

**BIOSURFACTANT PRODUCTION BY *PSEUDOMONAS AERUGINOSA* SP4
USING SEQUENCING BATCH REACTORS: EFFECT OF
OIL-TO-GLUCOSE RATIO**



Sira Pansiripat

A Thesis Submitted in Partial Fulfilment of the Requirements
for the Degree of Master of Science
The Petroleum and Petrochemical College, Chulalongkorn University
in Academic Partnership with
The University of Michigan, The University of Oklahoma,
Case Western Reserve University and Institut Français du Pétrole
2009

522087

Thesis Title: Biosurfactant Production by *Pseudomonas aeruginosa* SP4
Using Sequencing Batch Reactors: Effect of Oil-to-Glucose
Ratio

By: Sira Pansiripat

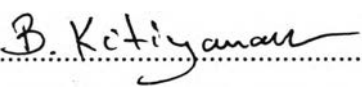
Program: Petrochemical Technology


Thesis Advisors: Asst. Prof. Boonyarach Kitiyanan
Assoc. Prof. Sumaeth Chavadej
Prof. Masahiko Abe
Assoc. Prof. Ratana Rujiravanit


Accepted by the Petroleum and Petrochemical College, Chulalongkorn
University, in partial fulfilment of the requirements for the Degree of Master of
Science.

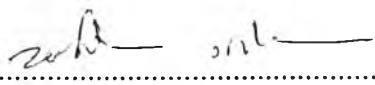

..... College Dean
(Asst. Prof. Pomthong Malakul)

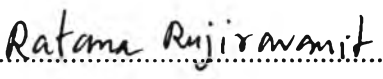
Thesis Committee:

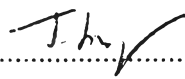

.....
(Asst. Prof. Boonyarach Kitiyanan)


.....
(Prof. Masahiko Abe)


.....
(Assoc. Prof. Sumaeth Chavadej)


.....
(Assoc. Prof. Suntud Sirianuntapiboon)


.....
(Assoc. Prof. Ratana Rujiravanit)


.....
(Asst. Prof. Siriporn Jongpatiwut)

ABSTRACT

5071024063: Petrochemical Technology Program

Sira Pansiripat: Biosurfactant Production by *Pseudomonas aeruginosa* SP4 Using Sequencing Batch Reactors: Effect of Oil-to-Glucose Ratio.

Thesis Advisors: Asst. Prof. Boonyarach Kitiyanan, Assoc. Prof. Sumeath Chavadej, Prof. Masahiko Abe, and Assoc. Prof. Rattana Rujiravanit, 89 pp.

Keywords: Biosurfactant/ Rhamnolipid/ *Pseudomonas aeruginosa*/ Sequencing Batch Reactor/ Palm oil/ Glucose

Sequencing batch reactors (SBRs) were used for producing biosurfactants by *Pseudomonas aeruginosa* SP4, which was isolated from a petroleum-contaminated soil in Thailand. The studied SBRs were operated at a constant temperature of 37°C under aseptic conditions with a 1,500 ml working volume, 500 ml feeding volume, and 500 ml decanting volume. Palm oil and a mineral medium were used as the carbon source and the nutrient source, respectively. Glucose (another type of carbon source) was added to the mineral medium for the enhancement of microbial growth which, in turn, increases the biosurfactant production. The oil-to-glucose ratio was varied in this work in order to determine the optimum ratio of oil-to-glucose for a maximum yield of biosurfactant. The results showed that the optimum ratio for biosurfactant production was 40/1. It provided a surface tension reduction of 58.5% and a surface tension of 29.9 mN/m, corresponding to a highest COD and oil removal of 85.1% and 77.7%, respectively. The finding exhibited that the biosurfactant was produced after 6 h of the aeration step caused the sharply decrease of surface tension before constant at around 28 to 31 mN/m. From the critical micelle dilution (CMD) method results, that the biosurfactant concentration was found to be 1.11 times CMC.

