Formulation of oil dispersants by mixing lipopeptide biosurfactant and non-ionic surfactants

Miss Parisarin Nawavimarn

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Hazardous Substance and Environmental Management
Inter-Department of Environmental Management
Graduate School
Chulalongkorn University
Academic Year 2018
Copyright of Chulalongkorn University

การผสมสูตรสารกระจายคราบน้ำมันที่ประกอบด้วยสารลดแรงตึงผิวชีวภาพชนิดลิโปเปปไทด์และสาร
ลดแรงตึงผิวชีวภาพชนิดไม่มีประจุ

น.ส.ปริสรินทร์ นววิมาน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาการจัดการสารอันตรายและสิ่งแวดล้อม สหสาขาวิชาการจัดการสิ่งแวดล้อม
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2561

Thesis Title	Formulation of oil dispersants by mixing lipopeptide bio surfactant and non-ionic surfactants
By	Miss Parisarin Nawavimarn
Field of Study	Hazardous Substance and Environmental Management
Thesis Advisor	Associate Professor Ekawan Luepromchai, Ph.D.
Thesis Co Advisor	Witchaya Rongsayamanont, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

.... Dean of the Graduate School

(Associate Professor Thumnoon Nhujak, Ph.D.)

THESIS COMMITTEE

.... Chairman

(Assistant Professor Chantra Tongcumpou, Ph.D.)

.... Thesis Advisor

(Associate Professor Ekawan Luepromchai, Ph.D.)

.... Thesis Co-Advisor

(Witchaya Rongsayamanont, Ph.D.)

.... Examiner

(Seelawut Damrongsiri, Ph.D.)

.... External Examiner

(Suwat Soonglerdsongpha, Ph.D.)

ปริสรินทร์ นววิมาน : การผสมสูตรสารกระจายคราบน้ำมันที่ประกอบด้วยสารลดแรงตึง
ผิวชีวภาพชนิดลิโปเปปไทด์และสารลดแรงตึงผิวชีวภาพชนิดไม่มีประจุ. (
Formulation of oil dispersants by mixing lipopeptide biosurfactant and no
n-ionic surfactants) อ.ที่ปรึกษาหลัก : รศ. ดร.เอกวัล ลือพร้อมชัย, อ.ที่ปรึกษาร่วม :
ดร.วิชญา รงค์สยามานนท์

      การรั่วไหลของน้ำมันในทะเลทำให้เกิดผลกระทบที่เป็นอันตรายต่อสิ่งแวดล้อม ซึ่งสามารถแก้ปัญหาโดยใช้สารกระจาย
คราบน้ำมันเพื่อช่วยส่งเสริมการบำบัดน้ำมันในทะเล สารกระจายคราบน้ำมันทางการค้าส่วนใหญ่ประกอบด้วยสารลดแรงตึงผิวและ
ตัวทำละลายที่สามารถก่อพิษต่อสิ่งมีชีวิตในทะเล งานวิจัยนี้มีจุดประสงค์เพื่อพัฒนาสารกระจายคราบน้ำมันที่ปราศจากตัวทำ
ละลาย โดยทำการผสมสารลดแรงตึงผิวชีวภาพชนิดลิโปเปปไทด์ที่ผลิตจากแบคทีเรีย *Bacillus subtilis* GY19 ร่วมกับสารลดแรง
ตึงผิวชนิดไม่มีประจุชนิดแอลกอฮอล์อีทอกซีเลทหรือดีไฮดอล (Dehydol LS7TH) ซึ่งเป็นสารลดแรงตึงผิวที่มีความเป็นพิษต่ำ ทั้งนี้
นำหลักการไฮโดรฟิลิคลิโพฟิลิคดีวีเอชั่น (HLD) หรือความแตกต่างของความชอบน้ำและไม่ชอบน้ำมาประยุกต์ใช้เพื่อผสมสูตรสาร
กระจายคราบน้ำมัน โดยสัดส่วนของลิโปเปปไทด์และดีไฮดอลจะคำนวณจากการพิจารณาค่าเทียบเท่าจำนวนคาร์บอนสายตรง
(EACN) ภายใต้สภาวะความเค็มของน้ำทะเล (34 ppt) พบว่าสัดส่วนลิโปเปปไทด์สอดคล้องกับค่าความไม่ชอบน้ำของ
ไฮโดรคาร์บอนในระบบ โดยสูตรสารกระจายคราบน้ำมันที่ผสมจากลิโปเปปไทด์และดีไฮดอลจะแสดงไมโครอิมัลชันที่มีความสมดุล
เมื่อสัดส่วนของลิโปเปปไทด์และดีไฮดอลคำนวณมาจากสมการ HLD สำหรับสารลดแรงตึงผิวมีประจุ สูตรสารกระจายคราบน้ำมันที่
ให้ค่าประสิทธิภาพการกระจายสูงประกอบด้วย ลิโปเปปไทด์ 6.6% ดีไฮดอล 11.9% และ เกลือ 3.4% ซึ่งประสิทธิภาพดีกว่าสาร
กระจายคราบน้ำมันทางการค้าคือ slickgone NS และ superdispersant-25 เมื่อทดสอบกับน้ำมันดิบชนิดเบาบงกช และน้ำมันเตา
อีก 2 ชนิด ได้แก่ น้ำมันเตาเอและน้ำมันเตาซี   การนำสารกระจายคราบน้ำมันที่ได้ไปประยุกต์ใช้ สามารถคำนวณอัตราส่วนระหว่าง
สารกระจายคราบน้ำมันและน้ำมัน (DOR) โดยใช้กราฟตอบสนองต่อพื้นที่ซึ่งได้จากการออกแบบการทดลองแบบบอกซ์-เบห์
นเคน แนวทางนี้สามารถใช้ได้กับปิโตรเลียมหลากหลายชนิดและภายใต้สภาวะความเค็มต่างๆ สูตรสารกระจายคราบน้ำมันที่ได้
จากการผสมลิโปเปปไทด์กับดีไฮดอลจัดว่าเป็นมิตรต่อสิ่งแวดล้อม เนื่องจากมีความเป็นพิษต่ำต่อแบคทีเรียย่อยสลายน้ำมันและ
สามารถส่งเสริมการเจริญของพืชได้ จึงสรุปได้ว่าการผสมสารลดแรงตึงผิวลิโปเปปไทด์และดีไฮดอลเพื่อพัฒนาสูตรสารกระจาย
คราบน้ำมันควรใช้การคำนวณจากสมการ HLD สำหรับสารลดแรงตึงผิวมีประจุ โดยสูตรกระจายคราบน้ำมันที่เหมาะสมมี
ประสิทธิภาพในการกระจายคราบน้ำมันที่สูง และมีราคาลดลงเนื่องจากใช้ลิโปเปปไทด์ในปริมาณที่น้อยเมื่อเปรียบเทียบกับงานวิจัย
ก่อนหน้า

สาขาวิชา	การจัดการสารอันตรายและสิ่งแวดล้อม	ลายมือชื่อนิสิต ....
ปีการศึกษา	2561	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

Parisarin Nawavimarn : Formulation of oil dispersants by mixing lipopeptide biosurfactant and non-ionic surfactants. Advisor: Assoc. Prof. Ekawan Luepromchai, Ph.D. Co-advisor: Witchaya Rongsayamanont, Ph.D.

Marine oil spill can cause various harmful impacts to the environment. To solve this problem, dispersant is usually applied to enhance petroleum remediation in seawater. The commercial dispersants mostly contain surfactant and solvent that can be toxic to marine lives. This study aimed to formulate solvent-free biosurfactant-based dispersants by mixing lipopeptides from *Bacillus subtilis* GY19 with fatty alcohol ethoxylate (Dehydol LS7TH), a low toxicity nonionic surfactant. Hydrophilic-lipophilic deviation (HLD) concept was applied. The fractions of lipopeptide and dehydol LS7TH were determined based on the equivalent alkane carbon number (EACN) of hydrocarbons under seawater salinity (34ppt). The molar fractions of lipoppeptide were corresponded with the hydrophobicity of hydrocarbons in the system. The lipopeptide-dehydol LS7TH formulations expressed microemulsion type III especially when the fractions of each surfactant were calculated from the HLD equation for ionic surfactant. The formulation consisted of 6.6% lipopeptide, 11.9% dehydol LS7 and 3.4% NaCl had the highest dispersion effectiveness (DE) with Bongkot light crude oil (BKC) and two fuel oils i.e. fuel A and fuel C, which was better than the commercial dispersants i.e. slickgone and superdispersant-25. To apply in the oil spill events, the dispersant to oil ratio (DOR) was determined by response surface plot from Box-Behnken design analysis. This approach could be applied to various petroleum types under wide range of salinity conditions. The lipopeptide-dehydol LS7TH formulation was considered environmental friendly since it had low toxicity with petroleum-degrading bacteria and also promoted the plant growth. In conclusion, this study recommended to use HLD equation for ionic surfactant to formulate the lipopeptide-dehydol LS7TH dispersant. The suitable formulation had high oil dispersibility, while the cost was reduced due to the lower amount of lipopeptide biosurfactant comparing to the previous report.

Field of Study:	Hazardous Substance and Environmental Management	Student's Signature ....
Academic Year:	2018	Advisor's Signature ....
		Co-advisor's Signature ....

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

LIST OF TABLES

LIST OF FIGURES

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

CHAPTER I

INTRODUCTION

1.1 State of problem

Petroleum contamination especially oil spill has become an important environmental concerns nowadays. The crude and refined oils can be released into terrestrial and aquatic environments during transportation, pipeline leakage including the inadvertent accidents (Mosaed *et al.*, 2015). In general, the spilled oil mostly contains with complex mixture of various chemicals and polycyclic aromatic hydrocarbons (PAHs), which is known as carcinogenic substance causing both acute and chronic negative effects to structural and functional properties of cell membranes in living organisms (Zheng *et al.*, 2014, Freitas *et al.*, 2016). In case of oil spill at the sea, the thin layer of petroleum on the water surface can inhibit the sunlight and oxygen exchange between air and water phases, and thereby damage several serious problems to the marine ecosystems.

Chemical dispersants are globally and routinely applied as an emergency response when oil spill occur. Most of conventional dispersants are mainly composed of 2-3 types of surface active agents (surfactants) mixed with chemical solvents (i.e. Ethylene glycol butyl ether, Glycol ethers and non-aromatic hydrocarbons). The dispersant will play an important role to enhance the dissolution

of oil into water (Kleindienst *et al.*, 2015), decrease oils accumulated on the water surface and reduce hydrocarbons transport to remote areas by breaking up of floating oil into small droplets in the water column (Zheng *et al.*, 2014, Brakstad *et al.*, 2018, Grote *et al.*, 2018). Therefore, they can enhance bioavailability and accelerate petroleum removal efficiently. However, using conventional chemical dispersants may cause harmful impacts to the environment due to the toxicity from solvents and chemical surfactants (Pi *et al.*, 2017). For example, Corexit 9500, a commercial dispersant, is significantly toxic to aquatic species and in cultured hepatic mammalian cells including central nervous system (LC$_{50}$ = 21–24 mg/L) (Zheng *et al.*, 2014). Slickgone NS, a less toxicity dispersant has the LC$_{50}$ of 0.5–0.8 mg/L with the copepods (Brakstad *et al.*, 2018). In order to minimize the toxicity and achieve environmentally friendly dispersant, less toxic surfactants and solvents have been interested recently. The dispersants composed of biosurfactants are a good alternative for petroleum remediation. There are numerous studies suggested that biosurfactants have good surface activities, effectiveness in extreme conditions, low toxicity, high dispersion efficiency and easily biodegrade in the environment (Chandankere *et al.*, 2013, Khondee *et al.*, 2015). To enhance the dispersion efficiency of surfactants, the mixing between different surfactants mostly perform higher efficiency when compare with the individuals. Mixed surfactant provides synergistic interaction between surfactants such as reduce interfacial tension (IFT) and CMCs lower than the single surfactant, in consequence enhance the dispersion

efficiency in the surfactant system. For environmental applications, ionic surfactant and nonionic surfactant are widely used because the mixture of these surfactants solutions have ability to form mixed micelles and facilitate the dispersion of the oil droplets and also form a continuous film, which stabilizes the new interface and prevents the coalescence of oil droplets (Song, 2013, Athas *et al.*, 2014).

To mix the surfactants, there are some practical formulation tools e. g. Hydrophilic-Lipophilic Balance (HLB) , Critical Packing Parameter (CPP) or Relative Solubility Number (RSN) (Abbott, 2016). These tools are basically applied based on the hydrophilic-lipophilic property of each surfactant and then briefly determine the emulsion types of surfactant in order to select the appropriate surfactant proportions. Nevertheless, these formulation tools still have many limitations such as the disturbance of turbidity and complex method. Hence, another theoretical method called hydrophilic-lipophilic deviation (HLD) concept is applied. The HLD concept is a semi-empirical equation for predicting emulsion behavior of mixed surfactant with considering the effects of salinity, surfactant structure, alcohol and equivalent alkane carbon number (EACN) of oil (Jin *et al.*, 2017). In this principle, there are two types of HLD equation applied for nonionic and ionic surfactants. Nonetheless, the HLD equation for the mixed nonionic and ionic surfactant system has not been reported. The HLD concept for anionic surfactant is recently applied to formulate biosurfactant-based oil dispersant by (Rongsayamanont *et al.*, 2017). In their study, the mixture of lipopeptide biosurfactant and anionic surfactant, SDHS,

showed higher dispersion efficiency than single surfactant alone. Therefore, it is possible to combine the lipopeptides with other surfactants to formulate oil dispersant product by using HLD concept equation.

According to the mixed surfactant efficiency and biosurfactant properties, this study aims to formulate oil dispersant by mixing lipopeptides produced by *Bacillus subtilis* GY19 and nonionic fatty alcohol ethoxylate surfactant without using any toxic solvents for enhancing petroleum remediation. The nonionic surfactant was selected because it generally has higher solubilization capacities, low toxicity, less sensitivity and economic benefits (Song, 2013, Cheng *et al.,* 2017). The oil dispersants were formulated using hydrophilic-lipophilic deviation (HLD) concept and compared between ionic and nonionic equations. Microemulsion study and oil dispersion efficiency were investigated with different types of hydrocarbons and petroleum. Furthermore, the factors affecting the dispersion efficiency of dispersant formulation were investigated through response surface model for applying in the real oil spill cases.

1.2 Objectives

The objectives of this study are to formulate oil dispersants by using the hydrophilic-lipophilic deviation (HLD) concept to calculate the proportions of

lipopeptides and fatty alcohol ethoxylate for oil spill remediation. The objectives can be divided as follows.

1. To calculate the molar fractions of lipopeptide biosurfactant and fatty alcohol ethoxylate surfactants based on the HLD concept and conduct the microemulsion study.

2. To evaluate the dispersion efficiency of dispersant formulations for remediate light crude oil and fuel oils in seawater condition.

3. To identify the factors affecting the dispersion efficiency of dispersant formulation by response surface methodology.

1.3 Hypothesis

1. The dispersant formulations from anionic-nonionic surfactants mixture using HLD equations will perform microemulsion type III (bicontinuous microemulsion).

2. The formulations with microemulsion type III, which expected to provide the lowest interfacial tension between oil-water phases, will have high oil dispersion efficiency with crude oil and fuel oils.

3. Dispersion efficiency of formulation might be significantly influenced by various factors e.g. salinity, dispersant to oil ratio and petroleum types.

1.4 Scope of study

1. Lipopeptide biosurfactants produced from *Bacillus subtilis* GY19 was used in this work to formulate oil dispersants. This biosurfactant is classified as an anionic surfactant and has relatively hydrophobic property.

2. Fatty alcohol ethoxylate surfactants, Dehydol LS7TH, was used as non-ionic surfactants in this study because they have low toxicity and are environmental friendly chemicals (Song, 2013).

3. Molar fractions of each surfactant were calculated from the HLD equations for ionic and nonionic surfactant and the equivalent alkane carbon number (EACN) was varied at 8, 10 and 12. Total concentrations of oil dispersants were varied at 0.1M and 0.3 M. Electrolyte, sodium chloride (NaCl), in this study was fixed at 3.4% (w/v) to represent seawater condition.

4. Three hydrocarbons i.e. octane, decane and dodecane, which have equivalent alkane carbon number (EACN) 8, 10 and 12, respectively, were used in microemulsion study.

5. Different types of petroleum oil such as light crude oil and fuel oils, were used in the baffle flask test for investigating the best oil dispersant formula for petroleum oil remediation.

6. Design of experiment (DOE) will be used for designing the experiment using response surface methodology to investigate the relationship between influenced factors affecting oil dispersion efficiency.

7. The correlation data will be interpreted by Box-Behnken design for recommending how to apply dispersant formulation for certain environmental conditions.

CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Hydrocarbons

2.1.1 General information

Petroleum hydrocarbons are the mixture of hydrocarbon compounds that combine with various types of chemical substances such as nitrogen, sulfur and oxygen. Petroleum can be in various forms such as solid, liquid or gas form and they also have different properties for example volatility and viscosity depending on the chemical components as shown in Table 1. Petroleum generally divided into 2 types (i) crude oil, which is an unrefined petroleum product and (ii) natural gas, which is hydrocarbon gas mixture. Crude oil can be refined to produce usable products such as gasoline, diesel and various forms of petrochemicals. The natural gas is primarily consisted of methane, but commonly included with varying amounts of higher alkanes, and sometimes a small percentage of carbon dioxide, nitrogen, hydrogen sulfide, or helium.

2.1.2 Crude oil

Crude oil is a mixture of wild ranges of hydrocarbons produced by the deposition of plants and animals for long periods. Crude oils exist in liquid form in

underground pools or reservoirs in small spaces within sedimentary rocks, and near the surface in tar (or oil) sands. Naturally, crude oil ranges in density and consistency, from very thin, light weight and volatile fluidity to an extremely thick, semi-solid heavy weight oil. Crude oil can be classified in 4 types as follow (Dept, 2009).

1) Very light oils / light distillates

These oils tend to be highly volatile and can evaporate within just a couple of days, which quickly diffuses and decreases toxicity levels. The example of this crude oil type are Jet Fuel, Gasoline, Kerosene, Light Virgin Naphtha, Heavy Virgin Naphtha, Petroleum Ether, Petroleum Spirit, and Petroleum Naphtha.

2) Light oils / middle distillates

In this type, the oils are moderate in volatilization, toxicity and less evaporation. The oils in this type can be fuel oils (Grade 1 and Grade 2), diesel fuel oils and most of domestic fuels including light crude marine gas oils.

3) Medium oils

The medium oils are low volatility, high toxicity and complex to cleanup and use long period for remediation. Most of commercial crude oil are classified in this crude oil type.

4) Heavy fuel oils

These crude oils type are very slow and low evaporation, high toxicity that impact to aquatic life when spill into the ocean. Moreover, these oils take long-term contamination and difficult to remediation. The oils in this type such as heavy crude oils, Grade 3,4,5 and 6 Fuel Oils (Bunker B & C) including intermediate and heavy marine fuels.

Nowadays, oil spill become a critical situation due to the increasing of petroleum usage. Oil spill may occur from natural causes, anthropogenic causes and also accidental spills. An oil spill usually describes as a release of a liquid petroleum hydrocarbon into the environment (especially marine areas) due to human activities or natural disasters (Li *et al.*, 2016). When the massive amount of spilled oil releases into the water phase, it can cause various serious effect to the marine environment both physical smothering, toxic effects to the aquatic life and socioeconomic (Nikolopoulou *et al.*, 2013, Grote *et al.*, 2018). Generally, there are 3 remediation technologies use for oil spill cases, which are physical/ mechanical, chemical and biological technologies.

2.2.1 Physical/mechanical technology

For this technology, the spilled oil will be removed by using equipment or materials without using or adding the chemical substances for example booms,

skimmers and sorbent materials (Al-Majed *et al.*, 2 0 1 2). Booms are a temporary floating barrier used to contain a spilled oil and used to reduce the spreading of pollutant to remote area due to the wind, wave and current; moreover, this equipment always use as the first response step that toward to easily oil spill remove in next step such as skimmers and using sorbent materials for cleaning oil in surface water.

2.2.2 Chemical technology

Dispersants become an important role for effective response when there is a large volume of petroleum oil spill in the environment. Dispersants are chemical agents, which are a mixture of surfactants and solvents (Mapelli *et al.*, 2017). These chemicals contain surfactant molecules that contain a hydrophilic part and a lipophilic part. When the dispersants are attached to oil slick in surface water, it will be reduced interfacial tension between oil and water; then, breaking up an oil slick into very small droplets and enhancing oil soluble in water phase (Figure 2.1). From these properties, it can also promote biodegradation by microorganisms.

One end of each dispersant molecule 'chain' attaches to water molecules while the other end of the 'chain' attaches to the oil droplets.

A little energy from wind and waves breaks the oil slick into smaller oil droplets surrounded by dispersant molecules as shown.
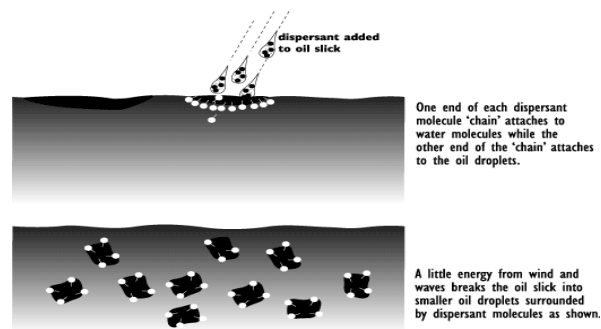
Figure 2.1 The work of dispersant in oil spill cases

(Hofer *et al.*, 2006)

However, there are several limitations about chemical or synthetic dispersants such as toxicity, the effect of biodegradation and long-term applications. Thus, biosurfactant-based dispersants with less toxicity and more environmental friendly, become popular and are selected to study in various researches (Li *et al.,* 2016).

2.2.3 Biological technology

Bioremediation technology is an important clean-up method for long-term remediation. This method is slow, natural process but it can completely remove oil in the water and lead to mineralization processes. Bioremediation techniques include biostimulation, bioaugmentation and biosurfactant application. Normally, biostimulation is applied for stimulating the degrading activity of indigenous bacterial by balancing C/N/P ratio, which is using as a substance for bacteria, after oil spill

occurrence. Bioaugmentation is also applied by adding indigenous microorganisms enriched from specific habitats of the contaminated site (e.g., surface or deep-sea water) to promote biodegradation process. In order to accelerate the activity of microorganisms, biosurfactants will be selected for increasing solubilization of oil droplet formation in water and the microorganisms will easily degrade oil in water portion (Mapelli *et al.,* 2017).

2.3 Surfactants

Surfactant become popular and is widely used for promoting oil spill clean-up because it effectively enhances bioavailability of oil. It is a group of surface active agent that has ability to reduce surface tension or interfacial tension between two liquid phases or between a liquid and a solid or liquid and air phases in order to enhance the mixing capacity (Lamichhane *et al.,* 2017). Nowadays, surfactants are selected for applying in various applications such as cleaning, wetting, dispersing, emulsifying, foaming and anti-foaming agents (Hirsch, 2015).

The structure of surfactant is consisted of hydrophilic and hydrophobic parts (Figure 2.2a). The presence of polar and nonpolar groups in each molecule is essential for detergency and solubility of surfactants. The surfactant molecules can form micelles, where the hydrophobic tails form the core and the hydrophilic heads

are immersed in the surrounding liquid in solution (Álvarez-Muñoz *et al.*, 2016) (Figure 2.2b).
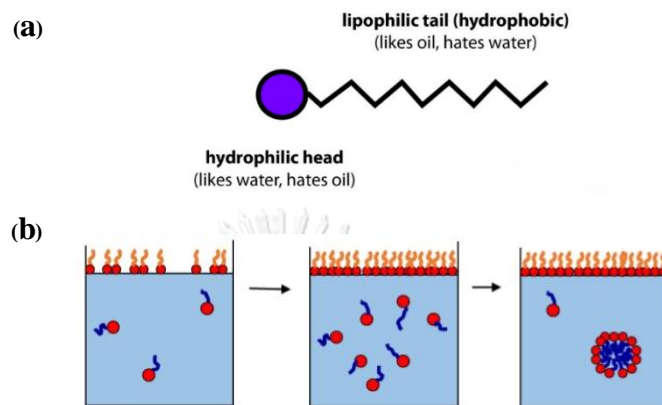
Figure 2.2 Characteristic of surfactant
(a) Surfactant structure and (b) Micelle formation
(Shahzad, 2017).

