

“I/We* hereby declare that I/we* have read this thesis and in my/our* opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering”

Signature :

Supervisor : Mr. Abd. Aziz Bin Mohd Azoddein

Date :

**THE EFFECT OF BIOSURFACTANT IN SOLVING OF PETROCHEMICAL
INDUSTRY WASTEWATER TREATMENT**

PETRUS BIN AKUN

**FACULTY OF CHEMICAL ENGINEERING AND NATURAL RESOURCES
UNIVERSITY COLLEGE OF ENGINEERING AND TECHNOLOGY
MALAYSIA**

NOVEMBER 2006

**THE EFFECT OF BIOSURFACTANT IN SOLVING OF PETROCHEMICAL
INDUSTRY WASTEWATER TREATMENT**

PETRUS BIN AKUN

**A thesis submitted in fulfilment of the
requirements for the award of the degree of
Bachelor of Chemical Engineering**

**FACULTY OF CHEMICAL ENGINEERING AND NATURAL RESOURCES
UNIVERSITY COLLEGE OF ENGINEERING AND TECHNOLOGY
MALAYSIA**

NOVEMBER 2006

NOVEMBER 2006

DECLARATION

I declare that this thesis entitled “The Effect of Biosurfactant in Solving of Petrochemical Industry Wastewater Treatment” is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature :

Name : PETRUS BIN AKUN

Date : 20 NOVEMBER 2006

DEDICATION

To my beloved mother, father, my siblings

My beloved friend

ACKNOWLEDGEMENT

Praise be to God for His help, bless and guidance that this research project has been a complete success.

First and foremost, I would like to extend my sincerest gratitude to my caring supervisor, Mr. Abd Aziz Bin Mohd. Azoddein for his guidance, critics, on-going support and courage. Without him, this research project would not have completed successfully. Thank you very much and I really do appreciate the critics and advise. Thank you very much for all the lecturer, Technical Unit and FKKSA Management.

Secondly, I would like to express my appreciation to my beloved family, my parents, and siblings for their continuous support, understandings and courage. May God bless you always?

For my beloved friends thank you very much for your support and assistance. Our friendship will last forever and deepest thanks for Siti Aisyah for being supportive ,encouragement and aid. I appreciate it very much and may God bless you always.

ABSTRACT

A research has been conducted to study the effect of biosurfactant in solving of petrochemical industry wastewater treatment. Biosurfactant is an alternative method to industrial wastewater treatment nowadays. Biosurfactant has the ability to treat wastewater by using naturally- occurring micro organisms to breakdown or to degrade hazardous substances into less or non toxic materials. Technologies using microorganisms and microbial products to remove metals have been successfully applied to waste streams. Metals that are widely concerned in waste treatment are mercury. In this study, biosurfactant is applied to wastewater to treat the mercury in the wastewater. Using current wastewater treatment pilot plant and HACH wastewater quality equipment technology in this project helps improve the progress of the research. Besides focusing onto mercury treatment, an observation to the values of BOD, COD and pH also will be made. Basically, the result obtained from the project will demonstrate that biosurfactant can be applied as one of the method to study the petrochemical wastewater treatment.

ABSTRAK

Satu kajian telah dijalankan untuk mengkaji kesan 'biosurfactant' ke atas rawatan sisa industri petrokimia. 'Biosurfactant' adalah satu kaedah alternatif dalam proses rawatan sisa masa kini. Ia mempunyai kemampuan untuk merawat sisa industri dengan menggunakan mikroorganisma sedia ada untuk menguraikan bahan toksik kepada bahan yang kurang atau tidak bertoksik. Teknologi yang menggunakan mikroorganisma dan hasil mikro untuk menyingkirkan logam telah berjaya dipraktikkan ke atas aliran sisa. Logam yang dititikberatkan dalam rawatan sisa ialah merkuri. Dalam kajian ini, 'biosurfactant' digunakan untuk merawat merkuri dalam bahan buangan. Loji rawatan sisa terkini dan peralatan kualiti sisa teknologi HACH yang digunakan telah meningkatkan kemampuan hasil kajian ini. Selain menumpukan kepada rawatan merkuri, pemerhatian turut dilakukan terhadap nilai BOD, COD dan pH sisa industri. Secara amnya, hasil yang diperolehi daripada kajian ini dapat menunjukkan bahawa 'biosurfactant' boleh digunakan sebagai satu kaedah untuk merawat sisa atau bahan buangan dalam industri petrokimia.

