decolourisation of various azo dyes by pure bacterial cultures</td>
</tr>
<tr>
<td>2.2</td>
<td>Decolourisation performance of different reactive dyes by various microbial consortia</td>
</tr>
<tr>
<td>2.3</td>
<td>Related researches on the azo dye degradation using fungi</td>
</tr>
<tr>
<td>3.1</td>
<td>Concentration of Nutrient Broth and Glucose for Different P5 Medium Strength</td>
</tr>
<tr>
<td>4.1</td>
<td>FTIR peaks for MCM-41 and their corresponding bonds</td>
</tr>
<tr>
<td>5.1</td>
<td>The actual and coded values of the design variables for the bacterial adsorption on MCM-41 granules</td>
</tr>
<tr>
<td>5.2</td>
<td>Experimental design for optimisation of bacterial adsorption on MCM-41 granules</td>
</tr>
<tr>
<td>5.3</td>
<td>Coded and actual value of the ranges selected for the variables for CCD</td>
</tr>
<tr>
<td>5.4</td>
<td>Experimental result for optimisation of bacterial adsorption on MCM-41</td>
</tr>
<tr>
<td>5.5</td>
<td>ANOVA for optimisation of bacterial adsorption on MCM-41 granules</td>
</tr>
<tr>
<td>5.6</td>
<td>Value from ANOVA for quadratic model of the design</td>
</tr>
<tr>
<td>FIGURES</td>
<td>TITLE</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>2.1</td>
<td>The structure of RBB</td>
</tr>
<tr>
<td>2.2</td>
<td>Microscopic study of the steps in biofilm formation by <em>V. cholera</em></td>
</tr>
<tr>
<td>4.1</td>
<td>Flow diagram of the synthesis of MCM-41</td>
</tr>
<tr>
<td>4.2</td>
<td>A flow diagram of the granulation process of MCM-41</td>
</tr>
<tr>
<td>4.3</td>
<td>FTIR Spectrogram of (a) calcined MCM-41 and (b) uncalcined MCM-41</td>
</tr>
<tr>
<td>4.4</td>
<td>X-ray diffractogram of synthesized ordered mesoporous silica type MCM-41</td>
</tr>
<tr>
<td>4.5</td>
<td>FESEM micrograph of MCM-41</td>
</tr>
<tr>
<td>4.6</td>
<td>Photograph of MCM-41(a) powdered and (b) granulated</td>
</tr>
<tr>
<td>5.1</td>
<td>A flow diagram showing the process of bacterial immobilisation onto granulated MCM-41</td>
</tr>
<tr>
<td>5.2</td>
<td>A graph illustrating the effect of agitation on bacterial adsorption on MCM-41 particles</td>
</tr>
<tr>
<td>5.3</td>
<td>Graph illustrating the effect of contact time on bacterial adsorption on MCM-41 particles</td>
</tr>
<tr>
<td>5.4</td>
<td>A graph illustrating the effect of temperature on bacterial adsorption on MCM-41 granules in normal saline at 100 rpm with 2 hours contact time where the bacteria were collected at its log phase.</td>
</tr>
<tr>
<td>5.5</td>
<td>Normal plot of residual for the optimisation cell adsorption</td>
</tr>
<tr>
<td>5.6</td>
<td>Outlier T plot for the optimisation of cell adsorption</td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>5.7</td>
<td>Cook’s Distance plot of experiments for the optimisation of cell adsorption</td>
</tr>
<tr>
<td>5.8</td>
<td>Leverage plot of experiments for the optimisation of cell adsorption</td>
</tr>
<tr>
<td>5.9</td>
<td>Predicted versus actual plot of experiments for the optimisation of cell adsorption.</td>
</tr>
<tr>
<td>5.10</td>
<td>Optimum conditions for cellular adsorption suggested by the generated model of Design Expert</td>
</tr>
<tr>
<td>5.11</td>
<td>3D surface plot for the cell adsorption on MCM-41 granules as a function of agitation and contact time</td>
</tr>
<tr>
<td>5.12</td>
<td>Contour surface plot for cell adsorption on MCM-41 granules as a function of agitation and contact time</td>
</tr>
<tr>
<td>5.13</td>
<td>3D surface plots for the cell adsorption on MCM-41 granules as a function of temperature and contact time</td>
</tr>
<tr>
<td>5.14</td>
<td>Contour surface plot for cell adsorption on MCM-41 granules as a function of temperature and contact time</td>
</tr>
<tr>
<td>5.15</td>
<td>3D surface plot for the cell adsorption on MCM-41 granules as a function of temperature and agitation</td>
</tr>
<tr>
<td>5.16</td>
<td>Contour surface plot for cell adsorption on MCM-41 granules as a function of temperature and agitation</td>
</tr>
<tr>
<td>6.1</td>
<td>Schematic diagram of the set-up used for the anaerobic decolourisation of RBB in packed bed column reactor</td>
</tr>
<tr>
<td>6.2</td>
<td>Concentration of azo dye RBB (100 ppm initial concentration) during the course of repeated batch cycles decolourisation using free cells of NAR1 consortium.</td>
</tr>
<tr>
<td>6.3</td>
<td>Concentration of azo dye RBB during the course of repeated batch cycles of decolourisation using MCM-41 immobilised NAR1 cells</td>
</tr>
<tr>
<td>6.4</td>
<td>Actual set up of continuous decolourisation process in Packed Bed Reactor</td>
</tr>
</tbody>
</table>
6.5 Decolourisation of 100 ppm RBB using a packed bed reactor at a flow rate of 0.1ml/min at a temperature of 45°C
6.6 The UV/Visible scans of RBB and its reduction products. The lines coloured red, green, yellow and blue represent the outcome of reduction at t= 0, 30, 60 and 90 mins., respectively
6.7 The structure of RBB
6.8 Structure of sulphanilic acid
6.9 Scanning Electron Micrograph of Enterococcus sp. C1 (a) 6000X magnification and (b) 25000X magnification
6.10 Scanning electron micrograph of Citrobacter sp. (L17) (a) 5000X magnification and (b) 10000X magnification
6.11 Micrographs of cells of Enterococcus sp. C1 and Citrobacter sp. L17 immobilised onto the support material MCM-41 indicated by arrows
6.12 Larger size matrix gives larger secondary pores in between the granules
6.13 Smaller size matrix gives smaller secondary pores
6.14 Comparison of two different matrix sizes (a) MCM-41 pellets with large pseudopores between the particles (b) Surfactant modified clinoptilolite particles of varying sizes which interlock very well with each other
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Abbreviation &amp; Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCD</td>
<td>Central composite design</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>L</td>
<td>Liter</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>RSM</td>
<td>Response Surface Methodology</td>
</tr>
<tr>
<td>rpm</td>
<td>Round per minute</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume per volume</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight per volume</td>
</tr>
<tr>
<td>µm</td>
<td>Micrometer</td>
</tr>
<tr>
<td>ºC</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>NA</td>
<td>Nutrient agar</td>
</tr>
<tr>
<td>OD</td>
<td>Optical Density</td>
</tr>
<tr>
<td>A&lt;sub&gt;600nm&lt;/sub&gt;</td>
<td>Absorbance at the wavelength of 600 nm</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>FESEM</td>
<td>Field Emission Scanning Electron Microscopy</td>
</tr>
<tr>
<td>XRD</td>
<td>X-Ray Diffraction</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Introduction