บทคัดย่อ

ศิริ พันศิริพัฒน์ : การผลิตสารลดแรงตึงผิวชีวภาพจาก *Pseudomonas aeruginosa* SP4 โดยเครื่องปฏิกรณ์แบบกะต่อเนื่อง: ผลของอัตราส่วนระหว่างน้ำมันและกลูโคส (Biosurfactant Production by *Pseudomonas aeruginosa* SP4 Using Sequencing Batch Reactors: Effect of Oil-to-Glucose Ratio) อ.ที่ปรึกษา : ศศ. ดร. บุญยรัชต์ กิตยานันท์ รศ. ดร.สุเมธ ชวเดช ศ. ดร.มะชะฮิโกะ อะเบะ และ รศ. ดร.รัตนา รุจิรวนิช 89 หน้า

เครื่องปฏิกรณ์แบบกะต่อเนื่องได้ถูกนำมาใช้ผลิตสารลดแรงตึงผิวชีวภาพชนิดแรมโนลิปิดจากเชื้อแบคทีเรียสายพันธุ์ *Pseudomonas aeruginosa* SP4 ซึ่งทำการคัดแยกมาจากแหล่งปิโตรเลียมที่มีดินปนเปื้อนน้ำมันเป็นเวลานานในประเทศไทย ซึ่งเครื่องปฏิกรณ์นี้ถูกดำเนินการทดลองในสภาวะที่ควบคุมอุณหภูมิที่ 37 องศาเซลเซียส และในสภาวะที่ปลอดเชื้อ โดยมีปริมาตรในการทำงานคือ 1,500 มิลลิลิตร ปริมาตรในการเติมสารคือ 500 มิลลิลิตร และปริมาตรในการตั้งสารผลิตภัณฑ์คือ 500 มิลลิลิตร โดยมีน้ำมันปาล์มเป็นแหล่งของธาตุคาร์บอนและสารอาหารแร่ธาตุเป็นแหล่งอาหารให้กับแบคทีเรีย นอกจากนี้ยังได้เติมกลูโคสซึ่งเป็นแหล่งของธาตุคาร์บอนลงในสารอาหารแร่ธาตุเพื่อเพิ่มการเจริญเติบโตของเชื้อแบคทีเรีย ทำให้ผลิตสารลดแรงตึงผิวชีวภาพได้มากขึ้น งานวิจัยนี้ได้ปรับเปลี่ยนอัตราส่วนระหว่างน้ำมันและกลูโคสเพื่อหาอัตราส่วนที่เหมาะสมในการผลิตสารลดแรงตึงผิวชีวภาพให้ได้มากที่สุด จากผลการทดลองแสดงให้เห็นว่าอัตราส่วนที่เหมาะสมสำหรับผลิตสารลดแรงตึงผิวชีวภาพคือ 40/1 ที่อัตราส่วนนี้สามารถลดแรงตึงผิวได้ถึง 58.5 เปอร์เซ็นต์ โดยมีค่าแรงตึงผิวเท่ากับ 29.9 มิลลินิวตันต่อเมตร ซึ่งสอดคล้องกับการย่อยสลายซีโอดีสูงสุดคือ 85.1 เปอร์เซ็นต์ และการย่อยสลายน้ำมันสูงสุดคือ 77.7 เปอร์เซ็นต์ จากการทดลองพบว่าสารลดแรงตึงผิวชีวภาพได้ถูกผลิตหลังจากช่วงเป่าอากาศ (aeration time) ไปแล้ว 6 ชั่วโมง ส่งผลให้แรงตึงผิวลดลงอย่างรวดเร็วจนกระทั่งคงที่ในช่วง 28 ถึง 31 มิลลินิวตันต่อเมตร จากวิธีเจือจางวิกฤตของไมเซลล์ (Critical Micelle Dilution) ทำให้พบว่าความเข้มข้นของสารลดแรงตึงผิวชีวภาพเป็น 1.11 เท่าของความเข้มข้นวิกฤตที่เกิดไมเซลล์ (Critical Micelle Concentration)

ACKNOWLEDGEMENTS

This thesis work was completed because the financial supported by the National Center of Excellence for Petroleum, Petrochemicals, and Advanced Materials; and The Research Unit of Applied Surfactants for Separation and Pollution Control, supported by Chulalongkorn University,

I would like to thank to all those who gave me the possibility to complete this thesis. All of my advisors, Asst. Prof. Boonyarach Kitiyanan, Assoc. Prof. Sumaeth Chavadej, Assoc. Prof. Ratana Rujiravanit and Prof. Masahiko Abe who advice and support through this research work.