TABLE OF CONTENTS

ACKNOWLEDGEMENT	I
ABSTRACT	II
ABSTRAK	III
TABLE OF CONTENTS	IV-VI
LIST OF FIGURES	VII
LIST OF APPENDIX	VI11

CHAPTER	TITLE	PAGE
1.0	INTRODUCTION	1
1.1	Research Background	1-3
1.2	Research Objectives	4
1.3	Research Scopes	4
2.0	LITERATURE REVIEW	
2.1	Introduction	5-6
2.2	Biosurfactant	6-8
2.2.1	General Use of Charge Types	8-9
2.2.2	General Effects of the Nature of the Hydrophobic Group	9
2.2.2.1	Length of the Hydrophobic Group	9
2.3	Environmental Effects of Biosurfactant	10
2.3.1	Biosurfactant Biodegradability	10-11
2.4	Petrochemical Wastewater Treatment System	11

2.4.1	Wastewater Treatment Principle	11-12
2.4.2	Waste treatment	13
2.4.2.1	Preliminary treatment	14
2.4.2.2	Primary Treatment	14
2.4.2.3	Secondary treatment	15
2.4.2.4	Final Treatment	5
2.5	Mercury	16
2.5.1	The structure of mercury	16-17
2.5.2	Some of Mercury Treatment	17-18
2.6	Chemical Oxygen Demand (COD)	18-19
2.6.1	COD Calculation	19
2.7	Biological Oxygen Demand (BOD)	20
2.8	pH	20-21
3.0	RESEARCH METHODOLOGY	
3.1.	Raw Material	22
3.1.1	Wastewater and Preparation of Synthetic Wastewater	22
3.1.2	Reagent and Apparatus	23
3.2	Methodology	23
3.2.1	Method of the Effect of Biosurfactant in Treating of Mercury Concentration	23-24
3.2.2	The Effect of Biosurfactant on COD	24
3.2.3	The Effect of Biosurfactant on BOD	24
3.2.4	The effect of biosurfactant on pH	25
4.0	RESULT AND DISCUSSION	
4.1	The effect of Biosurfactant in Mercury Concentration	26-28

4.2	The effect of Biosurfactant on COD	29-30
4.3	The effect of Biosurfactant on BOD	31-32
4.4	The effect of Biosurfactant on pH	33-34
5.0	CONCLUSION	35-36
	REFERENCES	37-38
	APPENDIX	40-57

LIST OF FIGURES

FIGURE NO	TITLE	PAGE
2.1	Wastewater Treatment System	11
2.2	Atomic bonding of mercury structure	16
2.3	Structure of Mercury	17
2.4	Example of Mercury Treatment	17
4.1	Graph for Mercury Analysis	27
4.2	Graph for Mercury Analysis (Linear)	28
4.3	Graph for COD Analysis	29
4.4	Graph For COD Analysis (Linear)	30
4.5	Graph for BOD analysis	31
4.6	Graph For BOD Analysis (Linear)	32
4.7	Graph for pH analysis	33
4.8	Graph for pH analysis (Linear)	34

LIST OF TABLE

TABLE NO.	TITLE	PAGE
TABLE 4.1	Mercury Analysis	26
TABLE 4.2	COD Analysis	29
TABLE 4.3	BOD Analysis	31
TABLE 4.4	pH Analysis	34

LIST OF APPENDIX

APPENDIX NO	TITLE	PAGE
APPENDIX A	Concentrated Biosurfactant	39
APPENDIX B	Synthetic Wastewater	40
APPENDIX C	Calorimeter Analysis	41
APPENDIX D	Cold Vapor Mercury Concentration Method	42-49
APPENDIX E	Dilution Method (BOD Analysis)	50-53
APPENDIX F	Reactor Digestion Method (COD Analysis)	54-57
APPENDIX G	Raw Material For Synthetic Wastewater	58