For chemical surfactants, they can be categorized into 4 types, which are nonionic, cationic, anionic and zwitterionic (amphoteric) based on the ionic charge of the hydrophilic group of surfactant (Table 2.1). The structure of surfactant shown in Figure 2.3.

Table 2.1 Types of chemical surfactant and their applications

Type of surfactant	Head charge	Component	Example	Applications
Anionic surfactant	Negative	Sulfate Sulphonates Phosphate esters Carboxylates	- Sodium / Ammonium Lauryl Ether Sulphate - Sodium /Ammomiun Lauryl Sulphate - Alpha Olefin Sulfonate (AOS) - Sarcosinate - Sulphosuccinate	Detergent
Non-ionic surfactant	No charge	Alcohol Phenol Ester Glycerides Ether Amide Ethylene oxide Carboxylic acids Halogen type	- Triton X-100 - Tween-80 - Decyl glucoside - Glyceryl laurate	Detergent
Cationic surfactant	Positive	Fatty amine salts Quateranry Ammonium salts	- Octenidine dihydrochloride - Cetyl trimethyl ammonium bromide (CTAB)	Textile Cleaner
Zwitterionic (amphoteric)	Two oppositely charged groups	Sulfobetaines Amino acid Phospolipid Alkyl betaines Alkyl dimethylamines	- Cocamidopropyl betaine - CHAPS	Cleaner

Source: Chen *et al.* (2015)
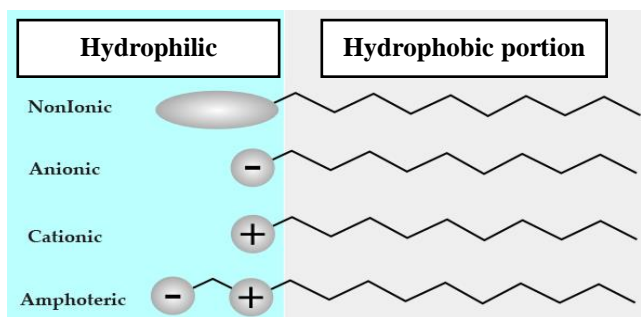
Figure 2.3 Surfactant type structures based on head polar of surfactants.
(Woltornist, 2016)

The surface activity of surfactant depends on the concentration of surface-active compounds until reaching the critical micelle concentration (CMC), where is the lowest concentration of surfactant monomers that can form micelle (Figure 2.4). At this point, micelle formation enables the decrease of surface and interfacial tension that enhances solubility and bioavailability of hydrophobic organic compounds. In addition, the formation of micelle will affect to microemulsion occurrence between liquid mixture of water and oil portions. Microemulsions are formed when one liquid phase is dispersed as droplets in another liquid phase base on hydrophilic-lipophilic balance (HLB) property.

Hydrophilic–lipophilic balance (HLB) is the balance of the size and strength of the hydrophilic and lipophilic moieties of a surfactant molecule. Normally, HLB values range from 0-20. When HLB value is 3.5 to 6.0, surfactants are more suitable to form water-in-oil (W/O) emulsions; while, the value is 8 to 18, the emulsion tends

to prefer oil-in-water (O/W) emulsion (Figure 2.5) (Pacwa-Plociniczak *et al.*, 2011, Yan Zheng, 2015).
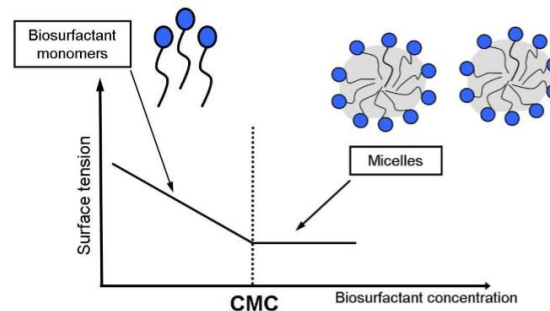
Figure 2.4 Correlation between surface tension and critical micelle concentration (Pacwa-Płociniczak, 2011)
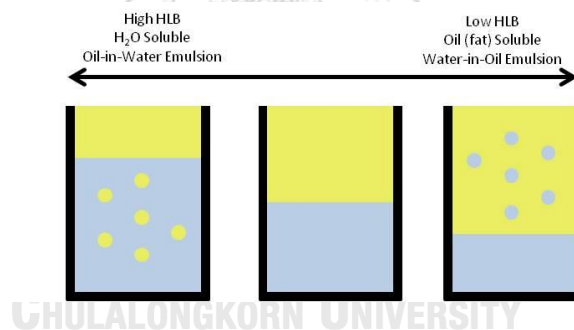
Figure 2.5 Microemulsion types; oil-in-water, bicontinuous and water-in-oil microemulsion

2.4 Biosurfactants

Biosurfactant is a surface-active substance produced by many types of microorganisms such as bacteria, fungi or yeasts (de Cassia *et al.*, 2014). According to amphiphilic property, biosurfactant can increase the surface area of hydrophobic and

also increase the water bioavailability of such substances and change the properties of the bacterial cell surface. Surface activity makes biosurfactants to be excellent emulsifiers, foaming, coating and dispersing agents (Pacwa-Plociniczak *et al.*, 2011). Biosurfactants have several advantages compared with chemical surfactants such as lower critical micelle concentration (CMC), easily biodegradation, less sensitive under wide range of environmental conditions, and low toxicity (Liu *et al.*, 2017, S.J *et al.*, 2018).

Biosurfactants are commonly classified into 2 groups based on the molecular weight; 1) low molecular weight biosurfactants, which are efficiently reduce surface tension (liquid-air) and interfacial (liquid-liquid) tension between two phases and 2) high molecular weight polymeric biosurfactants, which are more potentially stabilize oil-in-water emulsion. The biosurfactants were classified as shown in Table 2.2 (Karlapudi *et al.*, 2018).

Table 2.2 Classification of biosurfactants and their applications in environment.

Molecular weight	Biosurfactant		Microorganism	Application	Reference
	Group	Class			
Low	Glycolipids	Rhamnolipids	*Pseudomonas aeruginosa, Pseudomonas* sp.	Enhancement of the degradation and dispersion of different classes of hydrocarbons	(Kryachko *et al.*, 2013, Pi *et al.*, 2017)
	Fatty acids, phospholipids and neutral lipids	Spiculisporic acid	*Penicillium spiculisporum*	Removal of metal ions from aqueous solution	(Pacwa-Plociniczak *et al.*, 2011)
	Lipopeptides	Surfactin	*Bacillus subtilis*	Enhancement of the biodegradation of hydrocarbons and chlorinated pesticides	(Liu *et al.*, 2015, Rongsayamanont *et al.*, 2017)
		Lichenysin	*Bacillus licheniformis*	Enhancement of oil recovery	(Liu *et al.*, 2016)
High	Polymeric biosurfactants	Emulsan	*Acinetobacter calcoaceticus* RAG-1	Stabilization of the hydrocarbon-in water emulsions	(Pacwa-Plociniczak *et al.*, 2011)
		Biodispersan	*Acinetobacter calcoaceticus* A2	Dispersion of limestone in water	(Pacwa-Plociniczak *et al.*, 2011)

Generally, most of biosurfactants are anionic or neutral. Only a few biosurfactants are cationic that contain amine groups in their structure. The hydrophobic part of the molecule is long-chain fatty acids, hydroxy fatty acids or a-alkyl-b-hydroxy fatty acids and the hydrophilic portion can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol (Mulligan, 2005).

2.5. Hydrophilic-lipophilic deviation (HLD) concept

Hydrophilic-lipophilic deviation (HLD) is a semi-empirical dimensionless number for the change in chemical potential when a surfactant molecules transfer from the oil phase into the aqueous phase. HLD concept is an alternative technique that usually used for several objectives such as flow assurance and calculation for the suitable surfactant formation in petroleum works. For petroleum works, this concept can describe the effect of temperature, electrolyte concentration, type of oil, type of surfactant, and the type and concentration of co-surfactant, which can impact to microemulsion occurrence (Acosta, 2008).

In theory, HLD values can predict the type of microemulsion. The negative values of HLD show that the surfactant is more soluble in water and that it tends to form microemulsion Type I, while the positive HLD values are indicative of Type II systems. When HLD is zero (HLD= 0), the bicontinuous microemulsion occurs, where the emulsion appears in the middle layer between oil and water phases (Figure 2.5).

There are 2 types of HLD concept equations for ionic and nonionic surfactants as following (Nardello *et al.*, 2003, Acosta, 2008).

For ionic surfactant:

$$HLD = \ln(S) - k \times N_{C,O} - f(A) - \alpha_T \Delta T + Cc \qquad (Eq.1)$$

For nonionic surfactant:

$$HLD = (\alpha - EON) + b(S) - k(ACN) - f(A) + {}_{cT}\Delta T \qquad (Eq.2)$$

In case of nonionic surfactant (Eq.2), the characteristic curvature that represent surfactant property (Cc) of nonionic surfactant can be derived and calculated as shown in Eq.3 (Acosta, 2008)

$$Cc_n = \alpha - EON \qquad (Eq.3)$$

Where:

b,k	A constant depending on the type of surfactant
S	Represents the salinity (in wt% in the aqueous phase)
$N_{C,O}$ / EACN	An equivalent alkane carbon number
Cc	A characteristic curvature of surfactant base on hydrophilic/lipophilic property
f(A), ϕ(A)	Depend on the type and concentration of the co-surfactant added to the system
ΔT	$T - T_{ref}$, where T is the temperature of the system and T_{ref} is the reference temperature (25 °C)
α	The molecular structure of the tail (lipophilic) group of the surfactant
EON	The number of ethylene oxide (EO) groups in the surfactant molecule

2.6 Dispersant for oil spill remediation

One method to clean the petroleum contaminants is using of chemical dispersants. The dispersants enhance the dissolution of oil by reducing interfacial tension (IFT) between oil and water; then, allow the formation of very small oil droplets (Figure 2.6). Consequently, the microorganisms can easily degrade the spilled oil in seawater. The efficiency of dispersion depends on the nature of oil, the agitation, and temperature (Al-Sabagh *et al.*, 2007).
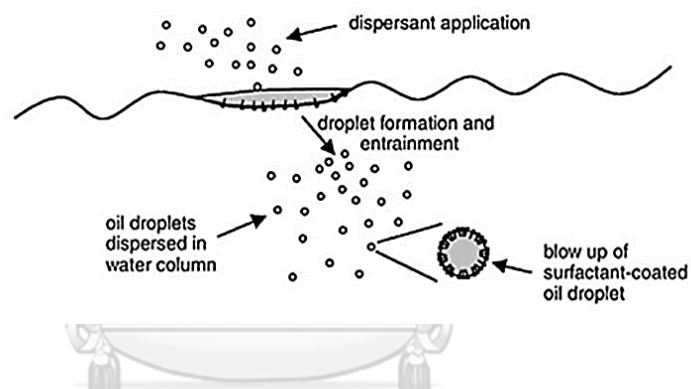
Figure 2.6 Dispersants and their interaction with oil in sea water; when adding dispersant, oil slicks will be broken up, dispersed and dissolve oil into water column. Then, surfactant molecules in dispersant will attach to oil droplets (National Research Council, 2005)

The dispersant can be classified in three classes based on generation and type as shown in Table 2.4.

Table 2.3 The generation of dispersant product

Generation of dispersant	Characteristics of dispersants
First generation	For industrial cleaners and degreaser - High aquatic toxicity to the environment - No longer use for oil spill response
Second generation	- Contain a hydrocarbon solvent with a low or no aromatic content. Typically, 15 to 25% surfactant mixed with solvents - Require high concentration for using
Third generation	- Contain a blend of two or more surfactants with glycol and light petroleum distillate solvents. - The most common surfactants used are non-ionic and anionic - Contain 25-65% surfactant combined with solvents - Require higher concentration than previous generation for oil spill applications

Source: Anish (2017)

In addition, the mixed surfactants have been used to produce oil dispersants. For example, Song (2013) studied about efficiency of oil dispersant formulated by mixing four kinds of surfactants, which are two kinds of nonionic surfactant (Tween 85 and GO440) and two kinds of glycolipid biosurfactants (rhamnolipid and sophorolipid) with less toxic solvent, ethylene glycol butyl ether using uniform design methods (UD). Afterward, twenty-four dispersant formulations were investigated for the optimal dispersant formulation by baffled flask tests with crude oil. Only 2 dispersant formulations showed high dispersion effectiveness up to 60% at the DOR of 1:25 and 150 rpm mixing speed. Furthermore, these formulations displayed higher dispersion effectiveness than Corexit 9500, which can disperse heavy and weathered oils less than 30%. In addition, they found that these two dispersant formulations expressed 40% dispersion effectiveness in cold weather (5 °C). Besides, pH and salinity was not a significantly affected to dispersion effectiveness of two formulations.

Shafira Adlina (2017) formulate a dispersant by mixing diethanolamide (DEA) (nonionic surfactant) with methyl ester sulfonate (MES) (anionic surfactant). Both surfactants were diluted in water to prepare a mixture solution at ratio of DEA and MES solution 9:1 to 1: 9). This oil dispersant product was characterized for density, surface tension, interfacial tension, pH, viscosity and droplet size. The test results show that a stable emulsion illustrated at the ratio system of DEA (1.5%) and MES

(0.9%) were equal at 9:1, 8:2, 7:3, 6:4 and 5:5. For surface tension, the dispersant product of DEA (1.5%) and MES (0.9%) ratio of 7:3 showed the lowest surface tension value as 23.57 dyne.cm^{-1} and interfacial tension at 0.20 dyne cm^{-1}. Moreover, the microcosm test of crude oil was evaluated and found that at ratio of crude oil: dispersant equal 1:1 can enhance the bioremediation process about 1.46 times compared to control without using oil dispersant formulation.

Currently, some studies try to decrease the chemical components in dispersant formulation in order to minimize toxicity of dispersant and promote the use of "green dispersant" for environmental remediation. Thus, the development of bio-based dispersant i.e. bio-dispersants become more interesting, such as the use of food-grade amphiphiles that are common additives in food and medicine, or microbial product from microorganisms as a main component in dispersant such as

Athas *et al.* (2014) used lecithin (L), which is phospholipid extracted from soybeans, and Tween 80 (T) and solvent to create a crude oil dispersant. The results displayed that the mixture performed O/W emulsion at the ratio of 60/40 of L/T (weight ratio) and the emulsion contain very small size of spherical oil droplets (about 5 μm in diameter) due to the tightly packing of micelle from synergism effect. They concluded that the smaller size and stability of crude oil droplets are important to promote the dispersion efficiency. Therefore, L/T mixture ratio could potentially be an alternative for the dispersion of oil spills in the ocean.

Not only Athas *et al.* (2014) studied the dispersant formulation from lecithin and Tween-80, but also have other researchers studied about the synergism effect between food-grade amphiphiles (lecithin+ Tween-80) to form stable emulsion with crude oil. Most of them found that the O/W emulsion was formed by LT mixtures at various concentration ratio and those mixtures occurred stable emulsion more than individual single lecithin, or Tween-80 alone (Riehm *et al.*, 2017, Rocchio *et al.*, 2017).

Jin *et al.* (2019) formulated an environmentally-friend dispersant by mixing soybean lecithin (L) and Tween-80 (T) for oil spill remediation. The L/T formulation was studied the stable of oil-water emulsion under various environmental conditions. The results showed that L/T formulation in the ratio of 6:4 expresses synergistic effect, which provided lowest interfacial tension as 0.075 mN/m. The emulsion index (EI_{24}) of the emulsion significantly increased with the increasing concentration. They found that the reduction of droplet diameter of light oil (diesel) is significantly higher than heavy oil due to the composition of oil type. In additions, the environmental factors such as temperature, pH, inorganic salt and dispersant to oil ratio (DOR) can influence to emulsion droplet diameter of L/T formulation. Moreover, PO^{2-} group in the Lecithin and the OH group of Tween 80 exhibit synergy for preventing the coalescence of droplets. It could be concluded that L/T formulation has potentially applied for oil spill remediation.

2.7 Lipopeptide biosurfactant

Lipopeptide biosurfactant is a microbial surface active compound produced by various types of bacteria, especially *Bacillus* species. The structure of lipopeptide biosurfactants consist of fatty acid chain connect with a peptide moiety and are classified into three families depending on their amino acids structure i.e. surfactins, iturins and fengycins (Bezza and Chirwa, 2015, Chen *et al.*, 2015). Currently, lipopeptides are extensively studied because they perform low critical micelle concentration (CMC) , good efficiency to reduce surface and interfacial tension. Moreover, they also have various functional properties such as emulsification, dispersing, foaming, viscosity reducer and solubilizing agents. From these properties, lipopeptides are used in many applications, particularly for petroleum industries and bioremediation.
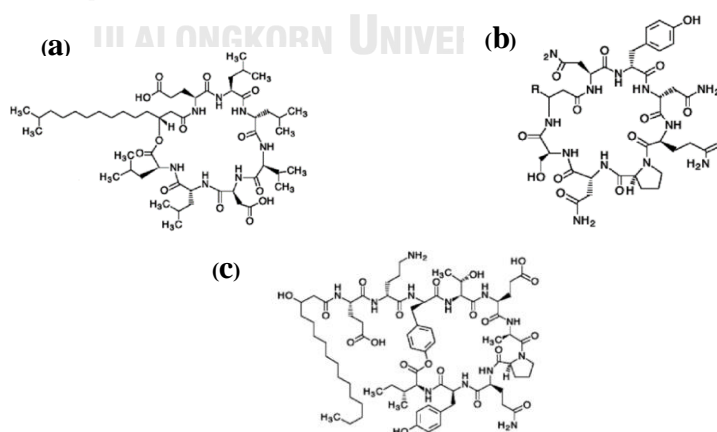
Figure 2.7 Lipopetide biosurfactant structure (a) surfactin structure (b) iturins structure and (c) fengycins structure

Currently, there are various researches about lipopetide biosurfactant.

For example, Bezza and Chirwa (2015) studied lipopeptide biosurfactant produced by *Bacillus subtilis* CN2, which isolated from contaminated soil. The results showed that lipopeptide biosurfactant increased biodegradation efficiency of used motor oil 2 times comparing with degradation without using biosurfactant. Moreover, it can lower the surface tension of culture broth from 72 to 32 mN/m and displayed the stable emulsification with hexane, cyclohexane, and used motor oil over 6 weeks of experiment. The research concluded that their biosurfactant can be applied for oil recovery and enhanced biodegradation in contaminated soil.

Mani *et al.* (2016) isolated bacterium from coastal sea sediment contaminated with petroleum hydrocarbons and found that a marine bacterium *Bacillus simplex* had ability to produce biosurfactant. The biosurfactant was classified as lipopeptide biosurfactant by FT-IR and NMR spectral analysis. Moreover, the purified lipopeptide biosurfactant was also investigated for crude oil recovery from the contaminated sand under various salinity conditions. The results revealed that this biosurfactant showed consistent and enhanced crude oil recovering efficiency over 84% under different salinity conditions (0–30%) comparing with synthetic surfactant and without surfactant. Thus, lipopeptide biosurfactant can enhance crude oil recovering efficiency at wide range of salinity conditions and is possible to apply in environmental applications even in hypersaline condition such as seawater.

Fooladi *et al.* (2016) characterized lipopeptide biosurfactant produced by *B. pumilus* 2IR, which was isolated from an oil field in Iran. This lipopeptide performed high surface activity and showed the lowest surface tension value at 32 mN/m with the highest oil spreading (3.2 cm), crude oil emulsification (60%) and hexadecane emulsification (68%). The study also revealed that this biosurfactant-producing bacterium was capable of consuming crude oil for the production of biosurfactant which can be advantageous for the in situ technology of the oil recovery from the abandoned oil reservoirs.

In this research, lipopeptide biosurfactant from *Bacillus subtilis* GY19 was produced following Khondee *et al.* (2015). The lipopeptide-producing bacterium was isolated from soil sample in Thailand and immobilized in chitosan flask before culturing in a productive medium containing waste glycerol and palm oil as carbon and energy sources. In addition, this lipopeptide performed the lowest surface tension at 26 mN/m and showed oil displacement efficiency at 67, 84 and 100% with diesel oil, light crude oil and heavy oil, respectively.

According to the good surface activity of lipopeptide biosurfactant produced by *Bacillus subtilis* GY19, it will be selected to use as a major component in the oil dispersant formulations in this study.

2.8 Nonionic surfactant

Nonionic surfactant is one of the surfactant type that has no charge in its head structure and do not ionize in aqueous solution. The hydrophilic portions of nonionic surfactants are usually made up from oxygen-containing groups such as alcohol, phenol, ether, ester, amid, or hydroxyl and polyoxyethylene glycol chain (Salager, 2002). According to the property of nonionize in solution, the oxygen group in hydrophilic head of nonionic surfactant will attach to hydrogen atom in aqueous phase. The micellization, forming of micelles, of nonionic surfactant is easier than ionic surfactant type because the aggregation mainly due to the hydrophobic attraction among non-polar chains whereas, hydrophilic chains are easily separated in an aqueous phase (Mao *et al.*, 2015). Therefore, nonionic surfactants normally provide low critical micelle concentration (CMC) that useful for applying during remediation, especially for soil washing (Li *et al.*, 2016, Zhong *et al.*, 2016, Cheng *et al.*, 2017).
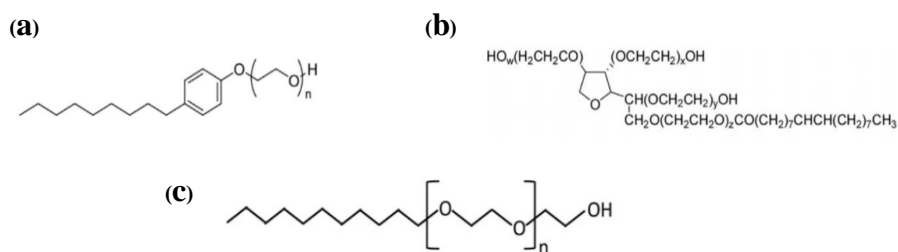
Figure 2.8 Nonionic surfactant structure

(a) Tergitol NP-10 (b) Tween-80 and (c) Dehydol structure

(Phasukarratchai *et al.*, 2012, Mao *et al.*, 2015)

There are various researchers on nonionic surfactant application, particularly in environmental remediation works such as;

Adrion *et al.* (2016) screened five nonionic surfactants (Brij 30, Span 20, Ecosurf EH-3, polyoxyethylene sorbitol hexaoleate (POESH) and R-95 rhamnolipid) for enhancing polycyclic aromatic hydrocarbons biodegradation in contaminated soil, which collected from manufactured-gas plant site in North Carolina. The results showed that Brij 30 perform the highest PAH desorption efficiency after 7 days adding surfactant. In addition, Brij 30, Span 20, and POESH were effective to enhance biodegradation of four- and five-ring PAHs, including five of the seven carcinogenic PAHs, with removals up to 80% compared with no-surfactant used. Moreover, the toxicity of these nonionic surfactant was investigated and found that only Brij 30 at the lower dose significantly reduced the genotoxicity in soil and the others tend to

increase toxicity in soil. Thus, this study concluded that Brij 30 can apply for PAHs cleanup in contaminated soil.

Arpornpong *et al.* (2018) formulated microemulsion-based products using 3 types of nonionic surfactants including Dehydol LS3, LS5 and LS7 for washing residual rice bran oil from spent bleaching earth (SBE), an industrial refining waste from bleaching process. The microemulsions in this experiment were calculated by HLD concept in order to predict microemulsion formulation and study the correlation of microemulsion and oil extraction efficiency. The results showed that Dehydol LS3 and LS5 systems achieve microemulsion type III at the middle phase between two liquid phases at the range of 10-20 wt% NaCl, which is in good agreement with predicted optimum salinity from HLD equation. Whereas, the optimum salinity for Dehydol LS7 to form type III microemulsion is at the range of 15-20 wt.% NaCl, which is higher than the values obtained from the theory. However, the oil extraction efficiency showed good performance (30.3%) and increased when NaCl concentration increased. Therefore, it is possible to use formulate nonionic using HLD concept for oil remediation.

From many studies, nonionic surfactants are widely applied for contaminated soil remediation and they performed good efficiency for petroleum hydrocarbon removal. However, the using of nonionic surfactant as dispersant is limited. Therefore, this study will use nonionic surfactants i.e. Dehydol LS5TH and LS7TH

which are low toxicity and produced in Thailand in order to minimize the cost and enhance the efficiency of lipopeptides for formulating oil dispersants.