All staffs of The Petroleum and Petrochemical College, Chulalongkorn University who assist and improve the instruments.

The previous student in this research, Ms. Onsiri Hauyyai, who provided useful information and instruction about the thesis.

Finally, I would like to give special thanks to my family whose encouragement enabled me to complete this research work.

TABLE OF CONTENTS

	PAGE
Title Page	i
Abstract (in English)	iii
Abstract (in Thai)	iv
Acknowledgements	v
Table of Contents	vi
List of Tables	ix
List of Figures	x
Abbreviations	xii
CHAPTER	
I INTRODUCTION	1
II LITERATURE REVIEW	3
2.1 Background on Surfactants and Biosurfactants	3
2.1.1 Surfactants	3
2.1.2 Biosurfactants	4
2.1.2.1 Types of Biosurfactants	4
2.1.2.1.1 Glycolipids	4
2.1.2.1.2 Lipoproteins or Lipopeptids	8
2.1.2.1.3 Phospholipids and Fatty Acids	9
2.1.2.1.4 Polymeric Biosurfactants	10
2.2 Factors Affecting Biosurfactants Production	13
2.2.1 Carbon Sources	13
2.2.2 Nitrogen Sources	16
2.2.3 Environmental Factors	17
2.3 Advantages and Disadvantages of Biosurfactants	19
2.3.1 Advantages	20
2.3.2 Disadvantages	20

CHAPTER	PAGE
2.4 Sequencing Batch Reactor on Biosurfactants Production	21
2.4.1 The Cyclic Process of SBR	21
III EXPERIMENTAL	26
3.1 Materials and Equipment	26
3.1.1 Equipment and Apparatus	26
3.1.2 Chemicals and Solvents	27
3.2 Methodology	28
3.2.1 Microorganism	28
3.2.2 Inoculums Preparation	28
3.2.3 Carbon Sources and Nutrients	28
3.2.4 Sequencing Batch Reactors (SBRs) Set up and Operation	29
3.2.4.1 Experimental Set up	29
3.2.4.2 SBRs Operation	31
3.2.5 pH Measurement	33
3.2.6 Suspended Solids (SS) Measurement	33
3.2.7 Mixed Liquor Suspended Solids (MLSS) Measurement	33
3.2.8 Biosurfactant Productivity Measurement	34
3.2.8.1 Surface Tension Measurement	34
3.2.8.2 Biosurfactant Concentration Measurement	34
3.2.8.3 Foam Layer Thickness Measurement	34
3.2.8.4 Critical Micelle Concentration (CMC) and Minimum Surface Tension Measurement	34
3.2.9 Chemical Oxygen Demand (COD) Measurement	35
3.2.10 Total Organic Carbon (TOC) Measurement	35
3.2.11 Total Nitrogen (TN) and Total Phosphorous (TP) Measurement	35
3.2.12 Palm Oil and Glucose Quantification	35

CHAPTER	PAGE
IV RESULTS AND DISCUSSION	37
4.1 Characterization of Mineral Medium, Palm Oil and Glucose	37
4.2 Effect of Oil-to-Glucose Ratios on Biosurfactant Production	38
4.2.1 Effect of Oil-to-Glucose Ratio on COD Removal	38
4.2.2 Effect of Oil-to-Glucose Ratio on Oil Removal	41
4.2.3 Effect of Oil-to-Glucose Ratio on Surface Tension and Surface Tension Reduction	42
4.2.4 Microbial Concentration	44
4.2.4.1 Mixed Liquor Suspended Solid (MLSS)	44
4.2.4.2 Suspended Solid (SS)	45
4.2.5 The Effluent pH	44
4.3 Measurement of Biosurfactant Concentration	49
V CONCLUSIONS AND RECOMMENDATIONS	51
REFERENCES	53
APPENDICES	56
Appendix A Experimental Data of Characterization of Mineral medium, Palm Oil, and Glucose	56
Appendix B Experimental Data of Biosurfactant Production at an Oil Loading Rate of 2 kg/m ³ d with Different Oil-to-Glucose Ratio	65
CURRICULUM VITAE	89

