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OPTIMIZATION OF IMMOBILIZATION MEDIA OF *THALASSOSPIRA PROFUNDIMARIS*: DIFFUSION AND STRENGTH STUDIES

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Bachelor of Engineering with Honours
(Chemical Engineering)
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This declaration is made on the 21 day of May 2018.

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21 MAY 2018
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OPTIMIZATION OF IMMOBILIZATION MEDIA OF *THALASSOSPIRA PROFUNDIMARIS*: DIFFUSION AND STRENGTH STUDIES

MUHD NURUL AZAM BIN MUHD DRUS

A dissertation submitted in partial fulfillment

of the requirement for the degree of

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Dedicated to my beloved parents and my family who always bestow me sustainable motivations and encouragements

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ABSTRACT

Many petroleum samples have abundant heterocyclic compounds. One example of petroleum samples is diesel where nitrogen, sulphur and aromatic compounds are the major impurities present in it. Heterocyclic compounds are inextricably into life processes in which a vast number of active heterocyclic compounds are being used. The problem to the industry is that the waste are discharged into the sea that could treat both marine life as well as human as consumer. Bacteria called *Thalassospira Profundimaris* could potentially degrade the carcinogenic compound of petroleum discharge. However, having the bacteria to work without protection could shorten the lifespan of the bacteria itself where free cell would offer a multitude of disadvantages such as substrate inhibition when using free cell suspension in wastewater. It also offers low stability, lower degradability of the cell in the presence of high concentration of pollutants from the systems. Cell immobilisation offers high mechanical strength, high metabolic activity, and resistance to toxic chemicals causing damage to the cell. The diffusion barriers within the biofilm against toxic compound could save the cell from damage as well as maintaining the microbial population in carriers without any loss. The cell immobilisation is used widely in wastewater industries whereby the media used widely are gellan gum, Ca-alginate, yeast and others. In this project, the focus is on the finding of the optimum strength and diffusivity of the media itself to increase the performance of the bacteria entrapped inside as well as withstanding the harsh environment around it. With various concentration of gellan gum and Ca-alginate, this project has proven that the concentration affects the porosity and the strength of the media. The project has also proven that increasing the concentration of media would form stronger media with lower diffusivity. Lower concentration forms soft media with higher diffusivity.

ABSTRAK

Kebanyakan sampel petroleum mempunyai banyak kompaun heterosiklik. Satu contoh bagi sampel ialah diesel. Diesel mengandungi nitrogen, sulfur, dan kompaun aromatic. Kesemua kandungan diesel ialah kotoran. Kompaun heterosiklik adalah sangat merbahaya kepada kehidupan. Walau bagaimanapun, bahan kumbahan utama industry petroleum dibuang kedalam laut dan akan mengganggu kehidupan marin dan jugak kehidupan manusia sebagai pemakan. Bakteria yang dipanggil *Thalassospira Profundimaris* mampu untuk memakan kompaun karsinogenik daripada limpahan petroleum. Walau bagaimanapun, bakteria dibiarkan untuk bekerja tanpa sebarang perlindungan akan memendekkan jangka hidup bakteria tersebut. Sel bebas banyak memberi keburukan kepada bakteria. Sel bebas adalah tidak stabil, kurang daya pemakanan sel bila berada di dalam larutan kotoran yang sangat tinggi. Immobalisasi sel membuat sel lebih kuat, tinggi metabolism dan senang menangkis kotoran yang merosakkan sel. Penghalang penyerapan dalam biofilm mampu untuk menyelamatkan sel daripada rosak dan mengekalkan populasi microbial didalam pembawa (medium) supaya sel tidak berkurangan. Immobalisasi sel digunakan secara meluas dan kebanyakan menggunakan gam gellan, Ca-alginate, yis dan sebagainya. Dalam projek ini, focus utama adalah untuk mengkaji kekuatan dan penyerapan yang paling optimum untuk menaikkan keupayaan bakteria untuk bekerja didalam persekitaran yang sukar. Dengan menggunakan pelbagai kepekatan gam gellan dan Ca-alginate, projek ini telah membuktikan yang kepekatan medium akan menyebabkan penyerapan dan kekuatan berubah. Projek ini juga telag membuktikan, peningkatan kepekatan medium akan membentuk medium yang lebih kuat dengan penyerapan yang lebih rendah. Kepekatan medium yang rendah membolehkan pembentukan medium menjadi lebih lembut dan mempunyai kadar penyerapan yang lebih tinggi.