2.9 Applications of mixed surfactants using HLD concept

Mixed surfactant systems have many advantages such as reducing the cost in surfactant manufacture processes, promoting a better performance of surfactant or exploiting synergistic behavior in mixed systems that can be applied in extended applications (Holland and Rubingh, 1992). There are many researches on the mixing of surfactants for environmental remediation (Table 2.4).
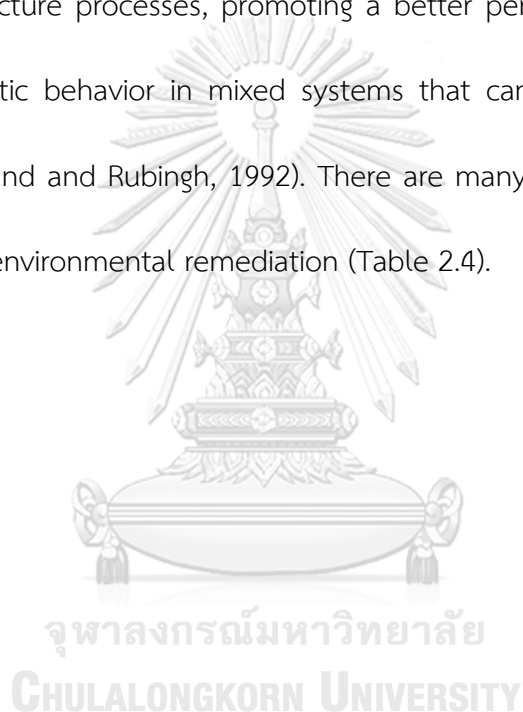
Table 2.4 The applications of mixed surfactants

Mixed surfactant	Surfactant type	Application	Reference
Sodium dodecyl benzene sulfonate (SDBS) and Tween 80	Anionic - Nonionic	Removal of phenanthrene and pyrene from contaminated soil	(Ni *et al.*, 2014)
Lipopeptides biosurfactant and sodium dihexyl sulfosuccinate (SDHS)	Anionic– Anionic	Oil spill dispersants	(Rongsayamanont *et al.*, 2017)
Hexadecylpyridinium bromide (HDPB) and Triton X-100	Cationic- Nonionic	Remediation of soil and groundwater contaminated by toxic organic compounds (adsorption on bentonite)	(Zhang *et al.*, 2012)
Tween 80 and Brij-35	Nonionic– Nonionic	Enhancing the solubility of polycyclic aromatic hydrocarbons (PAHs) (naphthalene and phenanthrene)	(Sales *et al.*, 2011)
Sodium dodecyl sulphate (SDS) and cetyltrimethylammonium bromide (CTAB)	Anionic- Cationic	Synergistic effect for enhance crude oil recovery	(Rashmi Kumari, 2018)
TX-100 and SDBS	Nonionic- Anionic	Enhanced solubilization and desorption of pyrene contaminated in soil	(Yanfu Wei a, 2015)
Tween 80 and sodium laurate	Nonionic- Anionic	Desorption of polycyclic aromatic hydrocarbons from soil	(Sales and Fernandez, 2016)
TX100-SDS and TX100-SDBS	Nonionic- Anionic	Synergistic effect on the water solubilization of three target PAHs	(Shi *et al.*, 2015)

Recently, HLD concept is one of useful tool for the mixing surfactants. It can be used for formulating optimal surfactant formulation and predicting emulsification of surfactant system in various applications. Example of researches are as followed;

Do *et al.* (2014) formulated a detergent for cleaning vegetable oils and semi-solid fats in cold temperature condition by mixing two anionic surfactants i.e. C10–18PO–2EO–NaSO4 and sodium dioctyl sulfosuccinate surfactant using HLD concept. Four kinds of vegetable oils, canola, jojoba, coconut and palm kernel oils were selected to study due to their melting points ranged from – 10 to 28 °C. The results showed that the mixtures had greater than 90 %   detergency efficiency at the condition of 0.5 %   NaCl above melting point of vegetable oils; while, detergency efficiency tended to decreased when the temperatures of system below the melting point.

Jin *et al.* (2017) predicted the optimum formulation for flooding a target reservoir crude oil using hydrophilic-lipophilic difference (HLD) and net average curvature (NAC) model. The microemulsion phase behavior was studied by mixing 4 types of sodium alkyl alkoxy surfate surfactant with sodium alkyl ethoxy surfactant (sodium laureth sulfate) and these formulations were investigated for the microemulsion type and equilibrium IFT. The results indicated that the predicted results from HLD-NAC calculation have good agreement with the measured equilibrium IFT. Comparing to experiment results, the HLD equation shows high accuracy in predicting optimum surfactant formulation for surfactant flooding. Thus, it

can conclude that HLD-NAC equation is not only reducing the surfactant formulation processes, but also predicting microemulsion phase behavior for environmental applications.

Budhathoki *et al.* (2016) studied the design of optimal phase microemulsion for promoting chemical enhance oil recovery (cEOR) in saline brine condition and used the HLD concept equation to find the optimal surfactant ratio. This work aimed to formulate alcohol-free surfactant formulation by mixing sodium alkyl alkoxy sulfate surfactant (anionic) and sodium alkyl ethoxy sulfate surfactant (anionic). The results showed that the optimal surfactant formulation provided the lowest interfacial tension (IFT) with crude oil at 0.004 mN/m and performed stability in salinity and temperature conditions. Moreover, the HLD parameters were correlated with the efficiency of surfactant mixture. This work demonstrated that using HLD concept for screening surfactant formulation for cEOR can be more efficient due to the reducing number of experiments and time to investigate in microemulsion study.

2.10 Design of experiment (DOE)

Design of experiment (DOE) is an advanced technique for design the experiment to achieve efficient results. Generally, the analytical experiment has been carried out by trial and error or one factor at a time on an experimental response. The experimental conditions from these conventional methods are normally

obtained from the conjugation of univariate with the response. Therefore, the univariate procedure may generate many errors if the response or optimization was affected by the other dependent variables (Ferreira *et al.*, 2007). Moreover, the amount of experimental condition from the conventional designs may generate various experimental numbers that increase time and expenses for investigating the research (Bezerra *et al.*, 2008). Currently, the advance mathematical and statistical techniques are applied for analyzing the chemical experiment such as response surface methodology (RSM). The multivariate experimental designs have been selected in order to optimize the chemical factors because these methods can reduce the number of experiment, minimize time and experimental cost in the research.

Response surface methodology (RSM) was developed by Box and his colleagues. This methodology consisted of a group of mathematic and advance statistic based on the fit of experimental results and empirical model equation (Bezerra *et al.*, 2008). To design the experiment by response surface methodology, there are many complex experimental designs such as Doehlert matrix (DM), Completely Randomized Design, CRD, central composite designs (CCD), Factorial Design and three-level designs such as the Box-Behnken design (BBD). These tools are selected based on the suitable number of factors including the level of interested factors.

Box-Behnken design are classified as rotatable or nearly rotatable, which estimates the first and second-order coefficients of mathematical model based on three-level factorial designs. The experimental point in Box-Behnken design are located on a hypersphere equidistant from the central point. This experimental design is usually applied for analysis the correlation between three independent factors and the optimization of independent factors with response. Box-Behnken design for 3 factors can be divided in 2 types i.e. three-variable factorial design (N = 3^k) and Box-behnken design (N = $2k(k-1) + C_p$), where k is the number of factors, C_p is the number of the central points as shown in Figure 2.9.
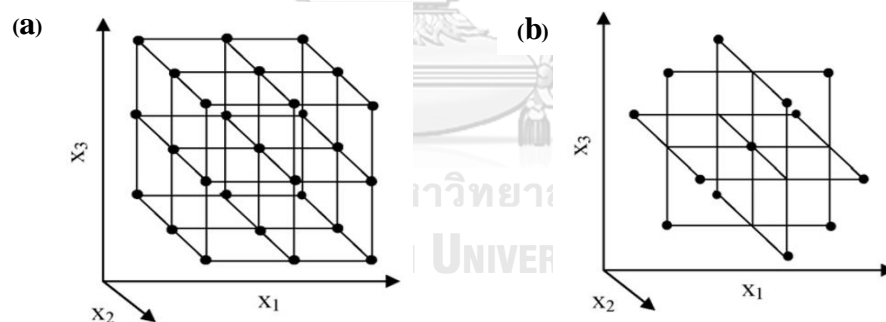
Figure 2.9 Experimental design based on Box-Behnken design (BBD) in three-level (a) three variables and (b) Box-Behnken design for the optimization of three variables (Bezerra *et al.*, 2008).

The experimental conditions from Box-Behnken design will express the correlated equation in form of the polynomial function and analyzed the fit of model by analysis of variance (ANOVA) to confirm the precision of model before apply in the real applications. In theoretical, ANOVA analysis can compare the variance of experimental results with variance from equation that impact to the response (dependent variable). Then, the results will be interpreted by surface response profiles (Figure 2.10).
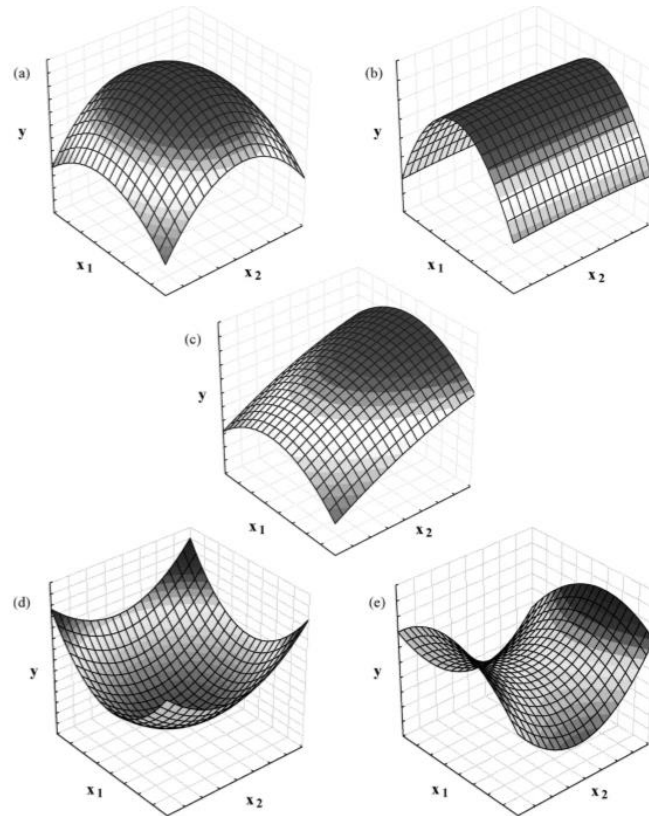
Figure 2.10 Response surface profile types

(a) maximum point inside the experimental region, (b) plateau: selected factors not impact to the response, (c) maximum outside the experimental region, (d) minimum point inside the experimental region, and (e) saddle surfaces: maximum and minimum point inside the experimental region.

(Bezerra *et al.*, 2008)

Currently, various researchers use Box-Behnken design for environmental applications for example:

Gaurav Dwivedi and Sharma (2015) implemented Box-Behnken design for optimizing the optimal condition from four process variables i.e. methanol/oil ratio, reaction time, reaction temperature and catalyst amount to obtain the maximum biodiesel yield produced from Pongamia oil. Twenty-nine experimental conditions were generated and the results were analyzed by the response surface regression using the polynomial equation. The results showed that the Pongamia biodiesel (PB) yields (%) from experimental response quite similar to the predicted responses from the model equation. Moreover, they found that all process variables significantly related with the PB yield. The PB yield reached the maximum efficiency at 98.4% when the optimal condition containing methanol/oil molar ratio at 11.06:1 using KOH as catalyst at 1.43% w/w in reaction time at 81.43 min and the temperature of system was 56.6°C.

Vecino et $al.$ (2015) studied the effect of extraction conditions i.e. salinity, extraction time and temperature on the emulsifying properties of the biosurfactant produced by $Lactobacillus$ $pentosus$ using Box-Behnken design analysis. The results displayed that the most influenced variables are extraction time, temperature and salt concentration, respectively. The maximum relative emulsion volume (EV) was observed at the extraction time is 120 min, salt concentration is 9 g kg^{-1} at the

temperature is 65 °C. Meanwhile, the emulsion stability (ES) reach to the highest efficiency at 120 min, 9 g kg^{-1} and 45°C for extraction time, salt concentration and temperature, respectively.

In this study, Box-Behnken design (13 experimental points) was selected to study the correlation between influenced factors and dispersion efficiency of dispersant in different salinity condition. In additions, optimization the suitable dispersant to oil ratio for applying in oil spill cases was be investigated. This experimental design is expected to provide more efficient and economical than full factorial experiment when applied in the large scale experiment.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

CHAPTER III

MATERIALS AND METHODOLOGY

3.1 Materials

3.1.1 Microorganism

Bacillus subtilis GY19 (MSCU0789) for biosurfactant production was isolated from soil in Thailand. The biosurfactants from this bacterium were classified as lipopeptides (Khondee *et al.*, 2015).

3.1.2 Chemicals

1) Hydrocarbons including octane, decane and dodecane were purchased from Sigma- Aldrich Co., LLC and the properties of each hydrocarbon are shown in the Table 3.1.
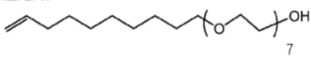
Table 3.1 The properties of hydrocarbons in this study

Hydrocarbons	EACN	Molecular formulation	Density (g/cm^3)	Solubility (mg/L)	Viscosity (mPa·s)
Octane	8	C_8H_{18}	0.703	0.66	0.542
Decane	10	$C_{10}H_{22}$	0.73	2.188	0.920
Dodecane	12	$C_{12}H_{26}$	0.75	0.005	1.34

Source: National Institutes of Health (2018)

2) Fatty alcohol ethoxylate surfactants (Dehydol LS7TH) were obtained from Thai Ethoxylate Company Limited, Thailand. These surfactants are nonionic surfactant produced in Thailand. The properties of nonionic surfactants are shown in the Table 3.2.

Table 3.2 The properties of nonionic surfactants in this study

Properties	Dehydol LS7TH
Chemical name	Fatty alcohol C12-14 7 moles ethoxylate
Molecular weight	494[a]
HLB	11.7 - 12.5[b]
Density (g/cm^3)	0.939[b]
Emulsion type	O/W[b]
Characteristic curvature (Cc) value	-1.1[a]
Structure	

[a] Data from Arpornpong et al., 2018
[b] Data from Thai Ethoxylate Company Limited

3) Two commercial dispersants i.e. slickgone NS type 2/3 and superdispersant-25 were obtained from Thai Oil PCL company, Thailand and were used for comparing oil dispersion efficiency with dispersant formulation. The compositions of commercial dispersant were shown in Table 3.3.

Table 3.3 Compositions of commercial dispersants in this study.

Dispersant name	Compositions
Slickgone NS	1-10% w/w Anionic surfactant and >50% kerosene
Superdispersant-25	1-10% Dioctyl sulfosuccinate and 10-30% 2-butoxyethanol

4) Two Light crude oils and 2 types of fuel oil were obtained from Thai Oil PCL and Bangchak Corporation PCL, respectively for representing the petroleum contaminated in the environment. The compositions of petroleum were analyzed by Thin layer chromatography (TLC), while other properties were provided by the manufacturer (Table 3.4).

Table 3.4 The properties of petroleum oils in this study

Type of oils	Hydrocarbon composition (%)				Viscosity (cP)	Density (g/cm^3)
	Saturates	Aromatics	Resin	Asphaltene		
Bongkot light (BKC)	100[a]	0	0	0	3.8[a]	0.84[a]
Arab light/Arab extra light blend (ARL)	31[a]	34[a]	20[a]	15[a]	3.8[a]	0.84[a]
Fuel A	14	11	46	29	72.4[b]	0.94[b]
Fuel C	16	13	46	26	171[b]	0.95[b]

[a] Data from Rongsayamanont et al, 2017

[b] Data was provided from manufacturer

3.2 Biosurfactant production and characterization

Lipopeptide biosurfactant in this study was produced by *Bacillus subtilis* GY19 using crude palm oil and waste glycerol as a carbon source. The lipopeptide productions were separated and preliminary purified by foam fractionation (Khondee *et al.,* 2015). Then, the foamate was lyophilized to obtain concentrated lipopeptides (Figure 4.1a). The lipopeptide sample is white-brown powder and contains 50% of lipopeptides (w/w). The lipopeptide characteristic curvature (Cc) value is 4.93 (Rongsayamanont *et al.,* 2017). The structure of lipopeptide consists of 7 amino acid group connect with fatty acid tail (Figure 3.1b). This biosurfactant expresses anionic charge in head structure. Therefore, it is classified as anionic surfactant.
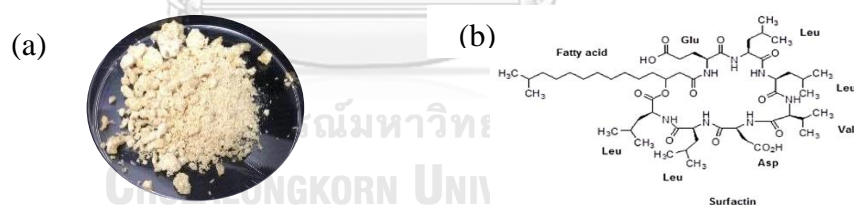
Figure 3.1 Characteristic of lipopeptide biosurfactant

(a) Lipopeptide powder (b) Structure of Lipopeptide biosurfactant produced from *Bacillus subtilis* GY19

3.3 Experimental setup

In this research, the experiment was divided into three phases, which are (i) Dispersant formulation and phase behavior study, (ii) Evaluation of oil dispersion

efficiency, and (iii) Correlation between influenced factors and oil dispersion efficiency as demonstrated in Figure 3.2.

Phase III
Correlation between influenced factors and oil dispersion efficiency

Identification of factors influencing the oil dispersion efficiency such as DOR and salinity

Design the experiment by using Box-Behnken design analysis

Identify the influenced parameters effecting with oil dispersion efficiency to predict the optimal condition by Response Surface Model (RSM) for real site applications

Toxicity test to confirm the real site application with bacteria and plants

Phase II
Evaluation of oil dispersion efficiency

Selected formulations with microemulsion type III

Oil dispersion efficiency test with different types of petroleum oil

Fuel oils

Light crude oil

Baffle flask test (DOR 1:25)

Selection of the high dispersion efficient formulations for all of petroleum

Phase I
Dispersant formulation and phase behavior study

Formulation of oil dispersants following HLD concept with varied EACN (8, 10, 12) and total concentrations (0.1 M and 0.3 M) at salinity 3.4% (w/v)

Ionic equation

Nonionic equation

Microemulsion study with different hydrocarbons

Selected microemulsion type III formulations for further studies

Figure 3.2 Experimental framework

3.4 Preliminary study

To select the nonionic surfactant in this study, two types of fatty alcohol ethoxylate in dehydol series, dehydol LS5TH and dehydol LS7TH were chosen because they have good surface activities, non-toxicity and could be produced in Thailand. Therefore, these nonionic surfactants were done the preliminary test to study to determine the suitable total concentration and dispersibility of these surfactant in mixture formulation.

Lipopetide biosurfactant were separately mixed with dehydol LS5TH and dehydol LS7TH for formulating oil dispersants. According to the system had nonionic surfactants, hydrophilic-lipophilic deviation (HLD) equation for non-ionic surfactant was applied in order to calculate molar fractions of each surfactant. The parameters in this equation such as electrolyte, salinity constant values were fixed and substituted followed Acosta (2008) and (Rongsayamanont *et al.,* 2017). The EACN was varied from 6 to 12 to select the appropriate EACN used in further study. Total concentration of dispersant was varied from 0.1-0.5 M for evaluating suitable mixed surfactant concentrations. Afterward, eighteen formulations (9 formulations from lipopeptide-dehydol LS5TH and 9 formulations from lipopeptide- dehydol LS7TH) were evaluated microemulsion with hexane, decane and dodecane, which has EACN 6, 10 and 12, respectively.

The results showed that molar fractions of lipopeptide increase when EACN was increased; while, molar fraction of nonionic surfactants decreased when EACN was increased (Table 3.5). Moreover, total concentration of oil dispersants was significantly affected to hydrophobicity of oil dispersant and impacted to microemulsion type. The more hydrophobicity of oil dispersant tended to perform microemulsion winsor type II (water in oil; W/O), of which the dispersant tended to dissolve in hydrocarbon phase and showed the excess water phase. However, lipopeptide-nonionic mixture could perform microemulsion type III (bicontinuous emulsion) at low total concentrations (Table 3.6) similar to lipopeptide alone and better than the result of dehydol LS5TH and dehydol LS7TH alone (provided type II microemulsion).

Table 3.5 Molar fraction of lipopeptide biosurfactant and different fatty alcohol ethoxylate (dehydol LS5TH and dehydol LS7TH) of different EACN.

	Molar fractions (Non-ionic equation)			
EACN	Formulation1		Formulation2	
	Lipopeptide	LS5	Lipopeptide	LS7
6	0.25	0.75	0.28	0.72
10	0.37	0.63	0.39	0.61
12	0.43	0.57	0.45	0.55

Table 3.6 Microemulsion type of dispersant formulations against with different hydrocarbons

EACN	Hydrocarbons	Conc.	Experiment			
			Lipopeptide-LS5	Lipopeptide-LS7	Lipopeptide alone	Dehydol alone
		0.1	III	III		
6	Hexane	0.3	II	II	III	II
		0.5	II	II		
		0.1	III	II		
10	Decane	0.3	II	III	III	II
		0.5	II	III		
		0.1	III	III		
12	Dodecane	0.3	II	II	III	II
		0.5	II	II		

In additions, the oil displacement efficiency of oil dispersants was investigated with different hydrocarbons based on the EACN in formulation by varying dispersant to oil ratio (DOR) at 1:2, 1:5 and 1:10. All of oil dispersants showed high oil dispersion efficiency over 50%; however, lipopeptide-dehydol LS7TH formulation seem to have higher dispersibility than lipopeptide-dehydol LS5TH formulation (Figure 3.3). The result was due to the more hydrophilicity of Dehydol LS7TH than Dehydol LS5TH. It is possible that more hydrophilicity of LS7TH could reduce the high hydrophobic

property of lipopeptide and enhance the balancing between hydrophobic-lipophilic properties of the surfactant mixture system.

(a)

(b)

(c)   EACN = 12
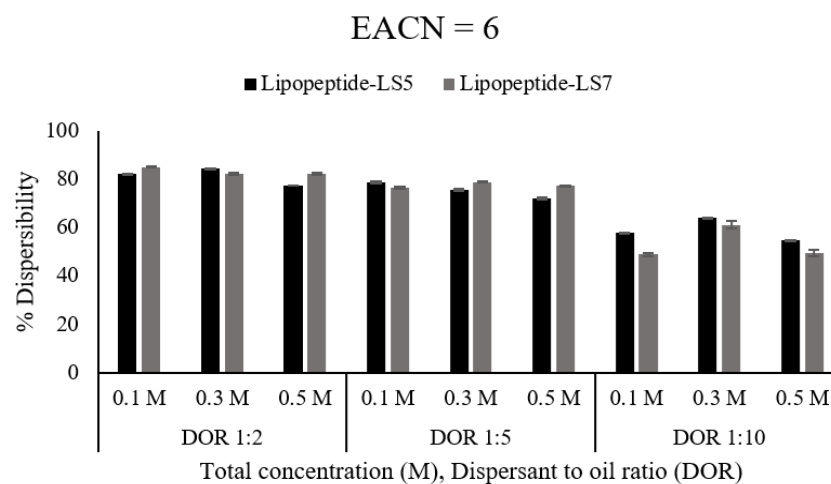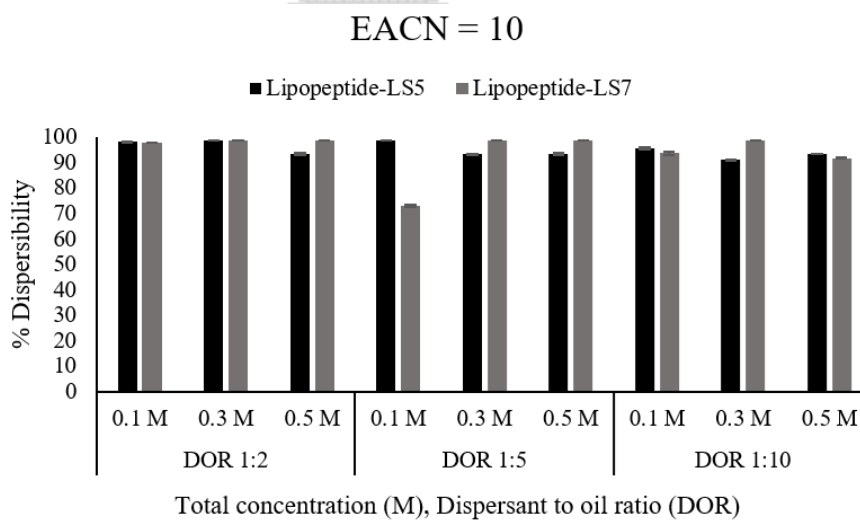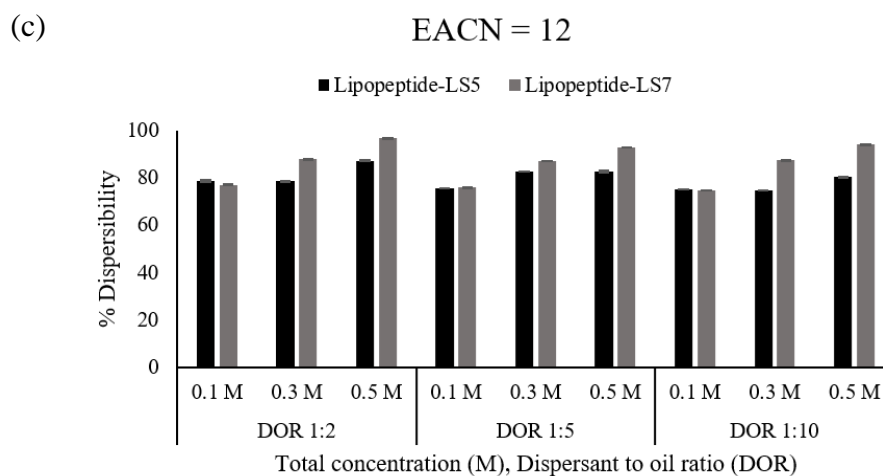
Figure 3.3 Dispersibility of lipopeptide-LS5TH formulation and lipopeptie-LS7TH

formulation calculated from different EACN against with different hydrocarbons

(a) formulations calculated from EACN 6 with hexane, (b) formulations calculated

from EACN 10 with decane and (c) Formulations calculated from EACN 12 with

dodecane.