LIST OF TABLES

TABLE		PAGE
2.1	Microbial source and properties of important types of Biosurfactants	7
2.2	Surfactant production by <i>Pseudomonas aeruginosa</i> 44T1 with different carbon sources	14
3.1	Six ports in the SBR lid	30
3.2	The operating conditions of SBRs	32
4.1	Characteristics of palm oil and glucose	37
4.2	Characteristics of mineral medium (include glucose) at different oil-to-glucose ratios	38
4.3	The average COD of influent, effluent, and COD removal during steady-state operation with different oil-to-glucose ratios	39
4.4	The average surface tension and surface tension reduction during steady-state operation (days 8-14) of oil-to-glucose ratio with different oil-to-glucose ratios	44
4.5	The average effluent pH during the steady-state operation at (days 8-14) at different oil-to-glucose ratios	47

LIST OF FIGURES

FIGURE	PAGE
2.1 Schematic representation of four different rhamnolipids produced by <i>P.aeruginosa</i> .	6
2.2 Trehalose dimycolate from <i>Rhodococcus erythropolis</i> , in which disaccharide trehalose is linked to two long-chain α -branched β -hydroxy fatty acids.	7
2.3 (a) Structure of free-acid and (b) lactonized forms of Sophorolipids from <i>Torulopsis bombicola</i> in which dimeric sophorose is linked to a long-chain (C18) hydroxy fatty acid.	8
2.4 Structure of cyclic lipopeptide surfactin produced by <i>Bacillus subtilis</i> .	9
2.5 Structure of phosphatidylethanolamine, a potent biosurfactant produced by <i>Acinetobacter</i> sp. R ₁ and R ₂ are hydrocarbon chains of fatty acids.	10
2.6 Structure of emulsan, produced by <i>Acinetobacter calcoaceticus</i> , in which fatty acids are linked to a heteropolysaccharide backbone.	11
2.7 SBR operation for each tank for one cycle for the five discrete time periods of Fill, React, Settle, Draw, and Idle. (Irvine and Ketchum, 2004)	22
3.1 Schematic diagram of SBR.	29
3.2 Sequencing batch reactors (SBRs).	31
3.3 SBR operation for each tank for one cycle for the four discrete time periods of Fill, React, Settle, and Draw.	31

FIGURE	PAGE
4.1 Effluent COD as a function of operation time with an oil loading rate of $2 \text{ kg/m}^3\text{d}$ at different oil-to-glucose ratios (days 2-14).	40
4.2 COD removal as a function of operation time with an oil loading rate of $2 \text{ kg/m}^3\text{d}$ at different oil-to-glucose ratios (days 2-14).	40
4.3 Oil removal percentage during steady-state operation with the different oil-to-glucose ratios.	41
4.4 Profile of surface tension at different oil-to-glucose ratios as a function of operation time with an oil loading rate of $2 \text{ kg/m}^3\text{d}$.	43
4.5 Profile of surface tension reduction at different oil-to-glucose ratios as a function of operation time with an oil loading rate of $2 \text{ kg/m}^3\text{d}$.	43
4.6 An average MLSS during steady state operation (days 8-14) at different oil-to-glucose ratios.	45
4.7 An average effluent SS during steady-state operation at (days 8-14) at different oil-to-glucose ratios.	46
4.8 Profile of pH at different oil-to-glucose ratios as a function of operation time with an oil loading rate of $2 \text{ kg/m}^3\text{d}$.	47
4.9 The average percentage of COD, oil, and surface tension reduction during steady-state operation at (days 8-14) at different oil-to-glucose ratios.	48
4.10 A surface tension profile at 40/1 oil-glucose-ratio with an oil loading rate of $2 \text{ kg/m}^3\text{d}$.	50
4.11 A surface tension of serial dilutions at aeration time of 6, 12, 18, 24, 30, 36, 42, and 48 h of 40/1 oil-to-glucose ratio with 2 d/cycle time at an oil loading rate of $2 \text{ kg/m}^3\text{d}$.	50

ABBREVIATION

CFU	Colony-forming unit
CMC	Critical micelle concentration
CMD	Critical micelle dilution
C/N	Carbon per nitrogen ratio
COD	Chemical oxygen demand
C/P	Carbon per phosphorus ratio
CSTR	Continuous-flow stirred tank reactor
HRT	Hydraulic retention time
MLSS	Mixed liquor suspended solids
MM	Mineral medium
OLR	Oil loading rate
SBR	Sequencing batch reactor
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
SS	Suspended solids