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Microbial surface active agents (biosurfactants) are important biotechnological products, with a wide range of applications in many industries. Their properties of interest are: *(i)* in changing surface active phenomena, such as lowering of surface and interfacial tensions, *(ii)* wetting and penetrating actions, *(iii)* spreading, *(iv)* hydrophilicity and hydrophobicity actions, *(v)* microbial growth enhancement, *(vi)* metal sequestration and *(vii)* anti-microbial action. Most of the applications today involve the use of chemically synthesized surfactants.

Most surfactants are produced from petroleum and require costly synthesis and purification steps. Despite their cost, new applications for surfactants have increased demand world-wide. One alternative to synthetic surfactants are microbial produced surfactants, also called biosurfactants. Like synthetic surfactants, biosurfactants reduce the surface and interfacial tensions of aqueous media. However, unlike many chemical formulations they are easily biodegraded. Biosurfactants are a unique class of compounds that have been shown to have a variety of potential applications, including remediation of organics and metals, enhanced transport of bacteria, enhanced oil recovery, as cosmetic additives and in biological control. Interest in microbial produced surfactants has led to a need for the further development of rapid and efficient qualitative and quantitative methods for screening and analyzing biosurfactant-producing organisms. Several methods exist to

measure surfactant concentrations in liquid media. These methods measure the surface force between a liquid and air (surface tension) or the force between two liquids (interfacial tension), which is then correlated to surfactant concentration. Currently, the most widely used method is the du Nouy ring method, which measures the force required to pull a platinum wire ring through a liquid-air or liquid-liquid interface. The method is widely used due to its accuracy, ease of use and the fact that it provides a fairly rapid measurement of surface and interfacial tension.

The widespread use of petroleum hydrocarbons has resulted in the contamination of valuable groundwater resources. Petroleum hydrocarbons may exist in the vadose and saturated zones as a free liquid or ganglia of residual hydrocarbon. Even if the free liquid hydrocarbon can be removed, substantial amounts of residual hydrocarbon remain entrapped by capillary forces and represent a long-term source of contaminate. Petroleum hydrocarbon continues to be used as the principle source of energy and hence an important global environmental pollutant. Apart from accidental contamination of the ecosystem, the vast amounts of oil sludge generated in refineries from water oil separation.

A biosurfactant is defined as a surface-active molecule produced by living cells, in the majority of cases by micro-organisms¹. They are amphiphilic molecules that tend to partition preferentially at the interface between fluid phases of different degrees of polarity and hydrogen bonding such as oil/water or air/water interfaces. In general bacteria make low molecular weight molecules that efficiently lower surface and interfacial tensions and high molecular weight polymers that bind tightly to surfaces.

Surfactants and emulsifiers are widely used in the pharmaceutical, cosmetic, petroleum and food industries. However, most of these compounds synthesized chemically are toxic to environments, not easily biodegradable and their manufacturing processes and by-products can be environmentally hazardous. Biodegradability, low toxicity, availability

of raw materials, biocompatibility and digestibility, which allows their application in cosmetics, pharmaceuticals and as food additives and their functionality under extreme conditions are some of the advantages shown by biosurfactants as compared to their chemically synthesized counterparts . Thus, these compounds have been tested in environmental applications such as bioremediation and dispersion of oil spill, enhanced oil recovery and transfer of crude oil, and are thought to be potential candidates to replace chemical surfactants in the future, especially in the food, cosmetics and health care industries, industrial cleaning and in agricultural chemicals . Nowadays environmental compatibility is becoming an increasingly important.

1.2 RESEARCH OBJECTIVE

The objectives of this study are to study the petrochemical wastewater quality based on the parameter that been decided before which is the mercury, BOD, COD and ph. The main objectives of this research project are focused more on the effect of biosurfactant in solving of petrochemical wastewater. Due to many compositions of petrochemicals wastewater, this research project focused more on the effect of mercury concentration from petrochemical wastewater by using biosurfactant. Another study is the effect on the BOD, COD and pH of petrochemical wastewater after treatment of biosurfactant.