From these preliminary results, dehydol LS7TH was selected for further

studies. Moreover, total surfactant concentration at 0.1 M and 0.3 M were used as

suitable total concentration because it performs high efficiency to form type III

microemulsion and has good oil dispersibility with hydrocarbons.

3.5 Phase I: Dispersant formulation and phase behavior study

3.5.1 Oil dispersant formulation

In this study, oil dispersants were formulated by mixing lipopeptides with Dehydol LS7TH. The hydrophilic-lipophilic deviation (HLD) equations for both ionic and non-ionic surfactants were used to calculate molar fractions of each surfactant. The equivalent alkane carbon number (EACN) were varied for 8, 10 and 12; moreover, total concentration of mixture also varied for 0.1 M and 0.3 M due to the optimum concentration for oil dispersion and microemulsion from the preliminary results and (Rongsayamanont $et\ al.$, 2017). Salinity (S) was fixed at 3.4% (w/v of NaCl) to represent seawater condition. To achieve solvent-free property, this study would not use chemical solvents or co-surfactants. The temperature was assumed in room temperature. Therefore, f(A) and $\alpha_T \Delta T$ in both equations were eliminated and the equations were rearranged to calculate molar fraction of each surfactant (Eq. 4 and 5). The amounts of lipopeptide biosurfactant and fatty alcohol ethoxylate surfactants of each formula were added into glass vial following molar fractions from calculation and mixing until homogeneous.

For ionic surfactant;

$$HLD = \ln(S) - K(EACN) + X_1(Cc_1) + X_2(Cc_2) \qquad (Eq.4)$$

For non-ionic surfactant;

$$HLD = b(S) - K(EACN) + X_1(Cc_1) + X_2(Cc_2) \qquad (Eq.5)$$

Table 3.5 Variables in HLD concept equation

Variables		Values
b	A constant depending on the electrolyte (0.13 when S in expressed as wt % NaCl)	0.13
S	Represents the salinity (in wt% in the aqueous phase)	3.4 and 0.5 for seawater and freshwater condition, respectively.
K	A constant number	0.17
EACN	An equivalent alkane carbon number	8, 10 and 12
X	Molar fraction of surfactant	-
Cc	A characteristic curvature of surfactant	-1.1 and 4.93 for LS7TH and lipopeptide biosurfactant, respectively (Rongsayamanont et al., 2017, Witthayapanyanon et al., 2008 and Arpornpong et al., 2018)

Source: Acosta (2008)

3.5.2 Microemulsion study with pure hydrocarbons

The formulations from the previous part were investigated for microemulsion formation. The equal volume of oil dispersant and different pure hydrocarbons (0.5 mL of each phase) were added into 1 mL glass tube and cover with caps. Octane, decane and dodecane have an equivalent alkane carbon number (EACN) 8, 10 and 12, respectively, that relate to EACN value used in HLD equations. The glass tubes were hand-shaken for 1 minute once a day for the first 3 days and left without disturbance for 2 weeks for reaching phase equilibrium.

The microemulsion can occur in three types (i) winsor type I (oil in water; O/W) microemulsion, which is in the equilibrium with an excess oil phase having vary low surfactant concentration. The second is (ii) winsor type III (bicontinuous) microemulsion, which occur the balance mixing of oil and surfactant solution in the middle phase and (iii) winsor type II (water in oil; W/O) microemulsion, which perform excess water phase in the system (Figure 3.4 and 3.5).
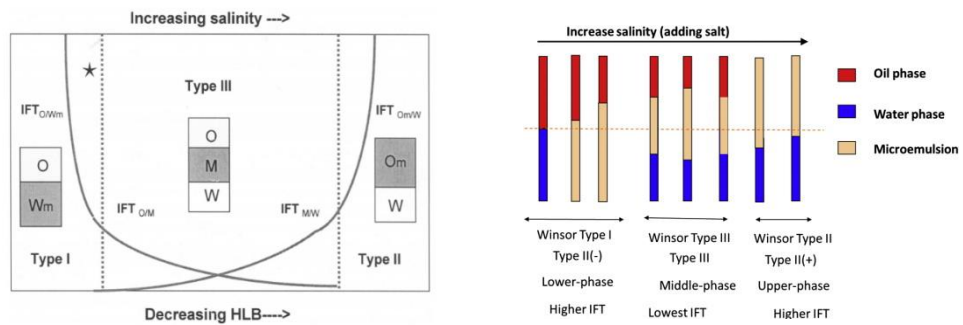
Figure 3.4 Phase behavior system.

Where O is oil; W is water; M is microemulsion

(Tongcumpoua *et al.*, 2003
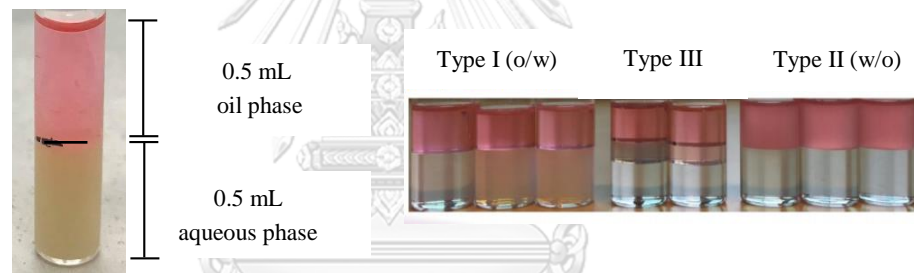
Figure 3.5 Phase behavior study

The microemulsion was evaluated by visual and laser light observation. The efficient dispersant formulations should perform microemulsion winsor type III. Then, the formulations with Type III microemulsion were selected to study the dispersion efficiency in the next steps.

3.6 Phase II: Evaluation of oil dispersion efficiency

3.6.1 Dispersion efficiency with different types of petroleum oils

To evaluate the dispersion efficiency of oil dispersants, the selected dispersant formulas were tested by a baffled flask test adapted from Venosa and Holder (2013) and Rongsayamanont *et al.* (2017) The synthetic seawater in this phase was prepared with salinity 34 ppt for representing seawater condition. Light crude oil and fuel oil were used to represent the variety of petroleum oil that possibly release from oil spill situation. Furthermore, oil dispersant formulations were compared the dispersion efficiency with the commercial dispersants (slickgone NS type 2/3 and superdispersant-25).

Briefly, the seawater 120 mL were added to a baffled flask followed by adding petroleum oil and dispersants, respectively. One hundred microliter of each petroleum oil were directly added onto the surface of the seawater and left it for dispersion on upper layer. A volume of 4 µL of dispersant were gently dropped on the center of oil slick (DOR is 1:25). The baffled flask was shaken on the orbital shaker and mixed for 10 minutes at 200 rpm. Then, the sample was left for 10 minutes before further analysis. The first 5 mL of sample were drained and discarded. The 30 mL of sample were collected and extracted by using dichloromethane (DCM). The residual petroleum oil concentration was analyzed using UV-visible spectrophotometer at the wavelength 340, 370 and 400 nm and

calculate the dispersion efficiency by this equation below (EPA 40 CFR Appendix C to

Part 300)

$$\% \ EFF_D = \%EFF_d - \%EFF_c \qquad (Eq.6)$$

Where

| EFF_D | % dispersed oil due to dispersant only |

| EFF_d | % dispersed oil with dispersant added |

| EFF_c | % dispersed oil with no dispersant added |

$$EFF \ (\%) \ = (\ C_{mean}/C_{TOT})\times100 \qquad (Eq.7)$$

Where

C_{mean} — Mean value for total mass of dispersed oil by spectrophotometric analysis

C_{TOT} — Total mass of oil initially added to the experimental
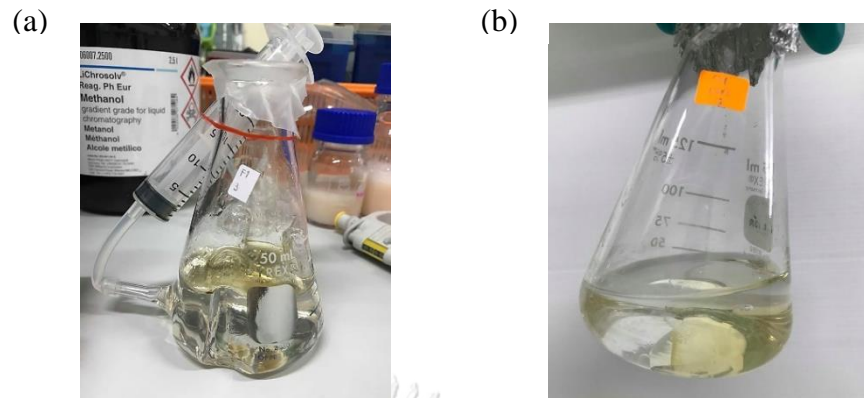
Figure 3.6 Baffle flask test

(a) Baffle flask for dispersion efficiency experiment and (b) Oil extraction by

DCM

From this experiment, the formulations that perform high oil dispersion efficiency was selected for investigating the influenced factors that impact to oil dispersion efficiency.

3.7 Phase III Correlations between influenced factors and oil dispersion efficiency

3.7.1 Investigation of influenced parameters impact to oil dispersion efficiency

The selected dispersant formula from previous phase was tested for oil dispersion efficiency under varying conditions. Baffle flask test was used as same

method as in the previous experiment. The experiment in this phase was design by STATISTICA10 program (StatSoft Tulsa, OK, USA) using Box-Behnken design analysis. The selected formulation with high oil dispersion efficiency with all petroleum in previously phase was tested again with fuel C in different DOR and salinity conditions. The salinity was varied for 0, 1.7 and 3.4 (% w/v of NaCl) based on the salinity in freshwater, brackish water and seawater, respectively (EPA, 2015). The amounts of surfactant formulations and petroleum oil (fuel C) were varied in the range of 2-20 µL and 20-200 µL, respectively covered the DOR ranges of 1:2 to 1:100 from the previous study (Rongsayamanont et al., 2017).

3 factor Box-Behnken design, 1 block , 15 runs			
Standard Run	Salinity (ppt)	Dispersant (uL)	Oil (uL) 3
1	0.00000	2.00000	110.0000
2	34.00000	2.00000	110.0000
3	0.00000	20.00000	110.0000
4	34.00000	20.00000	110.0000
5	0.00000	11.00000	20.0000
6	34.00000	11.00000	20.0000
7	0.00000	11.00000	200.0000
8	34.00000	11.00000	200.0000
9	17.00000	2.00000	20.0000
10	17.00000	20.00000	20.0000
11	17.00000	2.00000	200.0000
12	17.00000	20.00000	200.0000
13	17.00000	11.00000	110.0000
14	17.00000	11.00000	110.0000
15	17.00000	11.00000	110.0000

Figure 3. 7 Box-Behnken design for prepare the correlation study

3.7.2 Analyze the correlation by using Box-Behnken design

All data from the previous part (n = 45) were analyzed the correlation between influenced factors and oil dispersion efficiency by ANOVA using

STATISTICA10 (StatSoft Tulsa, OK, USA) and showed in surface response profile. Moreover, the regression equation of correlation between salinity (X_1), dispersant volume (X_2), oil volume (X_3) and dispersion efficiency (y) was obtained from this experiment. The acquired equation could help us identify the suitable dispersant volume and useful for applying in the real oil spill situation. For example, we can substitute the salinity of oil spilled site, volume of spilled oil and required dispersion efficiency into the equation. Then, the volume of dispersant would be calculated and recommended to apply in the treatment system. This analysis could reduce times and costs for oil spill treatment in the future.

3.7.3 Toxicity of dispersant formulation

The toxicity of selected dispersant solution was evaluated to confirm that the formulation is non-toxic to the environment. The dispersant formulation was tested compare with commercial dispersant, slickgone NS and superdispersant-25.

1) Minimum bactericidal concentration (MBC)

The toxicity of selected formulation on petroleum-degrading bacteria was determined by minimum bactericidal concentration (MBC) test on microtiter plate (96 well plates) modified by Siriratruengsuk (2016). Fifty microliter of 0.85% NaCl were added into each well. The testing solutions (concentrated dispersant formulation, slickgone NS and superdispersant-25) were prepared dilution series by adding 100 µL

of testing samples in the first well of each row. Then, 50 µL sample solutions were respectively pipetted to the next well (1:1 dilution) until the last well in each row. *Microbacterium saccharophilum* RK15 (MSCU1055) and *Gordonia amicalis* JC11 (MSCU0794) were used as representatives of petroleum-degrading bacteria. They were isolated from sea sand and fishery port seawater, respectively (Chanthamalee et al., 2012). The bacteria were grown separately and adjusted to the optical density (OD_{600}) of 0.1. During the test, 50 µL of bacterial solution were added into the testing solution in each well and incubated for 24 h. Afterward, the 5 µL of inoculums in each well were dropped on nutrient agar (NA) plate to determine the minimum bactericidal concentration (MBC), which represented the lowest concentration that bacteria could not grow compared with negative control (only NaCl solution, no tested solution) and positive control (only NaCl and inoculated solution, no tested solution).
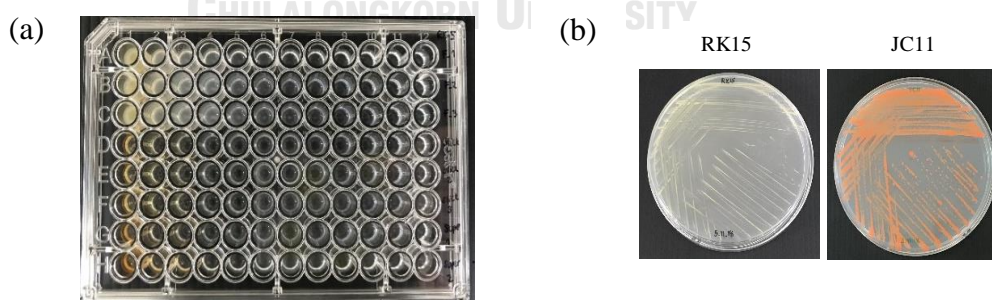
Figure 3.8 MBC test

(a) microtiter plate (96 well plates) and (b) colony of petroleum-degrading bacteria, Microbacterium *saccharophilum* RK15 and *Gordonia amicalis* JC11 on agar plate

2) Phytotoxicity

The phytotoxicity of dispersant formulation was investigated with green bean (*Vigna radiata* (L.) Wilczek) and duckweed (*Spirodela polyrhiza* (L.) Schleid) which rapidly grow and easily find in natural sources. For green bean, ten green bean seeds were immerged in distilled water for 3 hours and inoculated in petri-dish containing Whatman N° 1 filter paper. Testing solutions in this study contained 20 μL of each dispersant (lipopeptide-dehydol LS7TH formulation, slickgone NS and superdispersant-25) in 120 mL of distilled water to represent the highest concentration of dispersants in the baffle flask system (Part 3.6). Then, 5 mL of each testing solutions were sprayed onto the seeds and kept in dark place for 5-day. After 5-day incubation, seed germination and trunk elongation (≥5 mm) were determined using equation 8-9 (Luna *et al.*, 2013).

$$\text{Seed germination } (\%) = \frac{\text{number of seeds germinated in the solution}}{\text{number of seeds germinated in control}} \times 100 \qquad \text{(Eq.8)}$$

$$\text{Trunk elongation } (\%) = \frac{\text{mean trunk length in the solution}}{\text{mean trunk length in control}} \times 100 \qquad \text{(Eq.9)}$$
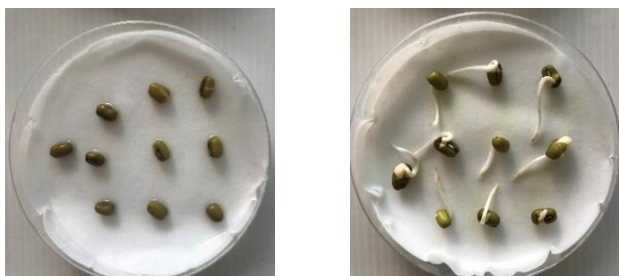
Figure 3.9 Seed germination and trunk elongation of green bean after sprayed with testing solutions and cultivated for 3 days

Duckweeds were used to represent the aquatic plant in natural fresh water. Ten colonies of duckweeds were grown in 120 mL sterile natural water, which collected from natural source, containing with different volume of testing solutions. For dispersant formulation, the added volume was varied at 2, 11 and 20 µL in order to represent the low, medium and high concentrations of dispersant in the baffle flask test (part 3.6). Meanwhile, the added volume of slickgone NS and superdispersant-25 was fixed at 20 µL to express the maximum concentration of commercial dispersant in the baffle flask system. The plants were observed for the morphology changes, root length and the number of leaf for 5 days compared with control (only water). All of the experiments were carried out in triplicates followed Siriratruengsuk (2016) and Wan (1990).

Figure 3.10 The characteristic of duckweed for phytotoxicity test

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Dispersant formulation by using HLD concept

Oil dispersants for the oil spill treatment are generally consisted of anionic and nonionic surfactants because these types of surfactants express good emulsifier with crude oil in seawater (Athas *et al.*, 2014). However, the conventional dispersants contain harmful solvent mixing with the surfactant mixture. Therefore, lipopeptide biosurfactant was selected as anionic surfactant due to its good surface activity and low toxicity (Fooladi *et al.*, 2016, Mani *et al.*, 2016) Nonionic surfactant in this study was dehydol LS7TH, which has good surface activity (CMC = 40 mg/L) and has low toxicity for environment applications. Two types of surfactants in this study were characterized and evaluated characteristic curvature (Cc value) as 4.93 and -1.1 for lipopeptide and dehydol LS7TH, respectively. From the previous study, the lipopeptide was considered as strongly hydrophobic due to the high positive Cc value (Rongsayamanont *et al.*, 2017). Therefore, the nonionic surfactant, dehydol LS7 TH, was selected to express as a hydrophilic moiety for balancing the surfactant mixture.

Lipopeptide and fatty alcohol etoxylate (dehydol LS7TH) were mixed at various proportions to formulate the dispersants without using any chemical

solvents. The surfactant mixtures were expected to have the balancing between hydrophilic and hydrophobic property and provide ultralow interfacial tension (IFT) against pure hydrocarbons (Acosta, 2008). The HLD concept was applied to calculate molar fractions of each surfactant with the different EACN of hydrocarbons. The total concentrations of surfactant mixture were also varied.

This study calculated the molar fractions of each surfactant from both ionic and nonionic HLD equations; then, investigated microemulsion against hydrocarbons and observed microemulsion type. Moreover, the molar fractions calculated from HLD equations were used to predict the mix micelle structure of lipopeptide and dehydol LS7TH. Finally, the suitable HLD equation for mixing anionic-nonionic surfactants were recommended.

4.1.1. Dispersant formulation calculated from HLD equation

In this experiment, the known parameters i.e. salinity, constant values (k,b), EACN and Cc values were substituted into Eq.4-5. The molar fractions were calculated from the optimal condition (HLD=0), which expected to give the bicontinuous microemulsion (Winsor type III). The molar fractions of each surfactant were shown in Table 4.1.

Table 4.1 Molar fraction of lipopeptide biosurfactant and fatty alcohol ethoxylate (Dehydol LS7TH) at different EACN.

	Molar fraction					
	Ionic equation			Non-ionic equation		
EACN	Lipopeptide (LP)	Fatty alcohol ethoxylate (dehydol LS7TH)	Molar fraction ratio (LS7/LP)	Lipopeptide (LP)	Fatty alcohol ethoxylate (dehydol LS7TH)	Molar fraction ratio (LS7/LP)
8	0.21	0.79	3.8	0.34	0.66	1.9
10	0.26	0.74	2.8	0.39	0.61	1.6
12	0.32	0.68	2.1	0.45	0.55	1.2

When calculated the molar fraction from HLD ionic and nonionic equations, the results showed similar trend that molar fractions ratio of lipopeptide and nonionic surfactant in the surfactant mixture increased when the EACN was increased. As a result of increasing EACN of hydrocarbons, the system needs more hydrophobic surfactant to balance the hydrophobicity of oil. Moreover, the increasing of lipopeptide in the system could increase the hydrophobicity in the mixture system. In order to achieve the optimal condition (HLD=0), hydrophilicity from nonionic surfactant was extremely required in the system. Therefore, the fractions of dehydol LS7TH were about 2-4 time higher than lipopeptide from the ionic equation, while there were about 1-2 time higher than lipopeptide from the nonionic equation

as shown in Table 4.1. The ratio of lipopeptide and dehydol LS7TH fractions was later used to predict the molecular structure of surfactant mixture.

According to the lipopeptide structure (Chen *et al.,* 2015), the repulsion force was occurred between negative charges in their head structure. The lipopeptide alone system could form large micelle structure, thus high surfactant concentration was required for forming micelle (Figure 4.1a). When nonionic surfactant was mixed with lipopeptide, a synergistic effect occurred by reducing the repulsion force between negative charges and enhancing the formation of mixed micelle rapidly. Thus, less amount of surfactants was required in the system to form the mixed micelle (Figure 4.1b and c). Lipopeptide-dehydol LS7TH mixture possibly formed the mixed micelle by the bonding of nonionic monomer with the hydrophobic site chain of amino acid (Madsen et al., 2001). The dehydol monomer could attach to leucine (Lue) and valine (Val) groups on surfactin head structure due to the similar hydrophobicity.

The predicted lipopeptide-dehydol mixed micelle structure were shown in Figure 4.1. The Figure 4.1a expresses the micelle structure of lipopeptide alone. The area between lipopeptide head was large due to the repulsion between anionic ion in its structure. Meanwhile, the synergistic effect was exhibited when lipopeptide was mixed with nonionic surfactant as show in Figure 4.1b and 4.1c. The Figure 4.1b illustrates the proposed micelle structure of lipopeptide-dehydol LS7TH formulation

when the formulation was calculated from ionic equation and Figure 4.2c displayed

the suggested structure of lipopeptide-dehydol LS7TH micelle when the formulation

was calculated from nonionic equation. The micelle structure of lipopeptide-dehydol

LS7TH formulation from ionic equation in Figure 4.1b is smaller than structure in 4.1c

because the head structure was tightly packed.