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LIST OF ABBREVIATIONS

cm	-	Centimeter
h	-	Hour
$MgCl.6H_2O$	-	Magnesium Chloride
KCl	-	Potassium Chloride
Na_2SO_4	-	Sodium Sulphate
NaBr	-	Sodium Bromide
$NaHCO_3$	-	Sodium Bicarbonate
H_3BO_3	-	Boric Acid
NaF	-	Sodium Floride
NH_4Cl	-	Ammonium Chloride
$Na_2HPO_4.7H_2O$	-	Sodium Phosphate
TAPSO	-	3-[N-Tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid
$CaCl_2.2H_2O$	-	Calcium Chloride
$SrCl_2.6H_2O$	-	Strontium Dichloride Hexahydrate
$FeCl_2.4H_2O$	-	Ferum Chloride
Ca-alginate	-	Calcium alginate

LIST OF SYMBOLS

A	-	Area
A_o		Initial Area
C	-	Concentration
C_s	-	Concentration at time
D	-	Diameter
E	-	*Young's Modulus*
$\in$	-	Engineering Stress
l	-	Thickness
L	-	Length
C_{s0}	-	Initial contaminants concentration
L_0	-	Initial Length
q_1	-	Positive nonzero root
α	-	Ratio of solution to amount of media
t	-	Time
Q	-	Volumetric Rate
σ	-	Tensile Stress
σ_y	-	Yield Stress

CHAPTER 1

INTRODUCTION

1.1 Introduction

The expansion of biotechnology and its development has revitalized enthusiasm on cells or enzymes immobilization. Immobilization is a term describing the entrapment or attachment of cell or particles (Lopez et al., 1997). Surprisingly, there have been few detailed and comparative studies on the methods of immobilization (Bickerstaff, 1997a). Immobilization can be applied to all types of biocatalyst including cellular organisms, animal and plant cells (Cláudia et al., 2013). Such immobilization have found many application, not only in the field of biotechnology but also in food, environment and other industries (Peinado et al., 2005).

Free cell would offer a multitude of disadvantages such as substrate inhibition when using free cell suspension in waste water (Elakkiya, Prabhakaran, & Thirumarimurugan, 2016). It also offers low stability, lower degradability of the cell in the presence of high concentration of pollutants from the systems (Elakkiya et al., 2016). Cell immobilisation offers high mechanical strength, high metabolic activity, and resistance to toxic chemicals causing damage to the cell. The diffusion barriers within the biofilm against toxic compound could save the cell from damage as well as maintaining the microbial population in carriers without any loss (Elakkiya et al., 2016).

More than a hundred immobilisation techniques have been worked out during the past forty three years (Zaborsky, 1973). They are divided into four groups known as encapsulation, cells containment behind a barrier, adsorption/adhesion and entrapment (Poulsen, 1984). Encapsulation is the process forming a continuous membrane around cells to immobilise the cells in which the inner matrix is protected by means of the outer membrane (Kailasapathy, 2002). The encapsulation of cell can be done by three methods such as emulsification, interface technique and coacervation (Elakkiya et al., 2016). The cell containment behind a barrier can be done by attachment of cells on a performed membrane. This immobilisation is intentionally to separate the cells and the products from the effluent and when the transfer of compounds is required. For example, the hollow fibre membrane reactor which is designed by using dual layer fibres. It composts of three compartments which are annual space, shell and lumen part (Yang, Teo & Ting, 2006). The adsorption of cells are done by absorbing most proteins where the enzymes is added to the support and mixed (Klibanov, 1983).

The entrapment of cell in polymeric gels is an approach where the enzyme is added to a solution of monomers before the gel is formed. The formation of gel is obtained by manipulating either temperature or adding a gel-inducing chemical where the cell is trapped inside the gel (Poulsen, 1984). There are several major methods of entrapment which are known as ionotropic gelation of macromolecules with multivalent cations, temperature-induced gelation, organic polymerization by chemical/photochemical reaction and precipitation from an immiscible solvent (Bickerstaff, 1997a). There are several types of immobilization that involve joining the cells to each other to form a large, three-dimensional complex structure is known as cross-linking (Broun, 1976). This technique is achieved by chemical or physical methods where normally for chemical methods, the crosslinking involves covalent bond formation between the cells (Bickerstaff, 1997a). Meanwhile, the physical crosslinking of cells by flocculation does lead to high cell densities (Bickerstaff, 1997a). The most popular cross-linkers are glutaraldehyde, dimethyl adipimidate and aliphatic diamines (Poulsen, 1984). The media used as immobilizers are identified as agar, agarose, Ca-alginate, carrageenan and gellan gum (Hulst et al., 1989). These biological cells are beneficial to be produced in capsules such as the use of erythrocytes (red blood cells) (Bickerstaff, 1997a).

1.2 Problem Statement

The entrapment of cells is beneficial for wastewater treatment system considering its efficiency and its cost-efficiency. However, the problem should be stated before having started using the cell immobilisation or specifically cell entrapments. The problem is that if the product from reaction accumulate rapidly, the problem associated with diffusion is more acute and may result to rupture.

The strength of the media to withstand the force in the sea water should also be considered as one of the main subjects. Alternatively, the concentration of media used in forming the immobilisation media is manipulated to obtain the optimum strength and diffusivity of the membrane media. The strength of the media is tested for its tensile strength and to which it would have withstood. Meanwhile, the diffusion rate of the media is tested for its ability to transfer the substrate to be degraded. The concentration of media of the beads affect the stiffness of the beads as well as its diffusion rates.