1.3 RESEARCH SCOPE

The scope of this research project will cover on how make treatment of synthetic wastewater (as an alternative to petrochemical wastewater) by using biosurfactant. This project was divided into three processes. The first project is to make sample preparation of synthetic wastewater. The second process is to make treatment to the synthetic wastewater by using biosurfactant. Make analysis of the wastewater quality was done at the final stage. Different method was used to a different parameter by using HACH technology (Waste water quality equipment). Result from this analysis would be analyzed to come up with conclusion.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Remediation soil contaminated with potentially toxic metal cations such as Pb^{2+} , Zn^{2+} , Cr^{3+} , Cd^{2+} , and Hg^{2+} has traditionally involved the excavation and transport of contaminated soil to hazardous waste sites for landfilling

Technologies using microorganisms and microbial products to remove metals have been successfully applied to waste streams such as sewage sludge, industrial effluents, and mine water. Approaches used in these systems exploit microbial-metal interactions to concentrate and separate metals from the waste stream. These interactions, which are described in several recent reviews, include metal binding to the cell surface or within the cell wall, translocation of the metal into the cell, volatilization of the metal as a result of a biotransformation reaction, and the formation of metal precipitates by reaction with extra cellular polymers or microbial produced anions such as sulphide or phosphate.

In situ bioremediation of metal-contaminated soils presents a more complex separation problem due to the presence of soil. The soil surface area as well as the mineral and organic matter composition of the soil will determine the amount of metal absorbed. Metal sorption occurs through one of three mechanisms: cation-exchange, metal-ligand complexation to soil, or metal complexation with soil organic matter. Sorption by any of these mechanisms effectively limits the availability of metals for removal by flushing. Another complicating factor is the selectivity of a soil for a metal. In many instances soils

are contaminated with a mixture of metals, and the relative affinity of the soil for any given metal in this mixture varies. Selectivity is both a function of ionic radius, for instance the sorption of $\text{Hg}^{2+} > \text{Cd}^{2+} > \text{Zn}^{2+}$, and of electron configuration, e.g., $\text{Cu}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Fe}^{2+} > \text{Mn}^{2+}$. Thus, the difficulty of removing specific metals from soil may vary. (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed&cmd=Retrieve&list_uids=7621801&dopt=Citation)

2.1 Biosurfactant

A biological Technique has potential for removal of metals from soil is the use of microbial produced surfactants (biosurfactants). Biosurfactants have the potential to impact the major factors that cause the removal of heavy metals from soil to be so difficult, namely, sorption, rate-limited mass transfer, and resistance to aqueous-phase transport. Biosurfactants are produced by plants, animals, and many different microorganisms. When considering approaches to remediation of contaminated sites, there are several apparent advantages to the use of biosurfactants rather than synthetic ones; they are biodegradable, they may be cost-effective, and it may be possible to produce them *in situ* at contaminated sites.

In general, surfactants are amphoteric molecules consisting of a nonpolar tail and a polar/ionic head. In aqueous solution, surfactants reduce surface tension by accumulating at interfaces and facilitating the formation of emulsions between liquids of different polarities. At low concentration, surfactants are present as individual molecules. However, as the concentration of the surfactant is increased, a concentration is reached where no further change in interfacial properties takes place. The amount of surfactant needed to reach this concentration is called the *critical micelle concentration* (CMC). At the CMC, surfactant

molecules aggregate to form structures such as bilayers, vesicles, or micelles. The type and size of aggregate formed depends on the surfactant structure and on the solution pH. Micelles are the smallest basic structure formed, generally less than 5 nm in diameter. A micelle is composed of a monolayer of surfactant molecules where the polar heads are oriented toward the surrounding aqueous solution and the nonpolar tails are oriented toward the hydrophobic center of the micelle. Vesicle structures are next in size and range from 10 nm to more than 500 nm in diameter. Vesicles are composed of surfactant bilayers, which are similar in structure to biological membranes. In aqueous solution, the polar surfactant heads of a bilayer face the outside while the nonpolar tails are sandwiched between the heads. Thus, the environment both inside and outside a vesicle is hydrophilic (aqueous) while the environment within the bilayer, composed of the nonpolar surfactant tails, is hydrophobic. Bilayers can also exist as flexible sheets or planar bilayers which are the largest of the basic surfactant structures. A bilayer sheet is essentially unlimited in size. If the solution on both sides of the bilayer is the same, the properties and behavior of the two bilayer surfaces will be identical. However, if the bilayer is at an interface, e.g., air-water or liquid-liquid, the bilayer may develop asymmetric properties. Typically, CMCs of biosurfactants range from 1 to 200 mg/l.