(a)

(b)

(c)
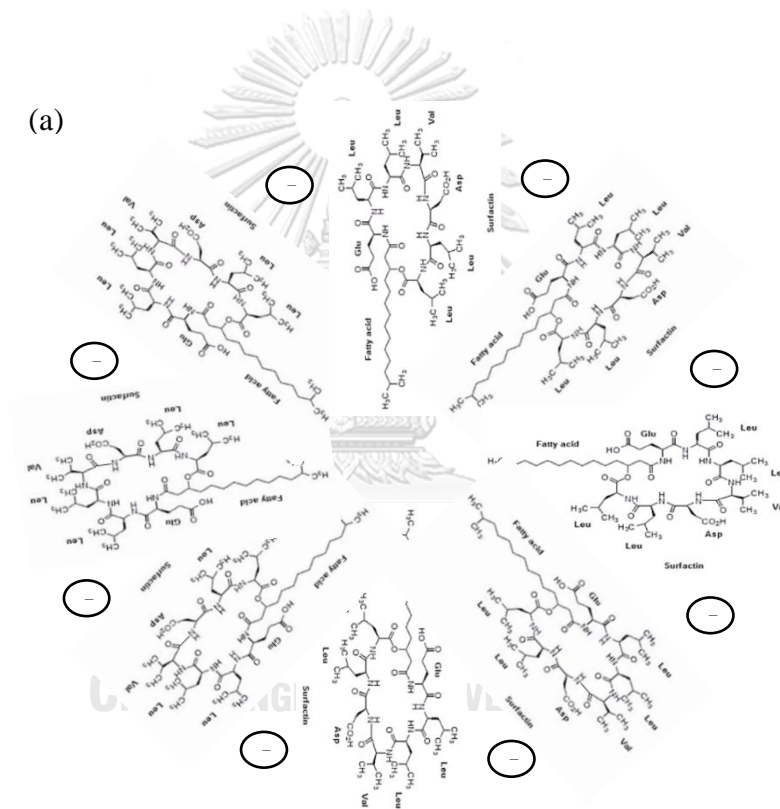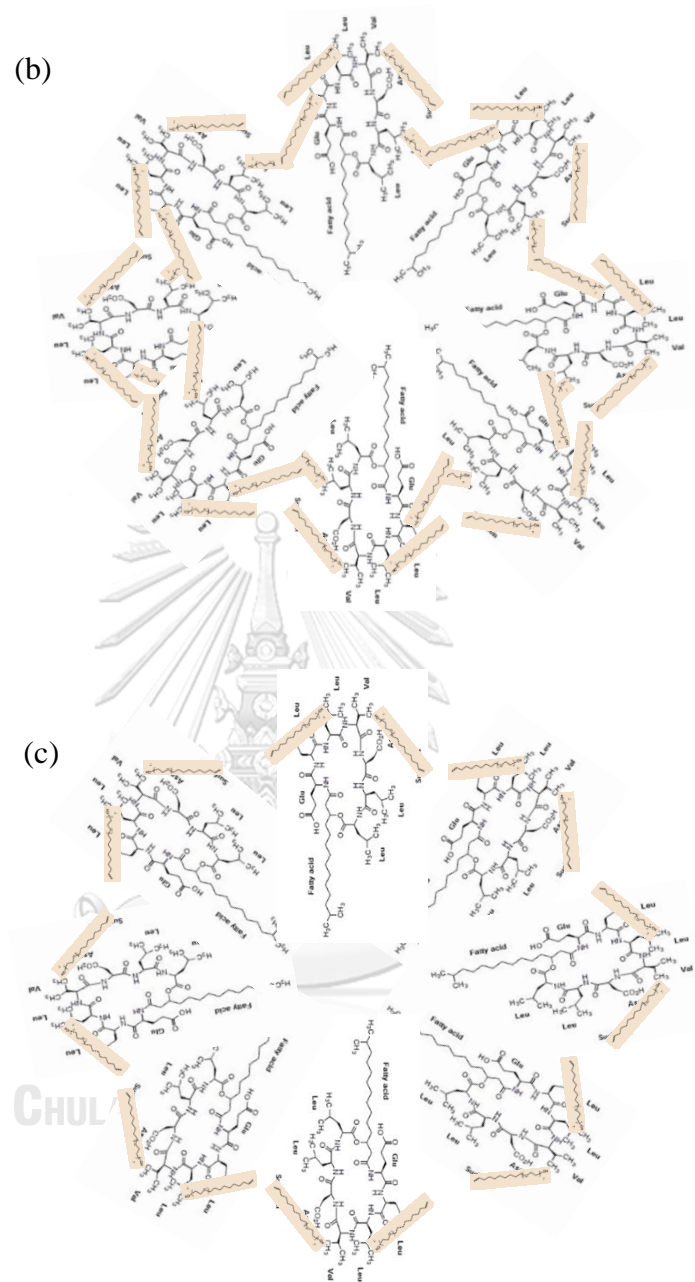
Figure 4.1 Predicted structure of micelle of lipopepitde – dehydol LS7TH mixture
(a) lipopeptide alone micelle (b) The fraction calculated from ionic equation (c) The
fraction calculated from nonionic equation.

Then, the molar fractions of each surfactant were calculated in mass ratio (%w/v) by varying the total concentrations at 0.1 M and 0.3 M and the EACN values at 8, 10 and 12. For ionic surfactant equation, the amounts of lipopeptide/dehydol LS7TH in mass ratio at the total concentration of 0.1 M were 2.2/3.97 %w/v, 2.73/3.72%w/v and 3.36/3.42%w/v for the EACN values of 8, 10 and 12, respectively. When increase the total concentration to 0.3 M, the amount of each surfactant was also approximately 3 times higher than that of the total concentration 0.1 M. The amounts of lipopeptide/dehydol LS7TH were 6.61/11.92 %w/v, 8.18 %w/v and 10.07/10.62 %w/v for the EACN values of 8, 10 and 12, respectively (Table 4.2).

For nonionic equation, the amounts of lipopeptide and dehydol LS7TH at 0.1 M were 3.57/3.32 %w/v, 4.09/3.07 %w/v and 4.72/2.77 % w/v for the EACN values of 8, 10 and 12, respectively. At total concentration of 0.3 M, the mass ratio of lipopeptide and nonionic surfactant were 10.7/9.96 % w/v, 12.27/9.21 %w/v and 14.16/8.30 % w/v the EACN values of 8, 10 and 12, respectively (Table 4.3). The mass proportions of lipopeptide surfactant were increased, while the mass proportion of dehydol LS7TH tended to decline when the total concentration of mixture system was increased. There results were similar to those using the ionic equation.

Table 4.2 The amounts of lipopeptide biosurfactant and dehydol LS7TH with different EACN values and total concentrations when calculated from the ionic equation.

EACN	Total concentration (M)	Ionic equation		
		Amount of all compositions (%w/v)		
		Lipopeptide	LS7	NaCl
8	0.1	2.2	3.97	
	0.3	6.61	11.92	
10	0.1	2.73	3.72	3.4
	0.3	8.18	11.17	
12	0.1	3.36	3.42	
	0.3	10.07	10.26	

จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

Table **4.3** The amounts of lipopeptide biosurfactant and dehydol LS7TH with different EACN values and total concentrations when calculated from the nonionic equation.

EACN	Total concentration (M)	Nonionic equation		
		Amount of all compositions (%w/v)		
		Lipopeptide	LS7	NaCl
8	0.1	3.57	3.32	
	0.3	10.7	9.96	
10	0.1	4.09	3.07	3.4
	0.3	12.27	9.21	
12	0.1	4.72	2.77	
	0.3	14.16	8.30	

When comparing the mass ratios from both equations, the amounts of lipopeptide and dehydol LS7TH in the mixed system were difference. The amounts of lipopeptide in ionic equation system were slightly lower than dehydol LS7TH in all formulations; meanwhile, the nonionic equation system contained more lipopeptide than dehydol LS7TH in all formulations. The different proportions of lipopeptide were influenced by different factors in the equation, which are ln(s) and b(s) as shown in eq.4-5. The ln(s) in ionic equation represented the charge shielding effect of electrolyte (Acosta, 2008), which the charge of surfactant head can shield each other

from the pull of electrolyte, resulting in the decreased attraction between surfactant and electrolyte in the system. Therefore, the high salinity in seawater system could not affect to the amount of lipopeptide in mixture system. Whereas, the b(s) factor in nonionic equation was accounted for the salting-out effect of nonionic surfactant. The high concentration of electrolyte in the system could impact to the hydrophobic group of surfactant and the large ionic charge could bind with water molecule (Ren *et al.*, 2016). In addition, the salting out effect could influence to protein structure of lipopeptide, of which the protein usually decreases in solubility at high salt concentration and tends to precipitate (Wingfield, 2016). Consequently, the nonionic system required more amount of lipopeptide to reach the equilibrium condition compared to the amount of lipopeptide in ionic system.

4.1.2 Microemulsion study of dispersant formulation against hydrocarbons

To prove the predicted microemulsion theory, the microemulsion test were set-up. This experiment expected to obtain the formulations that gave winsor type III emulsion at the middle phase and ultralow interfacial tension (IFT). Recently, numerous studies indicated that the oil/water interfacial tension (IFT$_{o/w}$) would reach the lowest value at the optimal condition (HLD = 0) and performed winsor type III emulsion; moreover, the solubility of oil in the system could be enhanced (Nardello *et al.*, 2003, Arpornpong *et al.*, 2018).

Twelve mixture formulations from the previous preparation were subjected to the phase behavior study and observed the winsor type emulsion with hydrocarbons based on each formulation. Octane, decane and dodecane were used to represent the EACN values as 8, 10 and 12, respectively. The results showed that all of the lipopeptide-dyhydol LS7TH formulations calculated from the ionic equation displayed winsor type III emulsion, except the 0.1 M formulation for EACN = 10. In contrast, the formulations calculated from nonionic equation gave the type III microemulsion only at low total concentration (0.1 M) and tended to shift to type II microemulsion (w/o) when the total concentration was increased (Table 4.4). It was possible that the formulations contained very high amount of lipopeptide, which were not the proper proportion due to the high hydrophobicity in the system. Thus, the microemulsion preferred to transition from winsor type I to III and reached to type II similar to Phan *et al.* (2011). On the other hand, the lipopeptide-dehydol LS7TH formulations calculated from ionic equation, of which the amount of lipopeptide was less than dehydol LS7TH, achieved the balancing condition and displayed microemulsion type III in the middle phase, which was agreed with the predicted result (HLD=0).

Table 4.4 Microemulsion types of dispersant formulations calculated from HLD

concept based on ionic and nonionic equations with different hydrocarbons.

Composition of formulation	EACN	Conc.	Expectation	Experiment	
				Ionic equation	Nonionic equation
	8	0.1		III	III
		0.3		III	II
Lipopeptide	10	0.1	III	II	III
+		0.3		III	II
LS7TH	12	0.1		III	III
		0.3		III	II

Table 4.5 Characteristic of microemulsion of lipopeptide-dehydol LS7TH formulations against different hydrocarbons

Hydrocarbons	EACN	Ionic		Nonionic	
		Total concentration (M)			
		0.1	0.3	0.1	0.3
Octane	8	Type III	Type III	Type III	Type II
Decane	10	Type II	Type III	Type III	Type II
Dodecane	12	Type III	Type III	Type III	Type II

4.2 Effectiveness of the selected lipopeptide-dehydol LS7TH formulations

The lipopeptide-dehydol LS7TH formulations, which formed the microemulsion type III in part 4.1 were then selected to evaluate the dispersion effectiveness with petroleum oils in a baffle flask test. From the results in part 4.1.2, eight formulations (5 formulations from ionic equation and 3 formulations from nonionic equation) were evaluated with three petroleum oils including Bongkot light crude oil, fuel A and fuel C. These oils are normally found in oil spill situations reported by Marine department of Thailand. The composition of each formulation were shown in Table 4.6. The Baffle flask test was modified from (Venosa and Holder, 2013) and Bioremediation Agent Effectiveness Test 40 CFR Appendix C to Part 300 from US.EPA. Following the standard method, the experiments were set in seawater condition and the dispersant to oil ratio (DOR) was fixed at 1:25. The dispersion effectiveness of each formulation was compared with commercial dispersants i.e. slickgone and superdispersant-25.

Table 4.6 Compositions of the selected lipopeptide-dehydol LS7TH formulations

Formulations	Name	Compositions
1	E8_ion_0.1	Lipopeptide 2.2%, dehydol LS7 4%, NaCl 3.4%
2	E8_ion_0.3	Lipopeptide 6.6%, dehydol LS7 11.9%, NaCl 3.4%
3	E8_non_0.1	Lipopeptide 3.6%, dehydol LS7 3.3%, NaCl 3.4%
4	E10_ion_0.3	Lipopeptide 8.2%, dehydol LS7 11.2%, NaCl 3.4%
5	E10_non_0.1	Lipopeptide 4%, dehydol LS7 3%, NaCl 3.4%
6	E12_ion_0.1	Lipopeptide 3.4%, dehydol LS7 3.4%, NaCl 3.4%
7	E12_ion_0.3	Lipopeptide 10.1 %, dehydol LS7 10.3%, NaCl 3.4%
8	E12_non_0.1	Lipopeptide 4.7%, dehydol LS7 2.8%, NaCl 3.4%

Remarks:

E = Equivalent alkane carbon number that substituted in to the HLD equation

Ion/non = Type of HLD equation (ion = ionic equation, non = nonionic equation)

0.1/0.3 = total concentration of mixture formulation (0.1 = total concentration is 0.1 M, 0.3 = total concentration is 0.3 M)

4.2.1. Dispersion effectiveness of the selected lipopeptide-dehydol LS7TH formulations with light crude oil

Bongkot light crude oil (EACN ~ 6) was selected as a representative for petroleum light crude oil. The results showed that the dispersion effectiveness of each formulation with BKC were difference. The dispersion effectiveness of all formulations ranged from 15 to 57% and only 3 formulations performed dispersion effectiveness greater than 45%. The highest effectiveness occurred at the formulation

5 (E10_non_0.1), which its effectiveness was higher than slickgone NS and superdispersant-25 at 41% and 27.6 %, respectively as shown in Figure 4.2.
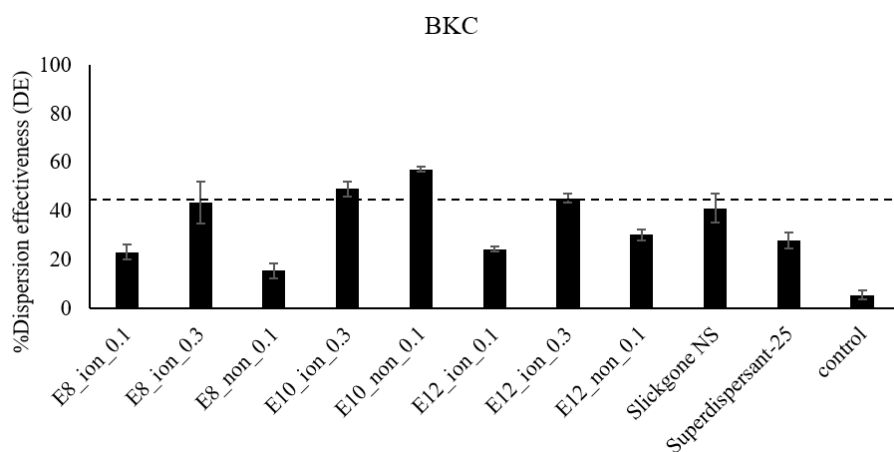
Figure 4.2 Dispersion effectiveness of the selected lipopeptide-dehydol LS7TH

formulations with Bongkot light crude oil compared with slickgone NS and

superdispersant-25
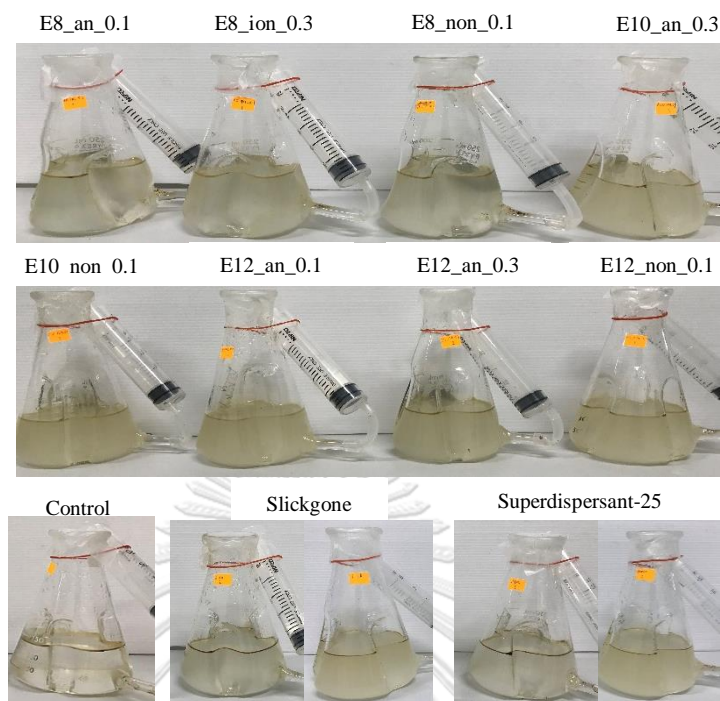
Figure 4.3 The dissolution of Bongkot light crude oil (BKC) and dispersant formulation (DOR 1:25) in baffle flask compared with BKC only (control), slickgone NS and superdispersant-25 in seawater condition.

There was only the study of (Rongsayamanont *et al.*, 2017) that used HLD concept for dispersant formulation. When compare the dispersion effectiveness with the previous study, lipopeptide-dehydol LS7TH formulation in this study exhibited lower dispersion efficiency with BKC than the formulation of lipopeptide-SDHS formulation in the previous study. It possible caused from the synergistic action between surfactant types. The mixture of anionic surfactant might have ability to dispersed the light crude oil more than the anionic-nonionic mixture in all formulation. For light crude oil, the large area of dispersant head structure may be

required for containing light crude oil to reach dispersion and solubilization mechanism into seawater. However, the efficiency of lipopeptide-SDHS formulation was dropped when the viscosity of oil was increased, thereby more total concentration of formulation was more required.

4.2.2. Dispersion effectiveness of the selected lipopeptide-dehydol

LS7TH formulations with fuel oils

To develop the dispersant formulation for applying in various petroleum type, eight formulations were tested the dispersion effectiveness with the different properties of petroleum. Fuel A and fuel C were selected to represent as commercial fuel oil, which had more viscosity and different hydrocarbon compositions compared with BKC. The results indicated that the dispersed oil could solubilize in seawater and displayed dispersion effectiveness ranged from 8.6% to 59.7% and 9.3% to 70.5% for fuel A and fuel C, respectively. The dispersion effectiveness of mixture formulations with both fuel oils showed the similar trend due to the similar hydrocarbon compositions of oils. According to the results, only two formulations, formulation 2(E8_ion_0.3) and 4 (E10_ion_0.3), had ability to dissolve fuel A and C into seawater more than 45% compare with other formulations.

For fuel A, formulation 2 (E8_ion_0.3) performed the highest dispersion effectiveness at 59.7% similar result with superdispersant-25 (58.7%). When compare the formulation with another commercial dispersant, formulation 2 had less effectiveness than slickgone, which expressed 76.9% as shown in Figure 4.4a. The results were contrasted with fuel C solubilization. For fuel C, the highest dispersion effectiveness was also obtained from formulation 2, which the effectiveness was 70.5% higher than slickgone NS and superdispersant-25 (Figure 4.4b).

(a)

(b)
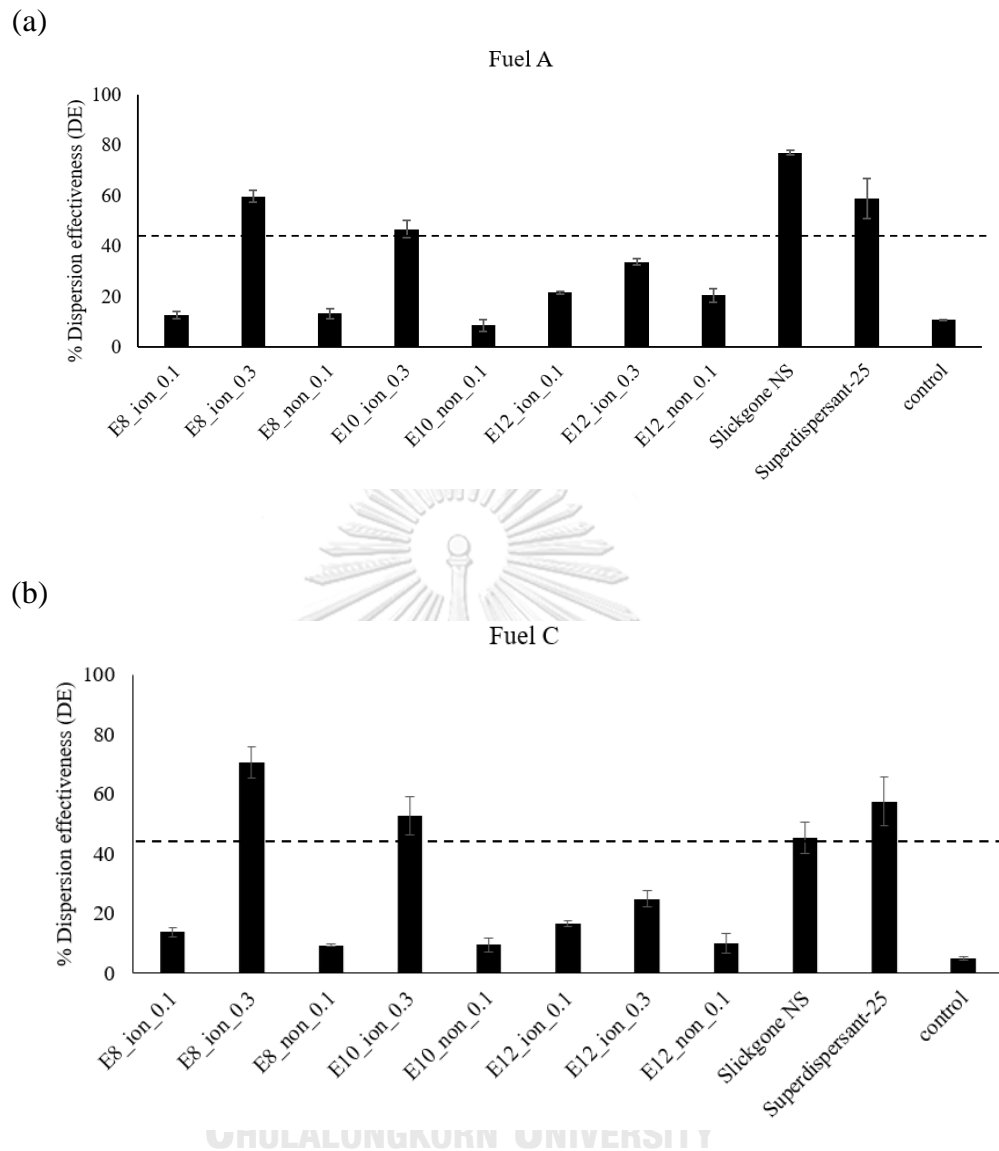
Figure 4.4 Dispersion effectiveness of the selected lipopeptide-dehydol LS7TH with

Fuel oils compared with slickgone NS and superdispersant-25

(a) fuel A and (b) fuel C.

E8_ion_0.1 E8 ion 0.3 E8 non 0.1 E10_ion_0.3

E10 non 0.1 E12 ion 0.1 E12 ion 0.3 E12 non 0.1

Control Slickgone Superdispersant-25
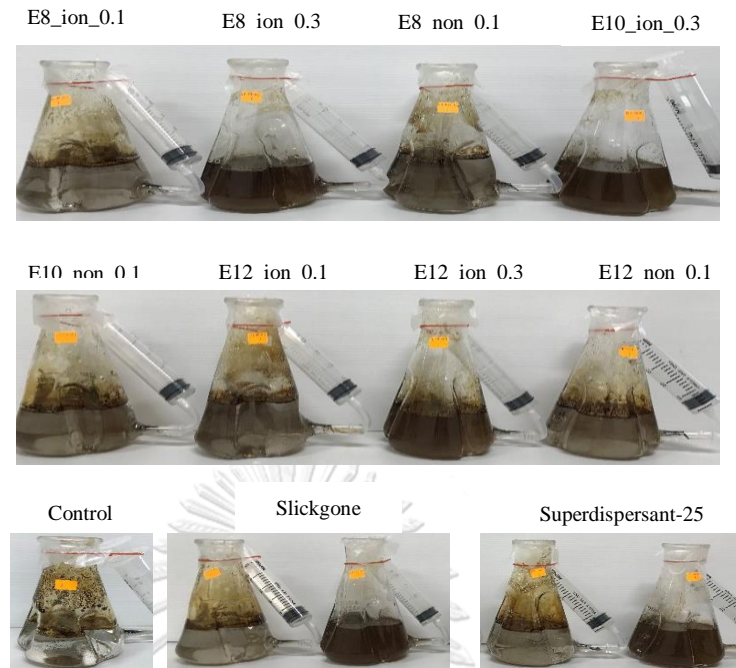
Figure 4.5 The dissolution of fuel A and dispersant formulation in baffle flask compared with fuel A only (control), slickgone NS and superdispersant-25 in seawater condition.

