UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF RHAMNOLIPID BIOSURFACTANT BY Pseudomonas aeruginosa USING SUNFLOWER OIL AND GLUCOSE AS CARBON SOURCES

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PRODUCTION OF RHAMNOLIPID BIOSURFACTANT BY *Pseudomonas aeruginosa* USING SUNFLOWER OIL AND GLUCOSE AS CARBON SOURCES

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162469

Thesis submitted to the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Bachelor Science (Honours) Biotechnology

UNIVERSITI PUTRA MALAYSIA

2015

FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES

UNIVERSITI PUTRA MALAYSIA

Date:

LETTER OF PERMISSION

It is certified that I, SARINAH BAHARUN (Matric Number: 162469) have completed this final year project entitled "**Production of Rhamnolipid Biosurfactant by *Pseudomonas aeruginosa* using Sunflower Oil and Glucose as Carbon Sources**" under the supervision of Dr. Helmi Wasoh @ Mohamad Isa from Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

I hereby give permission to my supervisor to write and prepare the manuscript of this research to be published in any form, if I have not done so within six months from this date with a condition that my name will be included as one of the authors of the article. However, the order of the names is depend on the discretion of my supervisor.

Sincerely,

…….....

(SARINAH BAHARUN)

FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
UNIVERSITI PUTRA MALAYSIA

APPROVAL SHEET

The thesis entitled "**Production of Rhamnolipid Biosurfactant by *Pseudomonas aeruginosa* using Sunflower Oil and Glucose as Carbon Sources**" was prepared by SARINAH BAHARUN (Matric Number: 162469) and submitted to Faculty of Biotechnology and Biomolecular Sciences as fulfilment of the requirement for the Degree of Bachelor Science (Honours) Biotechnology.

Approved by:

……..... Date:
(Dr.Helmi Wasoh @ Mohamad Isa)
Project Supervisor
Department of Bioprocess Technology
Faculty of Biotechnology and Biomolecular Sciences
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ABSTRACT

Abstract of thesis presented to the Faculty of Biotechnology and Biomolecular Sciences in fulfilment of the requirement for the Degree of Bachelor Science (Honours) Biotechnology

PRODUCTION OF RHAMNOLIPID BIOSURFACTANT BY *Pseudomonas aeruginosa* USING SUNFLOWER OIL AND GLUCOSE AS CARBON SOURCES

By:

SARINAH BAHARUN

JUNE 2015

Supervisor : Helmi Wasoh @ Mohamad Isa, PhD
Faculty : Faculty of Biotechnology and Biomolecular Sciences

Biosurfactants are surface active compounds and amphiphatic in nature which consist of hydrophilic head and hydrophobic tail. These properties allow them to accumulate at the interphase of two immiscible liquid with different polarity such as in oil/water system and reducing surface and interfacial tension to facilitate emulsification. Rhamnolipids are biosurfactants produced by *Pseudomonas* sp. that offer significant advantageous over synthetic surfactants. Its production become increasingly throughout the years, however, the production yield was very low which discourage investment in this field. Therefore, a study was conducted to investigate the effectiveness of sunflower oil and glucose in the production of rhamnolipids by *Pseudomonas aeruginosa* in shake flask fermentation. In this process, four different fermentation treatments were done for seven days at 30°C under 180rpm. The sampling was carried out in appropriate time intervals (24 hours) and monitored for cell growth and the rhamnolipids production. Colorimetric Orcinol analysis was used for the determination of rhamnolipids concentrations. The produced rhamnolipids were studied for emulsification activity against hydrophobic substrate by using emulsification index (E_{24}%) methods. In addition, oil displacement activity and the

thermal stability were also studied over a wide range of temperature (4°C - 120°C). All treatments allow the growth of *Pseudomonas aeruginosa*, however the utilisation of sunflower oil as carbon source (addition at 8 hours) and glucose as growth initiator were observed to be the best strategy for maximum rhamnolipids production. The maximum rhamnolipids production was achieved after 120 hours with 3.18 g/L of rhamnolipids. Diesel shows the highest emulsification activity among the substrate tested ranging from 55.56% - 60.00%. The oil displacement activity was correspond to rhamnolipids concentration and very good stability which up to 120°C (for 60 minutes). Therefore, from this research a good potential of rhamnolipids biosurfactants that may provide good application for industrial were successfully produced.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Fakulti Bioteknologi dan Sains Biomolekul sebagai memenuh keperluan untuk Ijazah Sarjana Muda Sains (Kepujian) Bioteknologi

