

**SEPARATION AND PARTIAL PURIFICATION OF BIOSURFACTANTS
BY FOAM FRACTIONATION TECHNIQUE**



Thitima Sarachat

A Dissertation 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,
and Case Western Reserve University

2009

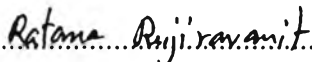
522075

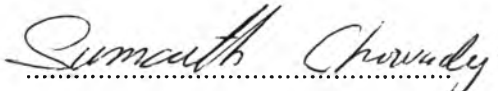
Thesis Title: Separation and Partial Purification of Biosurfactants by Foam Fractionation Technique
By: Thitima Sarachat
Program: Polymer Science
Thesis Advisors: Assoc. Prof. Ratana Rujiravanit
Prof. Masahiko Abe
Assoc. Prof. Sumaeth Chavadej


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

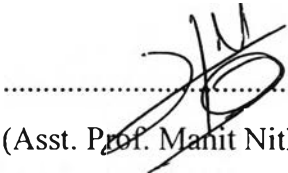

..... College Dean
(Asst. Prof. Pomthong Malakul)


Thesis Committee:


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


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


.....
(Prof. Masahiko Abe)


.....
(Asst. Prof. Manit Nithitanakul)


.....
(Dr. Manisara Phiriyawirut)

ABSTRACT

5072025063: Polymer Science Program

Thitima Sarachat: Separation and Partial Purification of Biosurfactants by Foam Fractionation Technique

Thesis Advisors: Assoc. Prof. Ratana Rujiravanit, Prof. Masahiko Abe, and Assoc. Prof. Sumaeth Chavadej 93 pp.

Keywords: Biosurfactants/ Foam Fractionation/ Biosurfactant recovery

Pseudomonas aeruginosa SP4, isolated from petroleum-contaminated soil in Thailand, was used to produce a biosurfactant from nutrient broth supplemented with palm oil. Normally, crude biosurfactant has been extracted from culture broth by organic solvent extraction. Owing to high cost and toxicity to environment and human health of organic solvents, foam fractionation, a surfactant-based separation, was used in this study to concentrate and remove the biosurfactant from cell-free nutrient broth solution. The effects of the air flow rate, column height, pore size of sinter glass disk, solution volume and the collecting time on enrichment ratio and percentage of biosurfactant recovery were investigated to determine the optimum condition for separating the biosurfactant from nutrient broth solution. It was found that increasing of either column height or pore size of sinter glass disk resulted in higher enrichment ratio but lower percentage of biosurfactant recovery. On a contrary, increasing of air flow rate resulted in a decrease in enrichment ratio but an increase in the percentage of biosurfactant recovery. The initial biosurfactant solution and biosurfactant in collapsed foam from foam fractionation process were fractionated by using HPLC-ELSD technique. The HPLC patterns of both biosurfactant samples are identical. However, the peak intensities of the biosurfactant in the collapsed foam were higher than those of the initial biosurfactant solution, indicating the higher biosurfactant concentration.

บทคัดย่อ

ฐิติมา สารชาติ : การคัดแยกและทำสารลดแรงตึงผิวชีวภาพให้บริสุทธิ์โดยวิธีการทำให้เกิดโฟม (Separation and Partial Purification of Biosurfactants by Foam Fractionation Technique) อ.ที่ปรึกษา : รศ.ดร.รัตนา รุจิรวนิช, ศ.ดร.มาชาฮีโกะ อาเบะ และ รศ.ดร.สุเมธ ชวเดช 93 หน้า

การผลิตสารลดแรงตึงผิวชีวภาพสามารถผลิตได้จากแบคทีเรียชนิด *Pseudomonas aeruginosa* SP4 ซึ่งทำการคัดแยกมาจากแหล่งปิโตรเลียมที่มีดินปนเปื้อนน้ำมันเป็นเวลานานในประเทศไทย โดยมีน้ำมันปาล์มเป็นแหล่งของธาตุคาร์บอนและสารอาหารแร่ธาตุเป็นแหล่งอาหารให้กับแบคทีเรียในการผลิตสารลดแรงตึงผิวชีวภาพ ซึ่งโดยปกติแล้วจะใช้สารเคมีอินทรีย์ในการสกัดสารลดแรงตึงผิวชีวภาพ นอกจากนี้จะมีราคาแพงแล้วยังก่อให้เกิดปัญหามลพิษและปัญหาสุขภาพจากการใช้สารเคมี ดังนั้นงานวิจัยนี้จึงได้นำการสกัดวิธีใหม่คือการทำให้เกิดโฟมมาใช้โดยนำมาคัดแยกและดึงสารลดแรงตึงผิวชีวภาพจากอาหารเลี้ยงเชื้อจนมีความเข้มข้นขึ้น