E8_ion_0. E8 ion 0. E8_non_0.1 E10_ion_0.3 E10_non_0.1

E12_ion_0. E12_ion_0. E12_non_0.1 Control

Slickgone Superdispersant-25
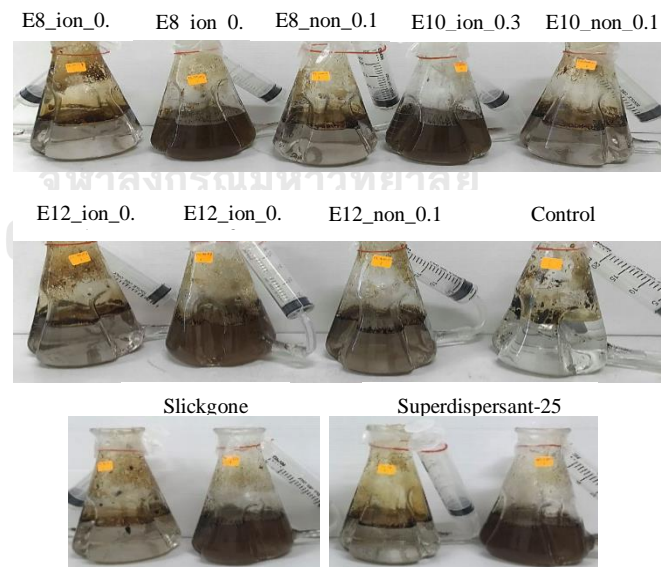
Figure 4.6 The dissolution of fuel C and dispersant formulation in baffle flask compared with fuel C only (control), slickgone NS and superdispersant-25 in seawater condition.

Type of oil is a crucial factor that directly impacts to the dispersion effectiveness due to the viscosity, hydrocarbon compositions or oil structure. When the dispersant contact to petroleum oils, there are two mechanisms occurred during baffle flask test. First mechanism is dispersion, which the surfactant mixture exhibited synergistic effect to reduce interfacial tension between oil and water. Then, the solubilization mechanism continuously occurred as a second mechanism that hydrophobic tails of surfactant could attach with oils and hydrophobic head of surfactant attached to the water. Therefore, mixed micelle could potentially break up the oil into small droplet and dissolve into water phase. Thus, the lighter viscosity oil tended to easily dispersed on the seawater surface before solubilized into the water column than heavy fuel oils.

Not only the nature oil properties, but various environmental factors such as mixing energy, dispersant to oil ratio (DOR), temperature or salinity also influence the dispersion effectiveness in the system (Song, 2013). According to the results, the ability to dissolve oil into water phase of the selected lipopeptide-dehydol LS7TH formulations were significantly difference. The lipopeptide-dehydol LS7TH formulations seem to exhibit high effectiveness with BKC more than fuel oils. It was possible that BKC has uncomplicated composition and low viscosity compared with fuel oil properties; then the micelle of mixture formulation could access and break up the dispersed oil into small droplets easily.

Comparing with the previous study, (Song, 2013) investigated dispersion effectiveness (DE) of anionic-nonionic mixture formulation by baffle flask test. The mixture formulations performed DE in the range of 30% to 60% for crude oil and the effectiveness were less than 30% when testing with heavy oil (Song, 2013)

In addition, the dispersibility of fuel oil in this study was compared to the study of (Rongsayamanont *et al.*, 2017), who tested the dispersion effectiveness of anionic mixture (lipopeptide-SDHS formulation) with light crude oils. They found that the lipopeptide-SDHS formulation provided high effectiveness approximately 90-97% for BKC and 60-80% for ARL. In addition, their formulations were obviously higher than slickgone NS and lipopeptide alone. Moreover, the efficiency of anionic-nonionic mixture (lipopeptide-dehydol LS7TH) was then studied and illustrated that the mixture of lipopeptide and dehydol LS7TH exhibited fuel oil dispersion effectiveness approximately 56 -72 %. The high effectiveness might cause from the composition in their formulation (lipopeptide 12.3%, dehydol LS7 9.2% and NaCl 3.4%), which suitable for high viscosity (630 cP) and EACN of fuel oil (EACN =10). Whereas, the fuel C in this study had lower viscosity (171 cP) and possible to had low EACN. Thus, the proper formulation was difference. However, the selected lipopeptide- dehydol LS7TH formulation (E8_ion_0.3) in this study also expressed high effectiveness with fuel oil, which closed to the previous report. Therefore, our formulation was better than the previous studies in term of provided high dispersion effectiveness, while the

less amount of lipopeptides was used, in addition, they could be applied for both light crude oil and fuel oil. This finding could be useful for economic selection and benefit to the real oil spill applications. The lipopeptide-dehydol LS7TH formulation (E8_ion_0.3), which consisted of 6.6% lipopeptide, 11.9% dehydol LS7 and 3.4% NaCl was chosen to study the correlation of influenced factors in the next experiment.

4.3 Correlations between influenced factors and oil dispersion effectiveness

In order to apply the selected lipopeptide-dehydol LS7TH formulation (E8_an_0.3) into the real environment, the correlation between influenced factors affected to dispersion effectiveness of this formulation was investigated. The salinity was varied at 0, 17 and 34 ppt to represent different type of natural water, freshwater, brackish water and seawater, respectively. Moreover, the dispersant to oil ratio (DOR) was varied cover the range of 1:2 to 1:100 by varying dispersant volume from 2 to 20 µL and oil volume from 20 to 200 µL to study the correlation between the amount of dispersant, oil volume and salinity for further applications.

4.3.1 Investigation of influenced parameters impact to oil dispersion effectiveness

To study the correlation of influenced factors to dispersion effectiveness, it is important to apply the design of experiment (DOE) in order to study the interested variable. In general, full factorial design was widely selected to obtain the highest

accuracy data. However, full factorial design had many limitations such as time, experimental cost including the number of experiment in the all experiments. To diminish these limitations, Response surface methodology (RSM) was selected to design the experiment in this phase. Due to the interested factors were independence each other, the experiment was set by Box-Behnken design and expressed in Table 4.7.

Table 4.7 The experimental design of 3 factors, which are salinity, dispersant volume and fuel C volume designed by Box-Behnken design for baffle flask test.

Experimental no.	Salinity (ppt)	Lipopeptide-dehydol LS7TH (μL)	Fuel C (μL)	Dispersion effectiveness (%)	
				Predicted response	Experimental response
1	0	2	110	57.7184	50.4
2	34	2	110	60.1392	57.5
3	0	20	110	89.0500	93.9
4	34	20	110	76.3976	79.5
5	0	11	20	58.3480	51.5
6	34	11	20	73.2021	67.5
7	0	11	200	64.0699	74.1
8	34	11	200	39.5184	43.4
9	17	2	20	62.8214	66.0
10	17	20	20	70.8569	83.1
11	17	2	200	32.8140	23.6
12	17	20	200	72.9026	65.4
13	17	11	110	62.3887	51.7
14	17	11	110	62.3887	64.2
15	17	11	110	62.3887	71.3

Fifteen experiments were tested in triplicates to evaluate the factors that affect to the dispersion effectiveness of mixture formulation. The results were analyzed and displayed by ANOVA Table (Figure 4.7a). The results illustrated that the dispersion effectiveness of the formulation was significantly influenced by the volume of dispersant and oil. The high effectiveness of the selected lipopeptide-dehydol LS7TH formulation happened when the system contains abundant of dispersant and low oil volume. The p value in ANOVA table was 0.000005 and 0.000406 for dispersant volume and oil volume, respectively, which was lower than significant value of 0.05 ($p < 0.05$). When considered the correlation between 3 interested factors and dispersion effectiveness in Figure 4.7b, the results showed that the relationship between salinity and oil volume and relationship between dispersant volume and oil volume displayed p value at 0.000704 and 0.003739, respectively. It concluded that these 3 factors were influenced the dispersion effectiveness of formulation. In addition, the correlation of 3 factors was interpreted by surface response profile (Figure 4.8).

Moreover, the high effectiveness also displayed when the volume of oil and salinity were varied. However, there was no correlation between salinity and dispersant volume in this experiment ($p > 0.05$). Therefore, it was possible to apply mixture formulation in the various salinity conditions.

(a)

Factor	SS	df	MS	F	p		
ANOVA; Var.:%DE; R-sqr=.79672; Adj:.70525 (optimize.sta) 3 3-level factors, 1 Blocks, 30 Runs; MS Pure Error=45.58076 DV: %DE							
(1)Salinity(L)	94.041	1	94.041	2.06317	0.169044		
Salinity(Q)	96.770	1	96.770	2.12304	0.163322		
(2)Dispersant(L)	2315.929	1	2315.929	50.80937	0.000002		
Dispersant(Q)	162.023	1	162.023	3.55464	0.076589		
(3)Oil (L)	781.856	1	781.856	17.15320	0.000682		
Oil (Q)	385.386	1	385.386	8.45502	0.009800		
1L by 2L	121.794	1	121.794	2.67206	0.120504		
1L by 3L	776.400	1	776.400	17.03349	0.000704		
2L by 3L	513.702	1	513.702	11.27015	0.003739		
Lack of Fit	577.622	3	192.541	4.22417	0.021024		
Pure Error	774.873	17	45.581				
Total SS	6653.456	29					

(b)

Factor	SS	df	MS	F	p		
ANOVA; Var.:%DE; R-sqr=.79672; Adj:.70525 (optimize.sta) 3 3-level factors, 1 Blocks, 30 Runs; MS Pure Error=45.58076 DV: %DE							
(1)Salinity L+Q	190.811	2	95.405	2.09310	0.153944		
(2)Dispersant L+Q	2477.952	2	1238.976	27.18200	0.000005		
(3)Oil L+Q	1167.242	2	583.621	12.80411	0.000406		
1*2	121.794	1	121.794	2.67206	0.120504		
1*3	776.400	1	776.400	17.03349	0.000704		
2*3	513.702	1	513.702	11.27015	0.003739		
Lack of Fit	577.622	3	192.541	4.22417	0.021024		
Pure Error	774.873	17	45.581				
Total SS	6653.456	29					

Figure 4.7 The analysis of the variable that influence the dispersion effectiveness of selected lipopeptide-dehydol LS7TH from Box-Behnken design.

The correlation of dispersant volume and dispersion effectiveness in this study was similar results with the previous study of (Srinivasan, 2007), who evaluated the dispersion efficiency of 3 commercial dispersants (Corexit 9500, Superdispersant 25 and AGMA Superconcentrate DR379) in the different conditions i.e. DORs, mixing

speeds, and temperatures. They found that baffle flask test at the higher mixing speeds (200 and 250 rpm) and higher DORs (4:100 and 2:100) achieved better dispersion efficiency in most cases with heavy fuel oils. The dispersion effectiveness of dispersants significantly increased by 50% when the DOR was increased from 2:100 to 4:100. This results caused form the higher volume of dispersant could more attach the oil and enhance the dispersibility and solublility in the system. Therefore, DOR is an important factor influencing dispersion effectiveness.

In additions, the correlation in this study also similar to Riehm *et al.* (2015) who confirmed that increasing of DOR can enhance the dispersion efficiency of mixed lecithin-tween 80 mixture with crude oil. The dispersant effectiveness at low DOR (1:100 and 1:200) posibly declines because the critical DOR may correspond to a critical micelle concentration for such dispersants in the crude oil. The higher amount of dispersant may occur the lower CMC and achieve better dispersibility more than less amount of dispersant. However, the others factors such as mixing speeds, times and viscosity of oil also affect to the dispersion effectiveness.

To confirm the suitable of model in this experiment, the difference between predicted results and empirical results was investigated and showed in the lack of fit value in ANOVA table. This model exhibited the lack of fit at 0.021024, slightly higher than significant value at 0.05. Therefore, Box-Behnken design could be applied for this experiment.

(a)

Fitted Surface; Variable: %DE
3 3-level factors, 1 Blocks, 30 Runs; MS Pure Error=45.58076
DV: %DE

(b)

Fitted Surface; Variable: %DE
3 3-level factors, 1 Blocks, 30 Runs; MS Pure Error=45.58076
DV: %DE

> 80
< 80
< 60
< 40

> 60
< 60
< 40

(c)

Fitted Surface; Variable: %DE
3 3-level factors, 1 Blocks, 30 Runs; MS Pure Error=45.58076
DV: %DE

> 80
< 80
< 60
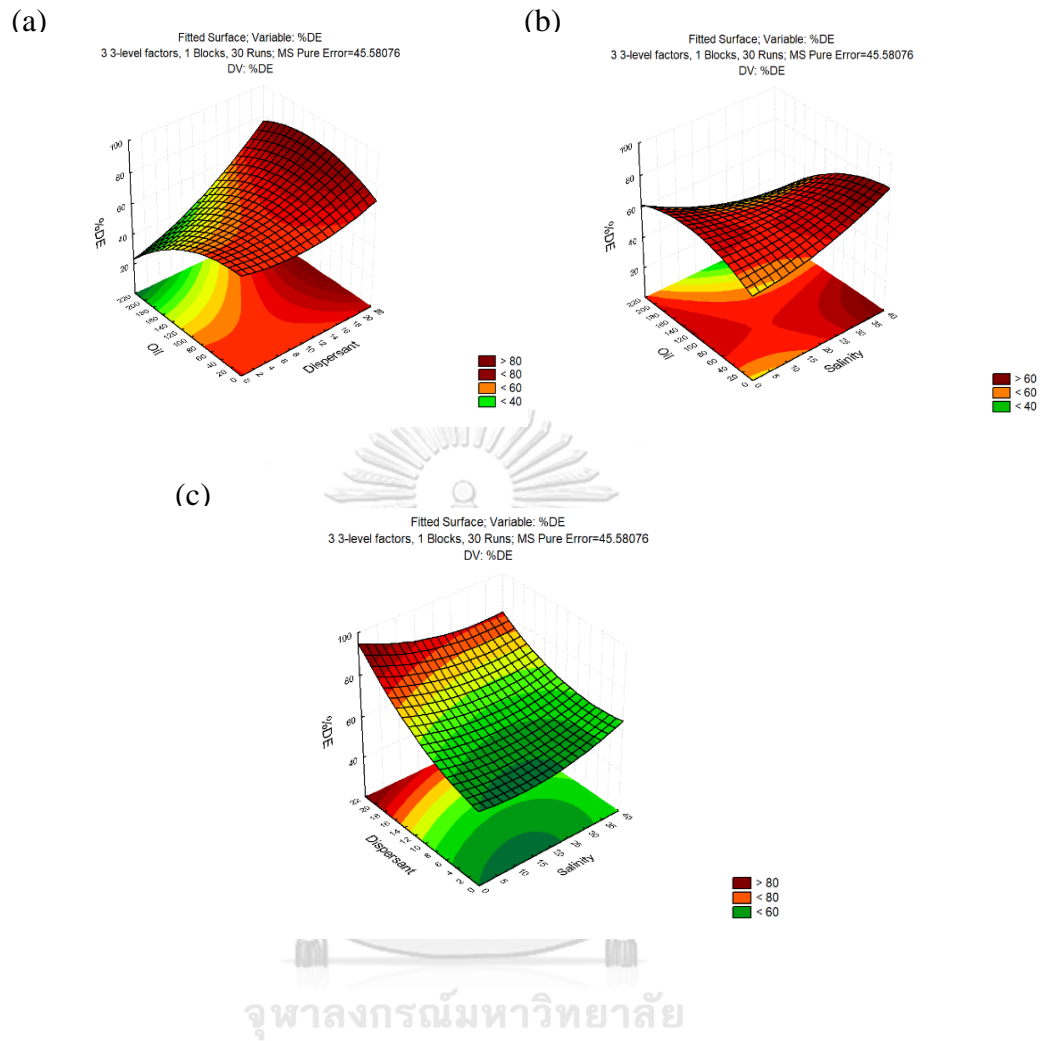
Figure 4.8 Surface response profiles of correlation between influenced factors to
dispersion effectiveness

(a) dispersant-oil, (b) oil-salinity and (c) dispersant-salinity

When considered the dispersion effectiveness of mixture formulation in different salinity conditions, surface response profiles were plot and the results showed in Figure 4.9. The formulation performed high effectiveness to disperse and break down the fuel C into the small droplets in wide range of salinity conditions. According to the results, the system containing seawater condition (3.4% NaCl) showed high dispersion effectiveness cover the large area of surface contour. This result indicated that our formulation provided dispersion effectiveness more than 80% in wide range of DOR. The effectiveness of formulation obviously dropped when the salinity of system was decreased.
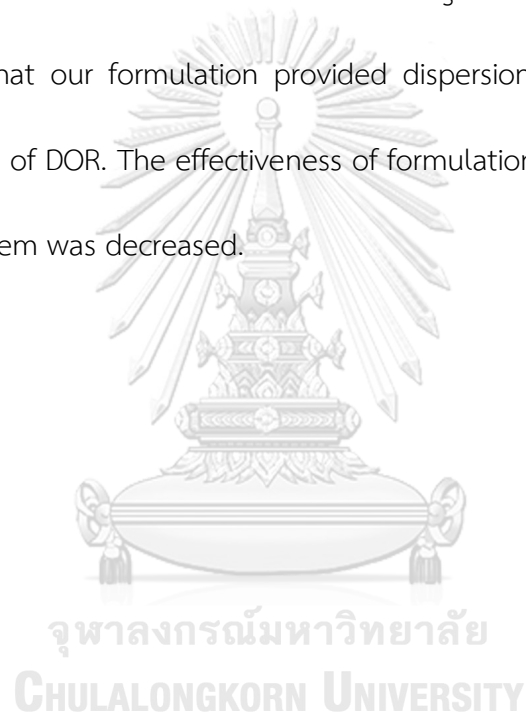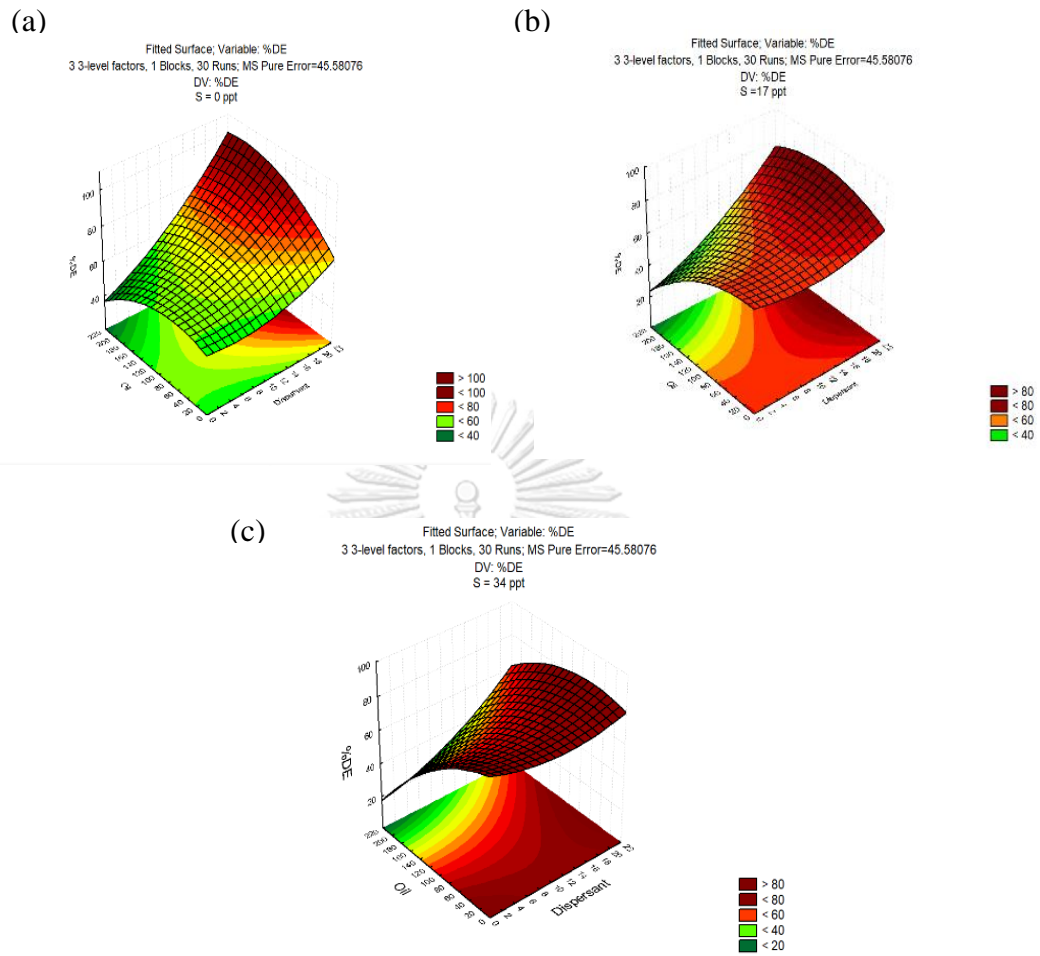
Figure 4.9 Surface response profiles of correlation between influenced factors to
dispersion effectiveness
(a) dispersant-oil, (b) oil-salinity and (c) dispersant-salinity

The correlation of variables was analyzed from the data in regression table (Figure 4.10). Correlation model was exhibited in term of polynomial equation as shown in Eq.10.

$$y = 53.63895 + 0.4203X_1 + 0.01253(X_1)^2 - 0.59013X_2 + 0.05783(X_2)^2 + 0.11918X_3 - 0.00089(X_3)^2 - 0.0255X_1X_2 - 0.00644X_1X_3 + 0.00989X_2X_3 \quad (Eq.\ 10)$$

The variable y in this equation was dispersion effectiveness of formulation. The variable X_1, X_2 and X_3 was salinity, dispersant volume and oil volume, respectively. This equation indicated the correlation between three factors with R^2 was 0.7962, which concluded that the predicted results from equation and experimental results were consistence.

	Regr. Coefficients; Var.:%DE; R-sqr=.79672; Adj:.70525 (optimize.sta) 3 3-level factors, 1 Blocks, 30 Runs; MS Pure Error=45.58076 DV: %DE					
Factor	Regressn Coeff.	Std.Err. Pure Err	t(17)	p	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	53.63895	7.554887	7.09990	0.000002	37.69953	69.57836
(1)Salinity(L)	0.42030	0.392670	1.07038	0.299412	-0.40816	1.24877
Salinity(Q)	0.01253	0.008597	1.45707	0.163322	-0.00561	0.03066
(2)Dispersant(L)	-0.59013	0.816040	-0.72316	0.479415	-2.31182	1.13157
Dispersant(Q)	0.05783	0.030672	1.88537	0.076589	-0.00688	0.12254
(3)Oil (L)	0.11918	0.081604	1.46042	0.162408	-0.05299	0.29135
Oil (Q)	-0.00089	0.000307	-2.90775	0.009800	-0.00154	-0.00024
1L by 2L	-0.02550	0.015601	-1.63464	0.120504	-0.05842	0.00741
1L by 3L	-0.00644	0.001560	-4.12717	0.000704	-0.00973	-0.00315
2L by 3L	0.00989	0.002947	3.35710	0.003739	0.00368	0.01611

Figure 4.10 The analysis of the regression coefficients between influence factors and dispersion effectiveness of mixture formulation from Box-Behnken design.

4.3.2 Investigation of model equation for other oils applications

The model equation (Eq. 10) was evaluated for the precision of the selected

lipopeptide-dehydol LS7TH formulation when applied to various types of petroleum.

The dispersant and oil volume in this experiment were selected from the

experimental condition that performed the highest dispersion effectiveness with fuel

C at the salinity condition (34 ppt) in part 4.3.1. Twenty µL of lipopeptide-dehydol

LS7TH formulation (X_2) and each 110 µL (X_3) of BKC, ARL, fuel A and fuel C (DOR =

1:5), were added into the baffle flasks containing 120 mL of synthetic seawater (X_1).