Synthetic dyes are extensively used in many industries such as, in various branches of the textile industry, the leather tanning industry in paper production, food technology, agricultural, light-harvesting arrays, photo electrochemical cells, and hair colourings (Van Der Zee and Villaverde, 2004, Ganesh et. al., 1994). Unfortunately, the exact amount of dyes produced in the world is not known. It is estimated to be over 10,000 tons per year. Exact data on the quantity of dyes discharged in the environment are also not available. It is believed that a loss of 1–2% in production and 1–10% loss in use are a fair estimate. For reactive dyes, this figure can be about 4%. Due to large-scale production and extensive application, synthetic dyes can cause considerable environmental pollution and are serious health-risk factors. Among the dyes used, azo dyes are the most important and widely used (Alexander et al., 2002). Azo dyes are characterised by the presence of one or more azo groups (-N=N-), known as the chromophore which gives the dye its colour and negative sulfonate groups (-SO\(_3\)) (Anjali et al., 2006). Azo dyes itself is hazardous; however, it can be more hazardous when the azo bonds are reduced to give amines which can be more carcinogenic than the parent structure (Anjali et al., 2006).

Currently, numerous methods can be applied for the wastewater treatment of colour removal either through chemical, physical or biological processes. The chemical and physical colour removal processes utilises a number of hazardous
chemical and the by-products are considered non-environmentally friendly. Biological techniques use microbes as powerhouse to directly utilise the azo dyes with no or minimal impact on the environment. Biological techniques for treating dyes employed the use of microorganisms to decolourise and biodegrade azo dyes under anaerobic, aerobic or combined anaerobic/aerobic treatment system. Microbial degradation and decolourisation of dyes is an environment friendly and cost competitive alternative to chemical decomposition processes (Swamy et al., 1999, Libra et al., 2004 and Rodriguez et al., 2002).

Many microorganisms belonging to different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolourise azo dyes (Khehra et al., 2005 and Chang et al., 2004). Anaerobic and microaerophilic microorganisms have been found to reduce azo bonds non-specifically in anaerobic conditions leading to dye decolourisation and in the process generate toxic aromatic amines which are mutagenic and carcinogenic (Coughlin et al., 1999). Hence, along with colour removal, complete degradation of azo dyes is the only solution for final elimination of xenobiotics from the environment. Apparently, there exists a need to develop novel biological decolourisation processes leading to more effective cleanup of azo dyes (Padmavathy et al., 2003).

