UNIVERSITI TEKNOLOGI MARA

A STUDY ON A STABILITY OF BIOSURFACTANT RHAMNO NR.22 FOR OIL AND GAS APPROACH

SYARIZAD SOFIA BINTI MOHD SHUKRI

Thesis submitted in fulfillment
of the requirements for the degree of
Bachelor of Engineering (Hons.) Chemical (Chemical Engineering)

Faculty of Chemical Engineering

June 2019

ABSTRACT

A Gram negative rod bacteria, *Pseudomonas aeruginosa* NR.22 (Ps.NR.22) has been cultured for biosurfactant Rhamno NR.22 production. This research focused on the application in oil and gas industry such as bioremediation and cleaning oil spill from crude oil shipping. The production of biosurfactant Rhamno NR.22 was observed by the 28% foaming formed in the cultured medium. It was characterized using FTIR analysis to observe the functional group and the component presence. O-H, C=C=O, C=C and C-N bonds were observed from the analysis concluded that the biosurfactant Rhamno NR.22 were presences. However, another non-related bond was detected as the biosurfactant Rhamno NR.22 produced was semi pure. The stability of the biosurfactant Rhamno NR.22 were tested by oil displacement test and determination of emulsification index in distilled water and brine solution with different range parameter of pH and temperature at 5, 7, 9 and 37ºC, 45ºC, 60ºC respectively. The results shows that pH 7 has the biggest displacement of oil with 9.6cm while the pH 5 recorded the lowest displacement of 7.5cm in distilled water. Therefore, the biosurfactant Rhamno NR.22 showed better stability at alkaline than acidic condition and the best stability when the pH is neutral. The findings shows that there was only slight change of displacement oil where the clear zone formed with less than 0.04cm changes. This biosurfactant can be categorized as thermostable as the thermal stability studies shows no significant effect on the biosurfactant performance. On the other side, other syhthetic surfactants exhibits a significant loss of activity a temperatures above 70ºC. It shown that the oil displacement in the brine solution was higher but not significant. The changes recorded for both pH and temperature stability are ±2%. The oil displacement test achieved with value almost the same in distilled water and brine solution showing that Rhamnolipid NR.22 is not a brine dependent. It was recorded that the biosurfactant Rhamno NR.22 has high emulsification activity with white layer fomed. The pH and temperature of emulsification index, E_{24} recorded above 50% and the highest at 37ºC and pH 7 with 60.78%. Biosurfactant Rhamno NR.22 proved to be encountered the oil field industry by the robust and stable over huge ranges of pH and temperature.

ACKNOWLEDGEMENT

Firstly, I wish to thank God for giving me the opportunity to embark on my Bachelor Degree and for completing this long and challenging journey successfully. My gratitude and thanks go to my supervisor Dr. Nik Raikhan Nik Him for her guidance and knowledge on bacteria to complete this research.

My appreciation goes to the lab asisstants, Encik Ridzuan and others from Faculty of Chemical Engineering who constantly gives help and provided the facilities such as lab equipment and assistance during testing and analysis.

I also would like to thank faculty and UiTM for this golden opportunity to learn on conducting research which gives me a lot of lesson and experience new things. Not to remember a special thanks to my colleagues and friends for helping me with this project.

Finally, this thesis is dedicated to my supermom and the loving memory of my very dear late father for the vision and determination to educate me. This piece of victory is dedicated to both of you. Alhamdulilah.

TABLE OF CONTENTS

CONFIRMATION BY PANEL OF EXAMINERS i
AUTHOR'S DECLARATION ii
ACKNOWLEDGEMENT iv
LIST OF TABLES vii
LIST OF FIGURES viii
LIST OF PLATES ix
CHAPTER ONE: INTRODUCTION 1
1.1 Research Background 1
1.2 Problem Statement 3
1.3 Objectives 4
1.4 Scope of work 4
CHAPTER TWO: LITERATURE REVIEW 6
2.1 Biosurfactant 6
2.1.1 Classification of Biosurfactant 7
2.1.2 Glycolipid biosurfactants 7
2.1.3 Availability of Feedstock and Impacts on Biosurfactant 8
2.2 Rhamnolipids 9
2.3 Economic Feasibility 11
2.4 Environmental Contamination 11
2.5 Application of Biosurfactant 12
2.5.1 Bioremediation 12
2.6 Toxicity of Biosurfactant 14
CHAPTER 3: METHODOLOGY 16
3.1 Process Flow Diagram 16
3.2 Materials 17
3.2.1 Biosurfactant Rhamno NR22 17
3.2.2 Preparation of Brine Broth 18
3.2.3 Petrol Oil 18
3.2.4 Crude Oil 18
3.3 Production of Biosurfactant Rhamno NR.22 19
3.3.1 Preparation of Pseudomonas agar 19
3.3.2 Microorganism and culture conditions 20
3.3.3 Extraction and purification of biosurfactant 22

CHAPTER ONE

INTRODUCTION

1.1 Research Background

Surfactant is a compound which lowers the surface tension between one medium with another medium such as liquid and liquid, liquid and solid also liquid and gas. An effective surfactant have a good solubilization capacity and can reduce the surface tension efficiently (Rikalović et al., 2015). Somehow some research and patents have been done for the production of biosurfactant as it can give promising production of biosurfactant based on its advantages an also findings for the alternatives of sustainability and new environmental legislation product. In Plate 1.1, it shows that the biosurfactant contains hydrophobic an hydrophilic moieties that are produced from varies types of microorganism such as bacteria, yeast and fungi (Syahriansyah et al., 2016) . This surfactants are synthesized from the living organism where plants produces saponins, microorganism produces glycolipids and animals produce bile salt. From the data recorded, there are 250 and more patents on these wonder biodegradable molecule so far (Randhawa et al., 2014).

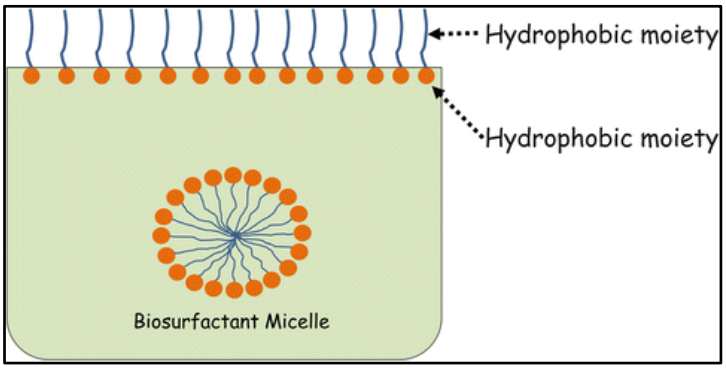

Plate 1.1 Hydrophobic and hydrophilic moieties of biosurfactant

Biosurfactants are divided into five major categories which are glycolipids, phospholipids and fatty acids, lipopeptides and lipoproteins, polymeric biosurfactants and particulate biosurfactants (Randhawa et al., 2014). Different types of biosurfactant produced based on its aerobic microorganism used and the carbon feedstock. Extensive amount of glycolipids was produced from the genus of *Pseudomonas aeruginosa* where the biosurfactants are classified as rhamnolipids (Silva et al., 2014). Rhamnolipids can