The dispersion effectiveness (y) from experiment were compared with predicted

results calculated from the response surface equation (Eq.10). The predicted

dispersion effectiveness (y) was calculated by substitute the known variables as

shown in Eq. 11. Finally, the predicted result was expressed as 76.4 %.

$$y = 53.63895 + 0.4203(34) + 0.01253(34)^2 - 0.59013(20) + 0.05783(20)^2$$

$$+0.11918X_3$$

$$- 0.00089(110)^2 - 0.0255(34)(20) - 0.00644(34)(110) + 0.00989(20)(110) \qquad \text{(Eq. 11)}$$

$$y = 76.3976$$

The results showed that the dispersion effectiveness of experiments was

closes to the predicted results from the equation (Table 4.8). This confirmed that the

lipopeptide-dehydol LS7TH formulation could provide the high effectiveness with

various types of crude oil including light and heavy crude oil at the DOR was 1:5

under seawater condition. Moreover, this correlate equation was also useful for predicting the required dispersant volume when we know the salinity condition and certain spilled oil volume to achieve the highest dispersion effectiveness for the oil spill treatment.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Table 4.8 Comparison between predicted and experimental values of %dispersion efficectiveness for the selected lipopeptide-dehydol LS7TH formulation

Oil types	Experimental condition			%Dispersion effectiveness (y)	
	Salinity (ppt) (X$_1$)	Dispersant volume (µL) (X$_2$)	Oil volume (µL) (X$_3$)	Predicted results	Experimental results
Bongkot light crude oil (BKC)					61.7
Arabian light/Arab extra blend (ARL)	34	20	110	76.4	82.0
Fuel A					91.7
Fuel C					86.6

4.4 Evaluation of the selected lipopeptide-dehydol LS7TH formulation toxicity

Numerous studies found that biosurfactant and nonionic surfactant had low toxicity and were suitable for applying as oil dispersant. Lechuga *et al.* (2016) studied toxicity of anionic and nonionic surfactants and found that the toxicity of nonionic surfactant increased with increasing alkyl chain length and decreased with increasing EO groups (Lechuga *et al.*, 2016). For lipopeptide, this biosurfactant was classified as

non-toxic to microorganisms and animals. Sahnoun *et al*. (2014) found that lipopeptide from *Bacillus subtilis* SPB1 had low toxicity with male mice (Sahnoun *et al.,* 2014). Rongsayamanot *et al*. (2017) also found that lipopeptide from *Bacillus subtilis* GY19 itself had low toxicity to both of copepod and whiteleg shrimp.

To confirm the low toxicity of the selected lipopeptide-dehydol LS7TH formulation, phytotoxicity and bacterial toxicity tests were preliminary carried out.

4.4.1 Phytotoxicity

For phytotoxicity test, the selected mixture formulation was investigated with green bean (*Vigna radiata* (L.) Wilczek) and duckweed (*Spirodela polyrhiza* (L.) Schleid). The experiment was divided into 2 set in order to study seed germination and the tolerance of aquatic plant. For green bean toxicity, the dispersant-testing solutions including lipopeptide-dehydol LS7 TH, slickgone NS and superdispersant-25 were prepared by adding 20 µL of testing samples into 120 mL of water. This condition was followed the highest volume of dispersant solution calculated from part 4.3.1. This ratio would represent the highest concentration of dispersant in the experimental condition. Moreover, this volume could cover the 1:1, 1:5 and 1:100 of dispersant to oil ratio in the seawater system for the real site applications. Hence, 10 seeds of green bean were sprayed the prepared formulation and cultivated in the dark place. Then, observed the seed germination daily for 3 days. The results were shown in Table 4.8 that green bean seeds could 100% germinate in all conditions

(Figure 4.11). When consider the length from trunk to root, the percentage of elongation was slightly less than the control at 97.5%, 89.6% and 92.7% for lipopeptide-dehhydol LS7TH formulation, slickgone NS and superdispersant-25, respectively. The trunk elongation of green bean at day 3 was shown in Figure 4.12.

Table 4.9 The percentage of seed germination and trunk elongation of green bean after sprayed with the dispersant-testing solution and cultivated for 3 days

Testing solutions	% Seed germination	%Trunk elongation
Lipopeptide-dehydol LS7T formulation	100	97.5
Slickgone NS	100	89.6
Superdispersant-25	92	92.7
Control (no dispersant)	100	100
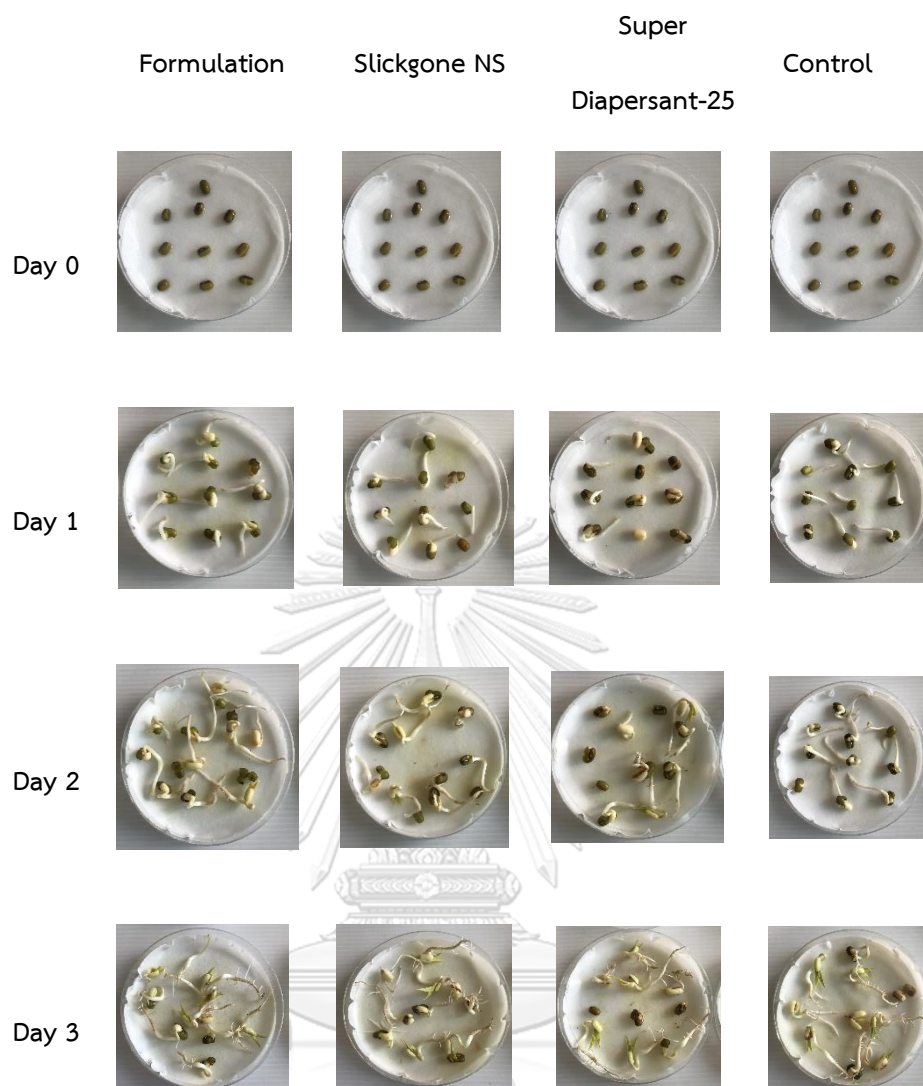
Figure 4.11 Seed germination of green bean at day 0, 1, 2 and 3 after sprayed the selected lipopetide-dehydol LS7TH formulation

compared with slickgone NS, superdispersant-25 and control (no dispersant)

Figure 4.12 Elongation of trunk to root of green bean at day 3 after sprayed the
selected lipopeptide-dehydol LS7TH formulation
compared with slickgone NS, superdispersant-25 and control (no dispersant)

To evaluate the phytotoxicity in aquatic condition, the experiment was preliminary examined the toxicity with duckweed (*Spirodela polyrhiza* (L.) Schleid) because it was generally found in the natural water and rapidly grow in short period. Duckweeds are the small floating plant and use as a representative of aquatic macrophyte for assessing the environmental safety of chemicals

(Wan, 1990, P. Ziegler, 2016). In this experiment, ten duckweed colonies were cultivated in sterile natural water collected from the natural source to maintain the nutrients in the system. The testing solutions were added in to the water with the different volume (2, 11 and 20 µL for dispersant formulation and 20 µL for commercial dispersants). This ratio was representing the low, medium and high concentrations of lipopeptide-dehydol LS7TH formulation in experimental conditions followed part 4.3.1. For slickgone NS and superdispersant-25, the highest volume (20 µL) were added due to less toxic to the duckweed root. Moreover, this volume could cover the high to low DOR in the seawater system, which might be applied for the real site applications.

Afterward, the changing of morphology and root length was observed after 5-day cultivation. The root elongation results of duckweed were shown in Table 4.10. From the results, it could be concluded that the selected dispersant formulation was non-toxic to the plant seeds and aquatic plants and also promoted plant growth. Consequently, the selected lipopeptide-dehydol LS7TH formulation would be possible to apply in the environment.

Table 4.10 The changing of duckweed morphology after cultivated in testing

solution for 5 days

Testing solutions	% Root elongation	Root germination	Leaf germination
2 µL of lipopeptide-dehydol LS7TH formulation	100	+	+
11 µL of lipopeptide-dehydol LS7TH formulation	89.3	+	+
20 µL of lipopeptide-dehydol LS7TH formulation	83.1	+	+
20 µL Slickgone NS	100	+	+
20 µL Superdispersant-25	100	+	+
Control (no added dispersant)	100	+	+

Remark:

Dispersant formulation = Lipopeptide-dehydol LS7TH formulation

Positive germination (+) = the regeneration of duckweed root or leaf could be observed.

Negative germination (-) = no regeneration of duckweed root or leaf could be observed.
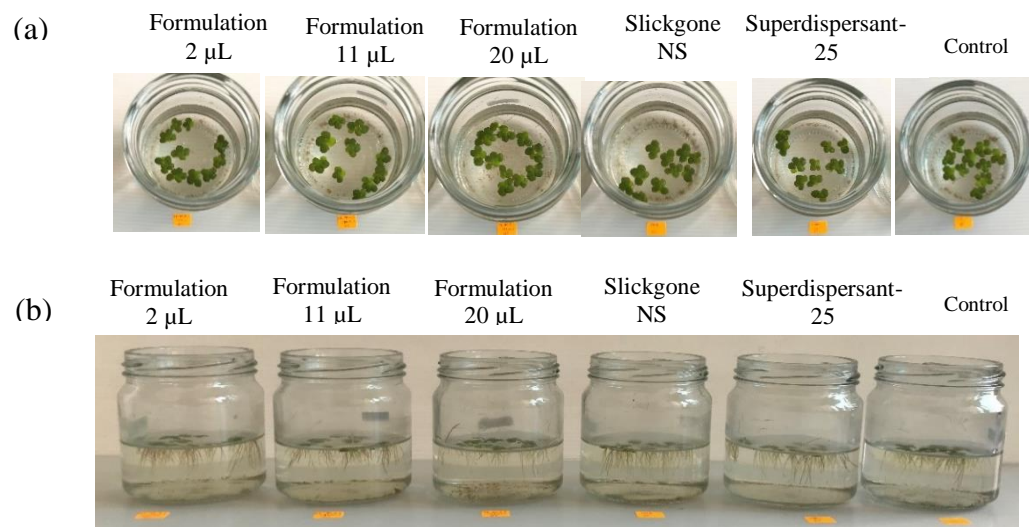
Figure 4.13 The characteristic of duckweed after cultivated in the testing solution for

5 days compared with control (a) leaf characteristic and (b) root length

4.4.2 Minimum bactericidal concentration (MBC)

Toxicity of chemical dispersants had been interested in nowadays. Numerous

researches discovered that the toxicity generally caused by the surfactant ingredient

and also the chemical solvents in dispersant. The surfactants can be persisted in the

environment and accumulate the toxic or harmful substance for long period The

toxicity of dispersant to microorganisms had been studied. For example, Corexit

9500, a crude oil dispersant, exhibited negative impact to energy transformation and

change the structure of marine zooplanktons and also inhibited the growth of

hydrocarbon degrading bacterial (Almeda *et al.*, 2014, Kleindienst *et al.*, 2015)

Lipopeptide biosurfactant, a well-known surfactin produced from *Bacillus subtilis,*

also has toxicity in itself because it has antimicrobial properties that useful for biocide (Couto *et al.*, 2016). Therefore, toxicity of the selected lipopeptide-dehydol LS7TH formulation to bacterial strains were investigated in this study and compared with slickgone NS and superdispersant-25.

Minimum bactericidal concentration (MBC) was applied in the experiment and identified the least concentration of antimicrobial agent required to kill microorganisms (Owuama, 2017). There were two types of petroleum-degrading bacteria, *Microbacterium saccharophilum* RK15 and *Gordonia amicalis* JC11. The concentration of testing solutions was diluted from 100% to 0.05% for each dispersant solution.

After incubated the bacterial strain for 72 h, the results indicated that the selected lipopeptide-dehydol LS7TH formulation did not inhibited all of petroleum-degrading bacteria and had less toxicity than slickgone NS and superdispersant-25. Each dispersant inhibited each bacterium at different concentrations as shown in Table 4.11. The selected lipopeptide-dehydol LS7TH formulation did not restrain RK15 growth at the volume of dispersant lower than 50% compared to slickgone and superdispersant, which inhibited RK15 when the volume of dispersant was higher than 1.6%. For JC11, the formulation began to inhibit bacterial growth at volume of formulation was more than 1.6%, less toxic than two commercial dispersants.

Table 4.11 Minimum bactericidal concentration (MBC) of dispersants on two types of petroleum-degrading bacteria.

Bacterial strains	Minimum bactericidal concentration (MBC)		
	Dispersant formulation	Slickgone NS	Superdispersant-25
Microbacterium saccharophilum RK15	> 50	> 1.6	> 1.6
Gordonia amicalis JC11	> 1.6	> 0.8	> 0.8

Remark:

Dispersant formulation = Lipopeptide-dehydol LS7TH formulation

From the results, it was concluded that lipopeptide-dehydol LS7TH formulation in this study had low toxicity due to no chemical solvents in the formulation. Meanwhile, slickgone and superdispersant contained kerosene and 2-butoxyethanol, respectively. Therefore, this dispersant formulation could be applied for oil spill remediation efficiently.

4.5 Dispersant formulation cost

The selected lipopeptide-dehydol LS7TH formulation (E8_ion_0.3) was examined for the cost of production by considering the cost of surfactant and other compositions in the formulation and showed in Table 4.12.

Table 4.12 Production cost of the selected lipopeptide-dehydol LS7TH at 1 L

production

.Dispersant	Composition	Volume (mL)	Cost (Baht/g)	Total cost (baht)	Formulation cost (baht/L)
Lipopeptide-dehydol LS7TH formulation in this study	Lipopeptides	66	1.5017[a]	99.11	
	Dehydol LS7TH	119	0.13[a]	15.47	124.65
	NaCl	34	0.00225[a]	0.07	
	Water	1000	0.0105	10	
Bio-based dispersant for fuel oil from previous study (Rongsayamanont et al., 2017)	Lipopeptides	123	1.5017	184.71	
	Dehydol LS7TH	92	0.13	11.96	206.74
	NaCl	34	0.00225	0.07	
	Water	1000	0.0105	10	
Slickgone NS					299[b]
Superdispersant-25					318.4[c]

Remark:

[a] Data from PTT report, 2017

[b] Data from Laem Chabang Port (2016)

[c] Data from SERPRO Spill management company

1. Lipopeptide-dehydol LS7TH formulation total concentration 0.3 M in this study consisted of lipopeptide 6.6% w/v, dehydol LS7TH 11.9% w/v and NaCl 3.4% w/v

2. The bio-based dispersant total concentration 0.3 M from previous study consisted of Lipopeptide 12.3 % w/v dehydol LS7TH 9.2 %w/v and NaCl 3.4 %w/v

The lipopeptide-dehydol LS7TH formulation was approximately 1.7 times cheaper than bio-based dispersant (lipopeptide and dehydol LS7TH mixture) from the previous study of Bioremediation Research Unit, Department of Microbiology, Faculty of Science, Chulalongkorn University (2017). In addition, this formulation also 2.6 times cheaper than two types of commercial dispersant, slickgone NS and superdispersant-25 (Table 4.9). The selected formulation was considered more environmentally friendly due to the absent of chemical solvent. Therefore, the dispersant formulation in this study was useful for reducing the remediation cost when the oil spilled occur and could be used as an environmentally friendly product.
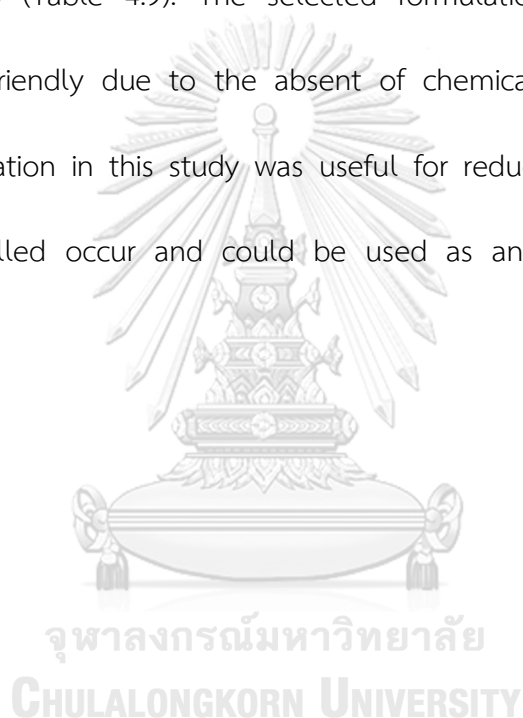
CHAPTER V

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

Since the oil spill has been concerned as a crucial environmental issue, the dispersants are widely used and play an important role for oil spill remediation. The conventional dispersants are mostly consisted of surfactants and harmful solvents, thereby toxic to the marine lives. The objectives of this study was to formulate the oil dispersant by mixing lipopeptide biosurfactant, a hydrophobic anionic surfactant, with low toxic nonionic surfactant, fatty alcohol ethoxylate (dehydol LS7TH). To reduce time and cost in formulation process, HLD concept was used to calculate the proportions of each surfactant based on EACN of hydrocarbons and seawater condition. Two types of HLD equations, ionic and nonionic, were compared for the calculation of surfactant proportions. The fractions of dehydol LS7TH from both equations were higher than lipopeptide fractions due to the balancing of hydrophobic-hydrophilic in the surfactant mixed system. Furthermore, the fractions of surfactants were increased with the higher EACN values because the increasing hydrophobicity of oil required more hydrophobicity portion of lipopeptide to reach the balanced system. In the nonionic HLD system, the salting-out effect was directly influenced to the protein structure of lipopeptide, thereby more amount of

lipopeptide was required when calculated in mass ratio. The phenomenon was opposite to the ionic HLD system, in which the required amount in mass ratio of lipopeptide was less than dehydol LS7TH due to the effect of charge shielding in the system.

The balancing of mixed surfactant at the equilibrium condition (HLD =0) could be monitored from the microemulsion occurrences, which related to the ultralow interfacial tension between oil and water phases. The results suggested that the lipopeptide-dehydol LS7TH formulations calculated from the ionic equation performed microemulsion type III with hydrocarbons rather than from the nonionic equation. The reason for this phenomenon was probably the synergistic effect between lipopeptide and dehydol LS7TH, which could reduce the repulsion between different molecular structures. Therefore, the mixed micelle could form with low concentration and remain stable to coalescence at equilibrium condition (HLD = 0).

The lipopeptide-dehydol LS7TH formulations with microemulsion type III were selected and applied as dispersants to seawater with petroleum light crude oil and fuel oils. All formulations had ability to disperse and solubilize the oil into seawater. However, the different compositions of oil affected to the dispersion efficiency of each formulation. Uncomplicated structure and low viscosity of light crude oil could be easily dispersed and solubilized more than fuel oils. The best

lipopeptide-dehydol LS7TH formulation contained 6.6% lipopeptide, 11.9% dehydol LS7 and 3.4% NaCl, which exhibited high dispersion effectiveness with both light crude oil and fuel oils under seawater condition. From these results, this study recommended to use HLD equation for ionic surfactant to formulate the lipopeptide-dehydol LS7TH dispersant.

To apply the lipopeptide-dehydol LS7TH formulation in the real oil spill cases, response surface methodology (RSM) analysis was used to identify the factors influencing the dispersion efficiency and to reveal the correlation as a model equation. The volume of dispersant and oil (DOR) was the main factors that impacted the dispersion efficiency. In addition, the different salinity conditions also correlated with the volume of dispersant. This results confirmed that lipopeptide-dehydol LS7TH formulation was not only exhibited high dispersion efficiency with various types of oil, but it could be applied in various environment such as freshwater, brackish water and seawater.

The lipopeptide-dehydol LS7TH formulation was non-toxic to plant seed germination and also enhanced plant growth when compared with commercial dispersants and control (no added dispersant). In addition, the dispersant formulation was obviously less toxic to petroleum- degrading bacteria than commercial dispersants. It could be concluded that lipopeptide-dehydol LS7TH formulation from

this study was environmentally friendly and had high efficiency to apply as oil dispersant for oil spill remediation.

5.2 Recommendations for future work

Formulation of lipopetide-dehydol LS7TH dispersant by HLD concept showed high dispersion efficiency with petroleum oils and could be used as environmentally friendly dispersant. To improve the formulations and further studies, the recommendations were listed as follow:

1. Due to the high hydrophobic property of lipopeptide biosurfactant, more hydrophilic nonionic surfactants such as dehydol LS9 and dehydol LS12 or biosurfactants from bacterial strains should be investigated as alternatives to formulate the dispersant.

2. The effect of HLD equation types (ionic and nonionic equations) in this study should be confirmed with other surfactants.

3. The EACN of hydrocarbons was an important variable in HLD equation for calculating fraction of each surfactant. Therefore, the EACN of various petroleum oils should be investigated for applying in the equation.

4. The total concentration of mixed surfactant should be lowered to minimize the amount of surfactant in mixed formulation.

5. The toxicity of lipopeptide-dehydol LS7TH formulation should be tested with marine organisms such as seaweed, whiteleg shrimp or mysidacea followed

standard toxicity test (WAF, CWAF) to confirm the low toxicity of dispersant formulation in real natural water.

6. According to the turbidity after dissolving lipopeptide powder, the interfacial tension (IFT) of mixed surfactant system cannot be determined. Thus, the other forms of lipopeptide such as solution or foamate should be applied in order to diminish this problem.

7. Dispersion effectiveness should be confirmed in larger scale experiments such as using real seawater, adding turbulence, sunlight, wave and temperature. In addition, the effect of environmental conditions to dispersion efficiency of the lipopeptide-dehydol LS7TH formulation should be studied.

8. Biodegradation of dispersed oil after adding the lipopeptide-dehydol LS7TH formulation should be evaluated by adding petroleum-degrading bacteria e.g. *Microbacterium saccharophilum* RK15 and *Gordonia amicalis* JC11 into the experimental system. The results will confirm the efficiency of oil spill remediation.

9. Shelf life of the lipopeptide-dehydol LS7TH formulation should be studied in order to evaluate its stability for future usage

REFERENCES

Acosta EJ (2008) The HLD–NAC equation of state for microemulsions formulated with nonionic alcohol ethoxylate and alkylphenol ethoxylate surfactants. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **320**: 193-204.

Adrion AC, Nakamura J, Shea D and Aitken MD (2016) Screening Nonionic Surfactants for Enhanced Biodegradation of Polycyclic Aromatic Hydrocarbons Remaining in Soil After Conventional Biological Treatment. *Environment Science Technology* **50**: 3838-3845.

Al-Majed AA, Adebayo AR and Hossain ME (2012) A sustainable approach to controlling oil spills. *J Environ Manage* **113**: 213-227.

Al-Sabagh AM, El-Hamouly SH, Atta AM, El-Din MRN and Gabr MM (2007) Synthesis of Some Oil Spill Dispersants Based on Sorbitol Esters and Their Capability to Disperse Crude Oil on Seawater to Alleviate Its Accumulation and Environmental Impact. *Journal of Dispersion Science and Technology* **28**: 661-670.

Almeda R, Hyatt C and Buskey EJ (2014) Toxicity of dispersant Corexit 9500A and crude oil to marine microzooplankton. *Ecotoxicol Environ Saf* **106**: 76-85.

Álvarez-Muñoz D, Llorca M, Blasco J and Barceló D (2016) Contaminants in the Marine Environment. *Marine Ecotoxicology,*p.^pp. 1-34.

Arpornpong N, Charoensaeng A, Khaodhiar S and Sabatini DA (2018) Formulation of microemulsion-based washing agent for oil recovery from spent bleaching earth-hydrophilic lipophilic deviation concept. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **541**: 87-96.