งานวิจัยนี้ได้มีการศึกษาถึงผลกระทบของตัวแปรต่างๆ ต่อการดึงสารลดแรงตึงผิวชีวภาพออกจากอาหารเลี้ยงเชื้อ ตัวแปรเหล่านั้นคืออัตราการไหลของอากาศ, ความสูงของคอลัมน์, ขนาดรูพรุนของตัวเป่าอากาศ, ปริมาณของสารละลายในคอลัมน์ และเวลาในการเก็บโฟมออกจากคอลัมน์ ในแต่ละการทดลองอัตราการดึงสารลดแรงตึงผิวชีวภาพ และอัตราส่วนของความเข้มข้นของสารลดแรงตึงผิวที่แยกออกมาจากโฟมต่อที่อยู่ในอาหารเลี้ยงเชื้อเริ่มต้น จะถูกคำนวณเพื่อวิเคราะห์ถึงความสามารถและประสิทธิภาพของกระบวนการคัดแยก และจากการทดลองพบว่าเมื่อเพิ่มความสูงของคอลัมน์หรือขนาดรูพรุนของตัวเป่าอากาศ ความเข้มข้นของสารลดแรงตึงผิวชีวภาพในโฟมจะเพิ่มขึ้นแต่อัตราการดึงสารลดแรงตึงผิวชีวภาพจะต่ำลง และในทางกลับกันการเพิ่มอัตราการไหลของอากาศส่งผลให้ ความเข้มข้นของสารลดแรงตึงผิวชีวภาพในโฟมลดลงแต่อัตราการดึงสารลดแรงตึงผิวชีวภาพเพิ่มขึ้น

นอกจากนี้ได้มีการศึกษาถึงส่วนประกอบของสารลดแรงตึงผิวชีวภาพเริ่มต้น และสารลดแรงตึงผิวชีวภาพที่ได้จากกระบวนการคัดแยกโดยทำให้เกิดโฟม โดยเครื่อง HPLC-ELSD และพบว่ารูปแบบกราฟมีลักษณะเหมือนกัน แต่ความสูงของกราฟในสารลดแรงตึงผิวที่ถูกคัดแยกแล้วจะมีความสูงของกราฟมากกว่าบ่งบอกได้ว่า สารลดแรงตึงผิวที่ถูกคัดแยกโดยวิธีทำให้เกิดโฟมจะมีความเข้มข้นสูงกว่าสารเริ่มต้น

ACKNOWLEDGEMENTS

First of all I would like to sincerely thank Assoc. Prof. Ratana Rujiravanit and Assoc. Prof. Sumaeth Chavadej, and Professor Masahiko Abe served as my thesis advisors, for their patient guidance, understanding and constant encouragement throughout the course of this research. Their positive attitude contributed significantly to inspiring and maintaining my enthusiasm in the field. I will always be proud to have been their student. I also would like to thank all of my teachers at the Petroleum and Petrochemical College for their generous help.

I am grateful for the partial scholarship and partial funding of the thesis work provided by the Petroleum and Petrochemical College; the Research Unit of Applied Surfactants for Separation and Pollution Control under the Ratchadapisek Somphot Fund, Chulalongkorn University; the Center for Petroleum, Petrochemicals, and Advanced Materials; and the faculty of engineering, Mahanakorn University of Technology.

I would like to thank all staff of the Petroleum and Petrochemical College, Chulalongkorn University for their assistance.

I feel fortunate to have spent 2 years with collection of graduated students who not only made the experience bearable, but also quite pleasant. Therefore, I simply say thanks to friends who made the two years such a memorable experience.

Finally, I would like to thank my family for their love, spirit and understanding during my studies and 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	xv
CHAPTER	
I INTRODUCTION	1
II LITERATURE REVIEW	4
2.1 Surfactants	4
2.1.1 Operation and Effects of Surfactants	4
2.1.2 Classification of Surfactants	5
2.2 Biosurfactants	6
2.2.1 Types of biosurfactants	7
2.2.1.1 Glycolipids	7
2.2.1.2 Lipoproteins or Lipopeptids	10
2.2.1.3 Phospholipids and Fatty acids	10
2.2.1.4 Polymeric Biosurfactants	11
2.3 Factors Affecting biosurfactants Production	12
2.3.1 Carbon Sources	12
2.3.2 Nitrogen Sources	15
2.3.3 Nutrition Sources	16
2.3.4 Environmental Factors	16
2.3.4.1 The pH	16