Biosurfactants are produced by many different bacterial genera. The chemical structure of biosurfactants varies widely, but all biosurfactants described thus far in the literature are anionic or nonionic. Biosurfactants can be classified into several broad groups: glycolipids, lipopeptides, lipopolysaccharides, phospholipids, and fatty acids/neutral lipids. The largest and best-studied group of biosurfactants are the glycolipids, which include the sophorose-, rhamnose-, trehalose-, sucrose-, and fructose-lipids. Both biosurfactant yield and composition are affected by growth conditions including carbon source, culture medium nutrients (e.g., nitrogen, phosphate, and iron), temperature, pH, and agitation. In addition, there are species level differences in the chemical structure of biosurfactants. For instance, the rhamnolipids produced by various

Pseudomonas sp. differ both in the number of rhamnose molecules (1 to 2) and the length of the lipid moiety. Biosurfactant molecular weights range from approximately 500 to 1500 mw, although some exceptions exist(*Francisco J. Ochoa-Loza, Janick F. Artiola, and Raina M. Maier**)

2.2.1 General Use of Charge Types

Most natural surfaces are negatively charged. Therefore, if the surface is to be made hydrophobic (water-repellent) by use of a surfactant, then the best type of surfactant to use is a cationic. This type of surfactant will adsorb onto the surface with its positively charged hydrophilic head group oriented toward the negatively charged surface (because of electrostatic attraction) and its hydrophobic group oriented away from the surface, making the surface water-repellent. On the other hand, if the surface is to be made hydrophilic (water-wettable), then cationic surfactants should be avoided. If the surface should happen to be positively charged, however, then anionics will make it hydrophobic and should be avoided if the surface is to be made hydrophilic.

Nonionics adsorb onto surfaces with either the hydrophilic or the hydrophobic group oriented toward the surface, depending upon the nature of the surface. If polar groups capable of H bonding with the hydrophilic group of the surfactant are present on the surface, then the surfactant will probably be adsorbed with its hydrophilic group oriented toward the surface, making the surface more hydrophobic; if such groups are absent from the surface, then the surfactant will probably be oriented with its hydrophobic group toward the surface, making it more hydrophilic.

Zwitterionics, since they carry both positive and negative charges, can adsorb onto both negatively charged and positively charged surfaces without changing the charge of the surface significantly. On the other hand, the adsorption of a cationic onto a negatively charged surface reduces the charge on the surface and may even reverse it to a positive charge (if sufficient cationic is adsorbed). In similar fashion, the adsorption of an anionic surfactant onto a positively charged surface reduces its charge and may reverse it to a negative charge. The adsorption of a nonionic onto a surface generally does not affect its charge significantly, although the effective charge density may be reduced if the adsorbed layer is thick. Differences in the nature of the hydrophobic groups are usually less pronounced than those in the nature of the hydrophilic group. Generally, they are long-chain hydrocarbon residues.

2.2.2 General Effects of the Nature of the Hydrophobic Group

2.2.2.1 Length of the Hydrophobic Group

Decreases the solubility of the surfactant in water and increases its solubility in organic solvents, Causes closer packing of the surfactant molecules at the interface (provided that the area occupied by the hydrophilic group at the interface permits it), Increases the tendency of the surfactant to adsorb at an interface or to form aggregates, called micelles, Increases the melting point of the surfactant and of the adsorbed film and the tendency to form liquid crystal phases in the solution, and Increases the sensitivity of the surfactant, if it is ionic, to precipitation from water by counterpart