PENGHASILAN RHAMNOLIPID BIOSURFAKTAN OLEH *Pseudomonas aeruginosa* MENGGUNAKAN MINYAK BUNGA MATAHARI DAN GLUKOSA SEBAGAI SUMBER KARBON

Oleh:

SARINAH BAHARUN

JUN 2015

Penyelia : Helmi Wasoh @ Mohamad Isa, PhD
Fakulti : Fakulti Bioteknologi dan Sains Biomolekul

Biosurfaktan adalah sebatian permukaan aktif dan amfifatik dalam alam semula jadi yang terdiri daripada kepala hidrofilik dan ekor hidrofobik. Ciri-ciri ini membolehkan mereka untuk berkumpul di antara fasa dua cecair yang tidak boleh bercampur dengan polariti yang berbeza seperti dalam sistem minyak/ air dan mengurangkan permukaan dan tegangan antara muka untuk memudahkan pengemulsian. Rhamnolipids adalah biosurfaktan dihasilkan oleh *Pseudomonas* sp. yang menawarkan kelebihan yang ketara berbanding sintetik surfaktan. Pengeluarannya sentiasa bertambah sepanjang tahun, walau bagaimanapun, hasil pengeluaran yang sangat rendah tidak menggalakkan pelaburan dalam bidang ini. Oleh itu, satu kajian telah dijalankan untuk mengkaji keberkesanan minyak bunga matahari dan glukosa dalam pengeluaran rhamnolipids oleh *Pseudomonas aeruginosa* secara penapaian dalam kelalang goncang. Dalam proses ini, empat rawatan penapaian yang berbeza telah dilakukan selama tujuh hari pada suhu 30°C dan goncangan 180 rpm. Pensampelan telah dijalankan pada selang masa yang sesuai (24 jam) dan dipantau untuk pertumbuhan sel dan pengeluaran rhamnolipids. Analisis permetrean warna Orcinol telah digunakan bagi menentukan kepekatan

rhamnolipids. Rhamnolipids terhasil dikaji untuk aktiviti pengemulsian terhadap substrat hidrofobik dengan menggunakan kaedah indeks pengemulsian (E24%). Di samping itu, aktiviti sesaran minyak dan kestabilan haba juga dikaji dalam pelbagai suhu (4°C - 120°C). Semua rawatan membolehkan pertumbuhan *Pseudomonas aeruginosa,* bagaimanapun strategi yang terbaik penggunaan minyak bunga matahari sebagai sumber karbon (penambahan pada jam ke 8) dan glukosa sebagai pemula pertumbuhan dikaji untuk pengeluaran rhamnolipids yang maksimum. Pengeluaran rhamnolipids maksimum dicapai selepas 120 jam dengan 3.18 g/L rhamnolipids. Diesel menunjukkan aktiviti pengemulsian yang tertinggi dalam kalangan substrat yang diuji iaitu terdiri dalam lingkungan 55.56% - 60.00%. Aktiviti sesaran minyak adalah sesuai dengan kepekatan rhamnolipids dan kestabilan yang sangat baik sehingga 120°C (untuk 60 minit). Oleh itu, kajian ini mempunyai potensi yang baik dalam menghasilkan rhamnolipids biosurfaktan yang boleh menyediakan aplikasi yang sesuai untuk industri.