1.3 Aim and Objectives

This study is aimed to produce the most robust and optimized immobilization media to increase the performance of carbazole degradation by *Thalassospira Profundimaris* MO2. The objectives are as listed below:

i. To investigate the optimum biodegradability of Carbazole by *Thalassospira Profundimaris* entrapped in various concentration of gellan gum and Ca-alginate.
ii. To investigate the optimum strength of different concentration of immobilized media of both gellan gum and Ca-alginate for production of immobilized *Thalassospira Profundimaris*.
iii. To identify the optimum diffusivity of substrate in immobilisation media for efficient degradation of Carbazole.
iv. To compare Ca-alginate and Gallen gum optimum immobilized media concentration for maximum degradation of Carbazole.

1.4 Scopes

The scopes of this thesis are as listed:

i. Identification of strength of different media concentration for both gellan gum and Ca-alginate.
ii. Identification of diffusion rate for different media concentration for both gellan gum and Ca-alginate.
iii. Evaluation on relation of both parameters studied, strength and diffusion rate for both gellan gum and Ca-alginate.
iv. Comparison on both gellan gum and Ca-alginate upon its robustness and strength.

CHAPTER 2

LITERATURE REVIEW

2.1 Carbazole

Many petroleum samples have abundant heterocyclic compounds (Singh, Srivastava, & Gupta, 2010). One example of petroleum samples is diesel where nitrogen, sulphur and aromatic compounds are the major impurities present in it (Singh et al., 2010). Heterocyclic compounds are inextricably into life processes in which a vast number of active heterocyclic compounds are being used (Mounika et al., 2015). Nitrogen containing xenobiotic compounds is a class of contaminants that is abundant in many petroleum samples (Singh et al., 2011). It is also wide spread in the environment (Singh et al., 2011). Carbazole is known as a nitrogen containing polycyclic aromatic compound. In industry, this aromatic heterocyclic aromatic compound is difficult to remove using conventional physio-chemical methods, is considered as the model compound in most of denitrification studies (Singh et al., 2010; Mounika et al., 2015). These N-containing synthetic compounds have also been broadly classified as the nitrite esters and compounds containing nitrogen-ring heterocycles (Singh et al., 2011). Generally, carbazoles are frequently used for manufactures of dyes, explosives, pesticides, medicines, plastics and is found in creosote, crude oil, tar sand sources, shale oil and wood preserving wastes (Singh et al., 2011). Practically, the structure of carbazole s tricyclic, containing of two six membered benzene rings that is fused on the side with a five membered nitrogen-containing ring (Mounika et al., 2015).

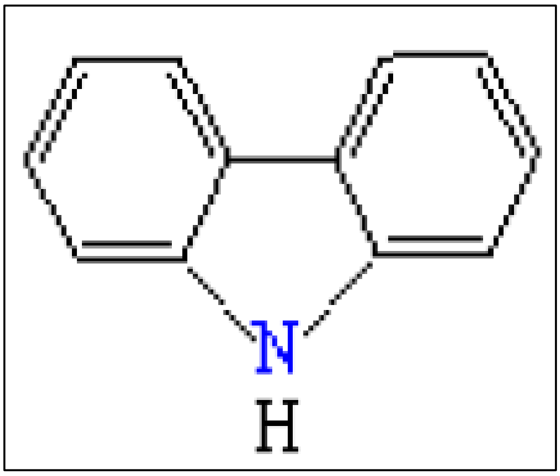

Figure 2.1: Carbazole, N-containing Heterocyclic Compounds (G. Singh et al., 2011)

Carbazole, or scientifically known as N-containing xenobiotic is both mutagenic and carcinogenic (Singh et al., 2011). A serious concern for proper disposal of this compound has been generated due to excessive use of these compounds that could lead to harmful effect on human health and environment (Singh et al., 2011). Thus, bioremediation and biorefining are the focal points into using microbes that is capable of degrading (Singh et al., 2010). According to Singh et al. (2010), some microbes are reported for its capability into degrading Carbazole *like Sphingomonas sp., Pseudomonas sp., Zanthomonas sp., Gordonia sp. Klbsiella sp., Burkholderia sp., Arthrobacter sp., Novosphingobium sp.* and *Thalisospira sp.*

2.2 *Thalassospira profundimaris*

The new innovation in bioremediation and biorefining is very beneficial in reducing any carcinogenic compounds in our daily life. As abovementioned, some microbes are reported for its capability into degrading Carbazole *like Sphingomonas sp., Pseudomonas sp., Zanthomonas sp., Gordonia sp. Klbsiella sp., Burkholderia sp., Arthrobacter sp., Novosphingobium sp.* and *Thalisospira sp*. which bring about the next topic which is microbes (Singh et al., 2011). Hydrocylic compounds are usually released into the marine environment as a result of anthropogenic activities (Zhao et al., 2010). These activities include marine seepage and accidental discharges during transport and disposal of petroleum products (Zhao et al., 2010). These carcinogenic compounds might have been transferred to humans through seafood consumption (Menzie et al., 1992). So, the removal of these compounds is significantly important. Gram-negative-staining cells