In this research, an azo dye Remazol Black B (RBB) or also known as C.I. Reactive Black 5, in particular was decolourised using selected bacterial consortium NAR-1 comprising of Enterococcus sp. (C1) and Citrobacter sp. (L17). Citrobacter sp. L17 was previously reported as Enterobacter cloacae L17 (Chan et al., 2012). However, genome sequence done on these bacteria reidentified the bacteria as Citrobacter sp. L17. Therefore, the bacterial strain Enterobacter cloacae L17 had been redesignated as Citrobacter sp. L17 in this thesis. Previous studies in this lab have shown this bacterial consortium has the ability to reduce RBB anaerobically in 45 mins under batch condition (Ilan Sollan, 2008). The biodegradation products of RBB following reduction process could include aromatic amines and other intermediates such as p-base, sulphanilic acid and triaminohydroxynaphthalene disulfonilic acid (TAHNDS). HPLC was used to determine the presence of
sulphanilic acid as it was previously reported to be a dead end product in RBB biodegradation (Alexander et al., 2002).

A type of mesoporous silica material called MCM-41 (Mobil Carrier Material; Mobil Catalytic Material) was utilised as a bacterial support for the degradation of RBB in this research. Mesoporous silica is a form of silica and a recent development in nanotechnology. The most common type of mesoporous nanoparticle is MCM-41 (Amit et al., 2006). Research continues on MCM-41 which has applications in catalysis, drug delivery and imaging (Brian et al., 2007). A procedure for producing mesoporous silica was patented around 1970 (Chiola et al., 1971). It went almost unnoticed and was reproduced in 1997 (Zu et al., 2007 and Direnzo et al., 1997). Mesoporous silica nanoparticles (MSNs) were independently synthesised in 1990 by researchers in Japan (Tsuneo et al., 1990). They were later produced at Mobil Corporation laboratories and named Mobil Crystalline Materials, or MCM-41 (Beck et al., 1992 and Trewyn, et al., 2007). The researchers who invented these types of particles planned to use them as molecular sieves. Today, mesoporous silica nanoparticles have many applications in biotechnology, medicine, biosensors, and imaging. In this research, a novel approach was taken whereby, MCM-41 was utilised as bacterial support in the bioremediation of azo dyes.

Response Surface Methodology (RSM) is important in designing, formulating, developing, and analyzing new scientific studies and products. It is also efficient in the improvement of existing studies and products. The most common applications of RSM are in industrial, biological and clinical science, social science, food science, and physical and engineering sciences (Nuran, 2007). Since the conventional method of optimisation, “one factor at a time” approach is laborious, time consuming and incomplete, RSM using CCD (as factorial experimental design) which involves full factorial search by examining simultaneous, systematic and efficient variation of important components was applied to model the cell adsorption process on MCM-41 granules. The method identifies possible interactions, higher orders effects and determines the optimum operational conditions. However, RSM using CCD is useful for small number of variables (up to five) but is impractical for a large number of
variables, due to high number of experimental runs required (Sharma and Satyanarayan, 2006).

Packed bed reactors are well-known to be utilised in various applications of biotechnology. Particularly, attached biofilm reactors present a higher potential for use than suspended growth biomass reactors because the former can retain higher concentrations of biomass with higher metabolic activity. Moreover, attached biomass is known to be more resistant to toxicity. So, the ultimate objective of this study was to evaluate the performance of a biofilm packed bed reactor for the removal of dye.

1.2 Problem Statement

Batch decolourisation of RBB by NAR-1 done in this lab had been successful but only small reaction volumes could be handled at one time. Therefore, a continuous decolourisation system requiring a support material for bacterial adhesion is necessary. A robust yet inert support without the need to be modified could simplify the process. Hence, MCM-41 was the choice of support material as it has met the above requirements. This was granulated, loaded into an upflow packed bed reactor and used in the immobilisation of NAR-1, followed by continuous RBB decolourisation process.

1.3 Objectives of Study

The objectives of this study were:

a. To synthesise and characterise ordered mesoporous silica (MCM-41) and granulate MCM-41 powder.

b. To optimise bacterial adsorption process on MCM-41 granules using conventional method and subsequently RSM.
c. To compare repetitive batch decolourisation of RBB using suspended bacteria or bacteria immobilised onto MCM-41.

d. To decolourise RBB continuously by NAR-1 immobilised onto MCM-41 granules in a packed-bed reactor.

1.4 Scope of Study

The scope of this research was to utilise ordered mesoporous silica (MCM-41) which was synthesized in this lab as support for immobilization of a bacterial consortium, NAR-1 for RBB decolourisation. The bacterial strains used were *Enterococcus* sp. C1 and *Citrobacter* sp. strain L17. The research also employed both conventional and RSM approaches to optimize batch immobilisation of NAR-1 onto MCM-41 granules. Additionally, the project concentrated on the feasibility of the packed-bed reactor for the attachment of bacterial cells and consequently continuous decolourisation of RBB.
REFERENCES