CHAPTER	PAGE
2.3.4.2 Temperature	17
2.3.4.3 Agitation and Aeration	17
2.4 Extraction of Crude Biosurfactant	18
2.5 Characteristics of Crude Biosurfactant	18
2.6 Foam	19
2.6.1 Foam Formations	19
2.6.2 Structure of Foam	20
2.6.3 Foam Stability	22
2.7 Principle of Foam Fractionation	25
2.8 Applications of Foam Fractionation process	27
III EXPERIMENTAL	31
3.1 Materials	31
3.1.1 Microorganisms	31
3.1.2 Media Used and Chemical Reagents	31
3.1.3 Instrument and Apparatus	31
3.2 Methodology	32
3.2.1 Inoculums Preparation	32
3.2.2 Preparation of Biosurfactant Solution	32
3.2.3 Foam Fractionation Apparatus	32
3.2.4 Experimental Methods	33
3.2.4.1 Effect of Operating Parameters	33
3.2.4.2 Measurement of Size of Air Bubbles in Foam Fractionation Column	34
3.2.4.3 Oil Removal	35
3.2.4.2 Anthrone Reaction	35
3.3 Characteristics of Crude Biosurfactant	36
3.3.1 High Performance Liquid Chromatography with Evaporative Light Scattering Detector	36

CHAPTER	PAGE
3.3.2 Mass Spectrometry	36
IV RESULTS AND DISCUSSION	37
4.1 Operating Limits	37
4.2 Effect of Air Flow Rate	38
4.3 Effect of Column Height	44
4.4 Effect of Pore Size of Sinter Glass Disk	47
4.5 Effect of Liquid Volume	54
4.6 Effect of Operation Time	56
4.7 Diameter of Bubbles	58
4.8 Fractions of Crude Biosurfactant	60
4.9 Chemical Structures of Biosurfactants	66
V CONCLUSIONS AND RECOMMENDATIONS	67
REFERENCES	69
APPENDICES	75
CURRICULUM VITAE	93

LIST OF TABLES

TABLE	PAGE
4.1 Effect of air flow rate on enrichment ratio, biosurfactant recovery (%) and wetness for the column height of 20, 40 and 60 cm.	41
4.2 Effect of air flow rate on oil removal (%) for the column height of 20, 40 and 60 cm.	41
4.3 Effect of air flow rate on enrichment ratio, biosurfactant recovery (%) and wetness for the sinter glass disk No.0, 1 and 3	49
4.4 Effect of air flow rate on oil removal (%) for the sinter glass disk No.0, 1 and 3	49
4.5 HPLC-ELSD isolated fractions of the crude biosurfactant produced by <i>Pseudomonas aeruginosa</i> SP4.	62
4.6 HPLC peak area ratio of isolated fractions produced by <i>Pseudomonas aeruginosa</i> SP4.	62

LIST OF FIGURES

FIGURE		PAGE
2.1	Schematic of a surfactant molecule (a) and a micelle (b)	4
2.2	Schematic diagram of the variation of surface tension, interfacial and contaminant solubility with surfactant concentration.	5
2.3	Schematic representation of four different rhamnolipids produced by <i>P.aeruginosa</i> .	8
2.4	Trehalose dimycolate from <i>Rhodococcus erythropolis</i> , in which disaccharide trehalose is linked to two long-chain α -branched β -hydroxy fatty acids.	9
2.5	Sophorolipid from <i>Torulopsis bombicola</i> in which dimeric sophorose is linked to a long-chain (C18) hydroxy fatty acid.	9
2.6	Structure of cyclic lipopeptide surfactin produced by <i>Bacillus subtilis</i> .	10
2.7	Structure of phosphatidylethanolamine, a potent biosurfactant produced	10
2.8	Structure of emulsan, produced by <i>Acinetobacter calcoaceticus</i> , in which fatty acids are linked to a heteropolysaccharide backbone.	11
2.9	The biosurfactant structure (Di-Rhamnolipid produced by <i>Pseudomonas</i> spp.)	12
2.10	Formation of foam (Rosen, 1988)	20
2.11	The structure of liquid foam	21
2.12	Schematic of foam structure in a column. (Tadros, 2005)	21
2.13	Stretch portions of foam lamellae, illustrating mechanism of film elasticity. (Rosen, 1988)	23
2.14	Marangoni Effect and Gibbs Film Elasticity	23