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LIST OF ABBREVIATIONS

°C	Degree celcius
%	Percentage
±	Plus minus sign
BS	Biosurfactants
$CaCl_2$	Calcium chloride
cm	Centimetre
g	Gram
g/L	Gram per litre
H_2SO_4	Sulphuric acid
KCl	Potassium chloride
K_2HPO_4	Dibasic potassiummonohydrogen phosphate
KH_2PO_4	Monobasic potassium dihydrogen phosphate
L	Litre
$MgSO_4$	Magnesium sulphate
mL	Milliltre
µL	Microlitre
NaCl	Sodium chloride

NaOH	Sodium hydroxide
$NaNO_3$	Sodium nitrate
nm	Nanometre
OD	Optical Density
R^2	Regression coefficient value
RLs	Rhamnolipids
Rpm	Revolutions per minute
V/V	Volume per volume
W/V	Weight per volume

CHAPTER 1

INTRODUCTION

In this modern year, the environment contamination by hydrocarbon substance is becoming increasingly concerned. One of the major sources of this contamination is oil spill. This usually happened during the transportation of the oil from one place to another place. The oil includes the crude oil or oil products such as diesel and oily waste. As the needs to clean up the oil either on the ocean or land, the demand on surfactants in the world is becoming increasingly important throughout the years. In the presence of surfactants, two immiscible liquid like water and oil will mix more easily. As a result, the cleanup process would be easier without the needs of use pump and suction which is high cost.

However, surfactant is chemically synthesised that the chemical themselves can cause harm to environment. Thus, as the increase of awareness on the need to protect the environment, the production of surfactants from microbial origin is developed as possible alternatives to replace the chemically synthesised ones. Biosurfactants can be produced extracellularly by microorganisms such as bacteria, fungi and yeast through biological processes. Rhamnolipids (RLs) are the best known biosurfactants produced by the Gram-negative bacterium *Pseudomonas aeruginosa*. Biosurfactants offer many advantageous over synthetic surfactants including its biodegradability and non-toxic to environment. Besides, it can be produced from renewable resources which are available in large quantities, thus help on sustainable production of biosurfactants. Sunflower oil is one of the examples that can be used as carbon source for the production of biosurfactants by bacteria. Sunflower oil produced by pressing the seed of sunflower. Sunflower oil is valued for its light taste and appearance, frying performance and health benefits. Sunflower oil is the mixture of monounsaturated and polyunsaturated fats with low saturated fat levels.

The cost of bioremediation process, waste treatments and other potential application is high. Due to this problem, the need for increasing the yield of biosurfactants is inevitable. There are multiple consecutive steps in downstream processing are needed in order to get pure surface active agents lead to high of the total production expenditure. Besides, the yield of bisurfactants produced is usually low. Carbon sources are one of the physicochemical factors that influence the production of biosurfactants. In addition to usual water soluble substrates such as glucose, there are varieties of unusual insoluble substrates have been used for the growth as well as biosurfactants production by microorganisms. The incorporation of using vegetable oils and glucose in industrial production media might potentially increase the yield of biosurfactants.

The purpose of the study is to investigate the effectiveness sunflower oil and glucose in the production of rhamnolipids biosurfactants by *Pseudomonas aeruginosa* in shake flask fermentation system. This will help in the verification of its potential industrial application in the environment.

1.1 Objectives

1.1.1 The general objective of this study is:

The general objective of this study is to study the effectiveness of sunflower oil and glucose in the production of rhamnolipids biosurfactants by *Pseudomonas aeruginosa* in shake flask fermentation system.

1.1.2 The specific objectives of the experiment are:

1. To determine the effectiveness of using sunflower oil (and glucose as co-substrate) as carbon source in rhamnolipids production by *Pseudomonas aeruginosa*.

2. To differentiate the production of rhamnolipids by *Pseudomonas aeruginosa* under single and dual substrate treatment.

3. To evaluate the efficiency of rhamnolipids produced in term of its surface activity and stability for further application in bioremediation.

1.2 Significant of Study

To investigate the utilisation of different carbon sources used for rhamnolipid biosurfactant production by *Pseudomonas aeruginosa.*

1.3 Hypothesis

A dual substrate system containing both glucose and sunflower oil as carbon sources would enhance the growth of *Pseudomonas aeruginosa* and rhamnolipid biosurfactant production.

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