FIGURE	PAGE	
2.15	Liquid drainage in lamellae by curvature effect.	24
2.16	Classification of bubble separation techniques (Carleson, T.E.,1989).	25
2.17	Principle of foam formation (Simmler,1972)	26
2.18	Experimental configurations for foam separation (Carleson, 1989)	26
3.1	Foam fractionation apparatus	33
4.1	Operational zone under operational condition; sinter glass disk no.1, initial concentration 362.1632 $\mu\text{g/l}$	38
4.2	Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 60 cm, sinter glass disk No.1, initial concentration 362.1632 $\mu\text{g/l}$.	42
4.3	Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 40 cm, sinter glass disk No.1, initial concentration 362.1632 $\mu\text{g/l}$.	42
4.4	Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 20 cm, sinter glass disk No.1, initial concentration 362.1632 $\mu\text{g/l}$.	43
4.5	Wetness of foam in the effect of air flow rate under operational conditions of foam height 20, 40 and 60 cm., sinter glass disk No.1, initial concentration 362.1632 $\mu\text{g/l}$.	43
4.6	Effect of foam height on enrichment ratio of Rhamnolipid	

FIGURE	PAGE
<p>biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min, sinter glass disk No.1, initial concentration 362.1632 $\mu\text{g/l}$.</p>	45
<p>4.7 Effect of foam height on biosurfactant recovery (%) of Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min, sinter glass disk No.1, initial concentration 362.1632 $\mu\text{g/l}$.</p>	45
<p>4.8 Effect of foam height on oil removal (%) with Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min, sinter glass disk No.1, initial concentration 362.1632 $\mu\text{g/l}$.</p>	46
<p>4.9 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 60 cm, sinter glass disk No.0, Initial concentration 434.4238 $\mu\text{g/l}$.</p>	50
<p>4.10 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 60 cm, sinter glass disk No.1, Initial concentration 434.4238 $\mu\text{g/l}$.</p>	50
<p>4.11 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 60 cm, sinter glass disk No.3, Initial concentration 434.4238 $\mu\text{g/l}$.</p>	51
<p>4.12 Wetness of foam in the effect of air flow rate under</p>	

FIGURE	PAGE
operational conditions of sinter glass disk No.0, 1 and 3.	51
4.13 Effect of porosity of the sintered glass disk on enrichment ratio of Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min; initial concentration 434.4238 $\mu\text{g/l}$.	52
4.14 Effect of porosity of the sintered glass disk on biosurfactant recovery (%) of Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min; initial concentration 434.4238 $\mu\text{g/l}$.	52
4.15 Effect of porosity of the sintered glass disk on oil removal (%) with Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min; initial concentration 434.4238 $\mu\text{g/l}$.	53
4.16 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution in the effect of solution volume under operational conditions of air flow rate 30 ml/min, foam height 60 cm, sinter glass disk No.0, Initial concentration 385.4343 $\mu\text{g/l}$.	55
4.17 Wetness of foam in the effect of solution volume under operational conditions of air flow rate 30 ml/min, foam height 60 cm, sinter glass disk No.0, Initial concentration 385.4343 $\mu\text{g/l}$.	55
4.18 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution in the effect of collection time under operational conditions of air flow rate 30 ml/min, foam height 60 cm, sinter glass disk No.0, solution volume 25 ml, Initial concentration 385.4343 $\mu\text{g/l}$.	57
4.19 Diameter of bubble at difference position of column,	

FIGURE	PAGE
Column height 60 cm, Air flow rate 30 ml/min, sinter glass disk No.0 and solution volume 100 ml.	59
4.20 Images of air bubbles in different position of column (a) in the free-cell culture medium (b) at interface of the free-cell culture medium, (c) in the middle of column and (d) at the top of column	59
4.21 HPLC fraction of crude biosurfactant	63
4.22 HPLC fraction of initial biosurfactant solution	63
4.23 HPLC fraction of collapsed foam from foam fractionation process	64
4.24 HPLC fraction of nutrient broth	64
4.25 Comparison of the peak area of initial biosurfactants solution, the collapsed foam biosurfactants and crude biosurfactants from HPLC histogram.	65

ABBREVIATION

CMC	Critical micelle concentration
C/N	Carbon per nitrogen ratio
NB	Nutrient broth
BM	Basal medium
DM	Defined medium
DO	Dissolved oxygen
HPLC	High performance liquid chromatography
GC-MS	Gas chromatography-mass spectroscopy
DADS	Dialkylated disulfonate
SDS	Sodium dodecyl sulphate
CPC	Cetylpyridinium chloride
OPEO ₁₀	Octylphenol polyethoxylate
CGA	Colloidal gas aphron
ELSD	Evaporative light scattering detector