Athas JC, Jun K, McCafferty C, Owoseni O, John VT and Raghavan SR (2014) An effective dispersant for oil spills based on food-grade amphiphiles. *Langmuir* **30**: 9285-9294.

Bezerra MA, Santelli RE, Oliveira EP, Villar LS and Escaleira LA (2008) Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta* **76**: 965-977.

Bezza FA and Chirwa EMN (2015) Production and applications of lipopeptide biosurfactant for bioremediation and oil recovery by Bacillus subtilis CN2. *Biochemical Engineering Journal* **101**: 168-178.

Brakstad OG, Ribicic D, Winkler A and Netzer R (2018) Biodegradation of dispersed oil in seawater is not inhibited by a commercial oil spill dispersant. *Mar Pollut Bull* **129**: 555-561.

Budhathoki M, Hsu T-P, Lohateeraparp P, Roberts BL, Shiau B-J and Harwell JH (2016) Design of an optimal middle phase microemulsion for ultra high saline brine using hydrophilic lipophilic deviation (HLD) method. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **488**: 36-45.

Chandankere R, Yao J, Choi MMF, Masakorala K and Chan Y (2013) An efficient biosurfactant-producing and crude-oil emulsifying bacterium Bacillus methylotrophicus USTBa isolated from petroleum reservoir. *Biochemical Engineering Journal* **74**: 46-53.

Chen W-C, Juang R-S and Wei Y-H (2015) Applications of a lipopeptide biosurfactant, surfactin, produced by microorganisms. *Biochemical Engineering Journal* **103**: 158-169.

Cheng M, Zeng G, Huang D, Yang C, Lai C, Zhang C and Liu Y (2017) Advantages and challenges of Tween 80 surfactant-enhanced technologies for the remediation of soils contaminated with hydrophobic organic compounds. *Chemical Engineering Journal* **314**: 98-113.

Couto CR, Jurelevicius DA, Alvarez VM, van Elsas JD and Seldin L (2016) Response of the bacterial community in oil-contaminated marine water to the addition of

chemical and biological dispersants. *J Environ Manage* **184**: 473-479.

de Cassia FSSR, Almeida DG, Rufino RD, Luna JM, Santos VA and Sarubbo LA (2014) Applications of biosurfactants in the petroleum industry and the remediation of oil spills. *International Journal of Molecular Sciences* **15**: 12523-12542.

Dept E (2009) A Detailed Guide on the Many Different Types of Crude Oil. p.^pp.

Do LD, Attaphong C, Scamehorn JF and Sabatini DA (2014) Detergency of Vegetable Oils and Semi-Solid Fats Using Microemulsion Mixtures of Anionic Extended Surfactants: The HLD Concept and Cold Water Applications. *Journal of Surfactants and Detergents* **18**: 373-382.

Ferreira SL, Bruns RE, Ferreira HS*, et al.* (2007) Box-Behnken design: an alternative for the optimization of analytical methods. *Anal Chim Acta* **597**: 179-186.

Fooladi T, Moazami N, Abdeshahian P, Kadier A, Ghojavand H, Wan Yusoff WM and Hamid AA (2016) Characterization, production and optimization of lipopeptide biosurfactant by new strain Bacillus pumilus 2IR isolated from an Iranian oil field. *Journal of Petroleum Science and Engineering* **145**: 510-519.

Freitas BG, Brito JG, Brasileiro PP, Rufino RD, Luna JM, Santos VA and Sarubbo LA (2016) Formulation of a Commercial Biosurfactant for Application as a Dispersant of Petroleum and By-Products Spilled in Oceans. *Frontiers in Microbiology* **7**: 1646.

Gaurav Dwivedi and Sharma MP (2015) Application of Box–Behnken design in optimization of biodiesel yield

from Pongamia oil and its stability analysis. *Fuel* **145**: 256–262.

Grote M, van Bernem C, Bohme B*, et al.* (2018) The potential for dispersant use as a maritime oil spill response measure in German waters. *Marine Pollution Bulletin* **129**: 623-632.

Hirsch M (2015) Surface Active Agents (Surfactants). p.^pp.

Holland PM and Rubingh DN (1992) Mixed Surfactant Systems. *Mixed Surfactant Systems,*p.^pp. 2-30.

Jin J, Wang H, Jing Y, Liu M, Wang D, Li Y and Bao M (2019) An efficient and environmental-friendly dispersant based on the synergy of amphiphilic surfactants for oil spill remediation. *Chemosphere* **215**: 241-247.

Jin L, Budhathoki M, Jamili A, Li Z, Luo H, Ben Shiau BJ, Delshad M and Harwell JH (2017) Predicting microemulsion phase behavior using physics based HLD-NAC equation of state for surfactant flooding. *Journal of Petroleum Science and Engineering* **151**: 213-223.

Karlapudi AP, Venkateswarulu TC, Tammineedi J, Kanumuri L, Ravuru BK, Dirisala Vr and Kodali VP (2018) Role of biosurfactants in bioremediation of oil pollution-a review. *Petroleum*.

Khondee N, Tathong S, Pinyakong O, Müller R, Soonglerdsongpha S, Ruangchainikom C, Tongcumpou C and Luepromchai E (2015) Lipopeptide biosurfactant production by chitosan-immobilized Bacillus sp. GY19 and their recovery by foam fractionation. *Biochemical Engineering Journal* **93**: 47-54.

Kleindienst S, Paul JH and Joye SB (2015) Using dispersants after oil spills: impacts on the composition and activity of microbial communities. *Nature Review Microbiology* **13**: 388-396.

Kryachko Y, Nathoo S, Lai P, Voordouw J, Prenner EJ and Voordouw G (2013) Prospects for using native and recombinant rhamnolipid producers for microbially enhanced oil recovery. *International Biodeterioration & Biodegradation* **81**: 133-140.

Lamichhane S, Bal Krishna KC and Sarukkalige R (2017) Surfactant-enhanced remediation of polycyclic aromatic hydrocarbons: A review. *Journal of Environmental Management* **199**: 46-61.

Li G, Guo S and Hu J (2016) The influence of clay minerals and surfactants on hydrocarbon removal during the washing of petroleum-contaminated soil. *Chemical Engineering Journal* **286**: 191-197.

Liu B, Liu J, Ju M, Li X and Yu Q (2016) Purification and characterization of biosurfactant produced by Bacillus licheniformis Y-1 and its application in remediation of petroleum contaminated soil. *Marine Pollution Bulletin* **107**: 46-51.

Liu Q, Lin J, Wang W, Huang H and Li S (2015) Production of surfactin isoforms by Bacillus subtilis BS-37 and its applicability to enhanced oil recovery under laboratory conditions. *Biochemical Engineering Journal* **93**: 31-37.

Liu Z, Li Z, Zhong H, Zeng G, Liang Y, Chen M, Wu Z, Zhou Y, Yu M and Shao B (2017) Recent advances in the environmental applications of biosurfactant saponins: A review. *Journal of Environmental Chemical Engineering* **5**: 6030-6038.

Luna JM, Rufino RD, Sarubbo LA and Campos-Takaki GM (2013) Characterisation, surface properties and biological activity of a biosurfactant produced from industrial waste by Candida sphaerica UCP0995 for application in the petroleum industry. *Colloids Surf B Biointerfaces* **102**: 202-209.

Mani P, Sivakumar P and Balan SS (2016) Economic Production and Oil Recovery Efficiency of a Lipopeptide Biosurfactant from a Novel Marine Bacterium Bacillus simplex. *Achievements in the Life Sciences* **10**: 102-110.

Mao X, Jiang R, Xiao W and Yu J (2015) Use of surfactants for the remediation of contaminated soils: a review. *Journal of Hazardous Materials* **285**: 419-435.

Mapelli F, Scoma A, Michoud G, Aulenta F, Boon N, Borin S, Kalogerakis N and Daffonchio D (2017) Biotechnologies for Marine Oil Spill Cleanup: Indissoluble Ties with Microorganisms. *Trends in Biotechnology* **35**: 860-870.

Mosaed HP, Sobhanardakani S, Merrikhpour H, Farmany A, Cheraghi M and Ashorlo S

(2015) The Effect of Urban Fuel Stations on Soil Contamination with Petroleum Hydrocarbons *Iranian Journal of Toxicology* **9**.

Mulligan CN (2005) Environmental applications for biosurfactants. *Environmental Pollution* **133**: 183-198.

Nardello Vr, Chailloux N, Poprawski Jrm, Salager J-L and Aubry J-M (2003) HLD concept as a tool for the characterization of cosmetic hydrocarbon oils. *Polymer International* **52**: 602-609.

Ni H, Zhou W and Zhu L (2014) Enhancing plant-microbe associated bioremediation of phenanthrene and pyrene contaminated soil by SDBS-Tween 80 mixed surfactants. *Journal of Environmental Sciences* **26**: 1071-1079.

Nikolopoulou M, Pasadakis N and Kalogerakis N (2013) Evaluation of autochthonous bioaugmentation and biostimulation during microcosm-simulated oil spills. *Marine Pollution Bulletin* **72**: 165-173.

Owuama CI (2017) Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method *African Journal of Microbiology Research* **11**: 977-980.

P. Ziegler KSSK-JA (2016) Duckweeds for water remediation and toxicity. *Toxicological & Environmental Chemistr* **98**: 1127-1154.

Pacwa-Plociniczak M, Plaza GA, Piotrowska-Seget Z and Cameotra SS (2011) Environmental applications of biosurfactants: recent advances. *International Journal of Molecular Sciences* **12**: 633-654.

Pi Y, Bao M, Liu Y, Lu T and He R (2017) The contribution of chemical dispersants and biosurfactants on crude oil biodegradation by Pseudomonas sp. LSH-7′. *Journal of Cleaner Production* **153**: 74-82.

Rashmi Kumari AK, R. Nagarajan & Jitendra S. Sangwai (2018) Synergistic effect of mixed

anionic and cationic surfactant systems on the interfacial tension of crude oil-water and enhanced oil recovery. *Journal of Dispersion Science and Technology*.

Riehm DA, Rokke DJ, Paul PG, Lee HS, Vizanko BS and McCormick AV (2017) Dispersion of oil into water using lecithin-Tween 80 blends: The role of spontaneous emulsification. *Journal of Colloid and Interface Science* **487**: 52-59.

Rocchio J, Neilsen J, Everett K and Bothun GD (2017) A solvent-free lecithin-Tween 80 system for oil dispersion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **533**: 218-223.

Rongsayamanont W, Soonglerdsongpha S, Khondee N, Pinyakong O, Tongcumpou C, Sabatini DA and Luepromchai E (2017) Formulation of crude oil spill dispersants based on the HLD concept and using a lipopeptide biosurfactant. *Journal of Hazardous Materials* **334**: 168-177.

S.J G, Banat IM and Joshi SJ (2018) Biosurfactants: Production and potential applications in microbial enhanced oil recovery (MEOR). *Biocatalysis and Agricultural Biotechnology* **14**: 23-32.

Salager J-L (2002) SURFACTANTS Types and Uses. p.^pp. FIRP BOOKLET # E300-A, Mérida-Venezuela.

Sales PS and Fernandez MA (2016) Synergism in the desorption of polycyclic aromatic hydrocarbons from soil models by mixed surfactant solutions. *Environ Sci Pollut Res Int* **23**: 10158-10164.

Sales PS, de Rossi RH and Fernandez MA (2011) Different behaviours in the solubilization of polycyclic aromatic hydrocarbons in water induced by mixed surfactant solutions. *Chemosphere* **84**: 1700-1707.

Shafira Adlina EH, Mohamad Yani (2017) Oil Spill Dispersant Formulation from Diethanolamine (DEA) and Methyl Ester Sulfonate (MES) for Bioremediation

Process. *Chemical and Process Engineering Research* **53**: 1-12.

Shi Z, Chen J, Liu J, Wang N, Sun Z and Wang X (2015) Anionic-nonionic mixed-surfactant-enhanced remediation of PAH-contaminated soil. *Environ Sci Pollut Res Int* **22**: 12769-12774.

Song D (2013) Development of High Efficient and Low Toxic Oil Spill Dispersants based on Sorbitol Derivants Nonionic Surfactants and Glycolipid Biosurfactants. *Journal of Environmental Protection* **04**: 16-22.

Srinivasan QL, 1 George A. Sorial,1* Albert D. Venosa,2 and Joseph Mullin3 (2007) Dispersant Effectiveness of Heavy Fuel Oils Using the Baffled Flask Test. *ENVIRONMENTAL ENGINEERING SCIENCE* **24**: 1307-1320.

Vecino X, Barbosa-Pereira L, Devesa-Rey R, Cruz JM and Moldes AB (2015) Optimization of extraction conditions and fatty acid characterization of Lactobacillus pentosus cell-bound biosurfactant/bioemulsifier. *J Sci Food Agric* **95**: 313-320.

Venosa AD and Holder EL (2013) Determining the dispersibility of South Louisiana crude oil by eight oil dispersant products listed on the NCP Product Schedule. *Mar Pollut Bull* **66**: 73-77.

Wan W (1990) Literature Review on Duckweed Toxicity Testing *ENVIRONMENTAL RESEARCH* **52**: 7-22.

Wingfield PT (2016) Protein Precipitation Using Ammonium Sulfate. *Curr Protoc Protein Sci* **84**: A 3F 1-9.

Yan Zheng MZ, Zonghui Ma, Benrong Xin, Ruihua Guo, Xuebing Xu (2015) Sugar Fatty Acid Esters. *Polar Lipids* 215–243.

Yanfu Wei a XL, Le Tonga, Chuling Guoa,b,*, Zhi Dang (2015) Enhanced solubilization and desorption of pyrene from soils by saline anionic–nonionic surfactant systems. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*

468: 211–218.

Zhang Y, Zhao Y, Zhu Y, Wu H, Wang H and Lu W (2012) Adsorption of mixed cationic-nonionic surfactant and its effect on bentonite structure. *Journal of Environmental Sciences* **24**: 1525-1532.

Zheng M, Ahuja M, Bhattacharya D, Clement TP, Hayworth JS and Dhanasekaran M (2014) Evaluation of differential cytotoxic effects of the oil spill dispersant Corexit 9500. *Life Sci* **95**: 108-117.

Zhong H, Wang Z, Liu Z, Liu Y, Yu M and Zeng G (2016) Degradation of hexadecane by Pseudomonas aeruginosa with the mediation of surfactants: Relation between hexadecane solubilization and bioavailability. *International Biodeterioration & Biodegradation* **115**: 141-145.
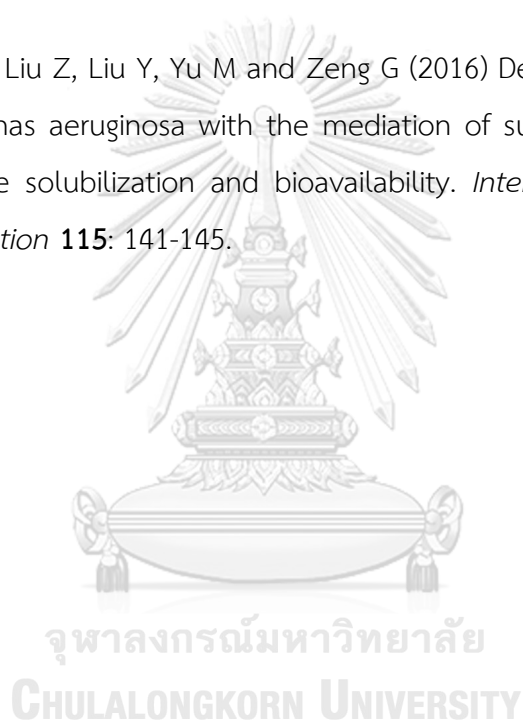
APPENDIX A

MEDIA

Table A.1 LB broth (Luria-Bertani broth) (per 1 Liter)

Ingredient	Amount (g)
Tryptone	10
Yeast Extract	5
Sodium Chloride	10

Suspend/dissolve all in 1 L of purified water, and adjusted pH to 7.0

Table A.2 Marine broth (Difco™ Marine Broth 2216)

Ingredient	Amount (g)	Ingredient	Amount (g)
Peptone	5	Yeast Extract	1
Ferric Citrate	0.1	Sodium Chloride	19.45
Magnesium Chloride	5.9	Magnesium Sulfate	3.24
Calcium Chloride	1.8	Potassium Chloride	0.55
Sodium Bicarbonate	0.16	Potassium Bromide	0.08
Strontium Chloride	0.034	Boric Acid	0.022
Sodium Silicate	0.004	Sodium Fluoride	0.0024
Ammonium Nitrate	0.0016	Disodium Phosphate	0.008

Dissolve the following in 1000 ml of distilled water and adjust pH to 7.6, then, mix thoroughly.

APPENDIX B

SUPPLEMENTARY DATA OF PRELIMINARY RESULTS

Table B.1 Dispersibillity of lipopeptide- dehydol LS5TH (F1) formulation calculated from

EACN = 6 in different total concentration with hexane by Oil displacement test.

Sample	DOR	Diameter (cm)				Average	SD	% dispersibility
		Before	After					
			1	2	3			
	1:2	9	7.4	7.6	7.1	7.4	0.3	82.1
F1 0.1 M	1:5	8.6	7.1	6.9	6.3	6.8	0.4	78.8
	1:10	9	5.3	5.0	5.3	5.2	0.2	57.9
	1:2	9	7.5	7.4	7.9	7.6	0.3	84.3
F1 0.3 M	1:5	8.6	6.8	6.6	6.1	6.5	0.4	75.7
	1:10	9	6.0	5.6	5.7	5.8	0.2	64.1
	1:2	9	7.0	6.9	6.9	7.0	0.0	77.3
F1 0.5 M	1:5	8.6	5.8	6.2	6.7	6.2	0.5	72.2
	1:10	9	4.6	5.2	4.9	4.9	0.3	54.7

Table B.2 Dispersibillity of lipopeptide- dehydol LS7TH (F2) formulation calculated from EACN = 6 in different total concentration with hexane by Oil displacement test.

Sample	DOR	Diameter (cm)				Average	SD	% dispersibility
		Before	After					
			1	2	3			
F2 0.1 M	1:2	9	7.6	8.0	7.4	7.7	0.3	85.1
	1:5	8.6	6.6	6.2	7.0	6.6	0.4	76.6
	1:10	9	4.1	5.1	4.0	4.4	0.6	48.9
F2 0.3 M	1:2	9	7.0	7.9	7.3	7.4	0.4	82.1
	1:5	8.6	6.8	7.0	6.6	6.8	0.2	79.0
	1:10	9	7.0	4.0	5.5	5.5	1.5	61.2
F2 0.5 M	1:2	9	7.1	7.6	7.5	7.4	0.3	82.3
	1:5	8.6	6.9	6.4	6.7	6.6	0.3	77.3
	1:10	9	5.6	3.2	4.5	4.5	1.2	49.5

Table B.3 Dispersibillity of lipopeptide- dehydol LS5TH (F1) formulation calculated from EACN = 10 in different total concentration with decane by Oil displacement test.

Sample	DOR	Diameter (cm)				Average	SD	% dispersibility
		Before	After					
			1	2	3			
	1:2	9	8.9	8.7	8.9	8.8	0.1	98.1
F1 0.1 M	1:5	8.6	8.5	8.5	8.5	8.5	0.0	98.8
	1:10	9	8.9	8.7	8.3	8.6	0.3	95.8
	1:2	8.6	8.5	8.5	8.5	8.5	0.0	98.8
F1 0.3 M	1:5	9	8.3	8.7	8.2	8.4	0.2	93.3
	1:10	8.6	7.9	7.8	7.8	7.8	0.1	91.1
	1:2	9	8.17	8.2	8.9	8.4	0.4	93.6
F1 0.5 M	1:5	8.6	7.57	8.1	8.5	8.0	0.5	93.6
	1:10	9	8.47	8.5	8.3	8.4	0.1	93.5

จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

Table B.4 Dispersibillity of lipopeptide- dehydol LS7TH (F2) formulation calculated from

EACN = 10 in different total concentration with decane by Oil displacement test.

Sample	DOR	Diameter (cm)				Average	SD	% dispersibility
		Before	After					
			1	2	3			
	1:2	9	8.87	8.8	8.8	8.8	0.1	98.0
F2 0.1 M	1:5	8.6	6.77	6.0	6.1	6.3	0.4	73.3
	1:10	9	7.53	8.9	8.9	8.4	0.8	93.8
	1:2	8.6	8.5	8.5	8.5	8.5	0.0	98.8
F2 0.3 M	1:5	9	8.9	8.9	8.9	8.9	0.0	98.9
	1:10	8.6	8.5	8.5	8.5	8.5	0.0	98.8
	1:2	9	8.9	8.9	8.9	8.9	0.0	98.9
F2 0.5 M	1:5	8.6	8.5	8.5	8.5	8.5	0.0	98.8
	1:10	9	8.13	8.1	8.6	8.3	0.3	91.9

จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

Table B.5 Dispersibillity of lipopeptide- dehydol LS5TH (F1) formulation calculated from EACN = 12 in different total concentration with dodecane by Oil displacement test.

| Sample | DOR | Diameter (cm) | | | | Average | SD | % dispersibility |
| | | Before | After | | | | | |
			1	2	3			
F1 0.1 M	1:2	9.0	7.4	6.6	7.3	7.1	0.4	78.7
	1:5	8.6	6.5	6.5	6.6	6.5	0.1	75.7
	1:10	9.0	6.9	6.6	6.8	6.8	0.2	75.3
F1 0.3 M	1:2	8.6	6.9	6.6	6.8	6.8	0.1	78.7
	1:5	9.0	7.0	6.8	7.0	6.9	0.1	77.1
	1:10	8.6	6.6	6.3	6.4	6.4	0.1	74.8
F1 0.5 M	1:2	9.0	7.7	7.9	8.0	7.9	0.2	87.3
	1:5	8.6	7.5	7.0	6.8	7.1	0.4	82.8
	1:10	9.0	7.2	7.2	7.3	7.2	0.0	80.4

จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

Table B.6 Dispersibillity of lipopeptide- dehydol LS7TH (F2) formulation calculated from

EACN = 12 in different total concentration with dodecane by Oil displacement test.

| Sample | DOR | Diameter (cm) | | | | Average | SD | % dispersibility |
| | | Before | After | | | | | |
			1	2	3			
F2 0.1 M	1:2	8.6	6.5	6.8	6.6	6.6	0.1	77.1
	1:5	9.0	6.9	6.7	6.9	6.8	0.1	75.9
	1:10	8.6	6.6	6.2	6.5	6.4	0.2	74.8
F2 0.3 M	1:2	9.0	7.9	7.7	8.1	7.9	0.2	87.9
	1:5	8.6	7.6	7.4	7.5	7.5	0.1	87.1
	1:10	9.0	7.8	8.1	7.7	7.9	0.2	87.5
F2 0.5 M	1:2	8.6	8.2	8.4	8.4	8.3	0.1	96.8
	1:5	9.0	8.3	8.5	8.3	8.4	0.1	92.9
	1:10	8.6	7.8	8.2	8.3	8.1	0.3	94.2

จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

APPENDIX C

SUPPLEMENTARY DATA OF CHAPTER III
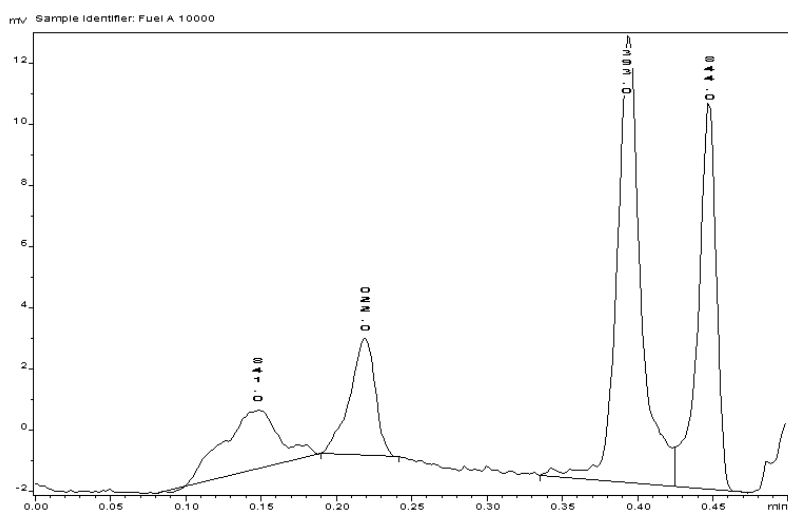
Figure C.1 Chromatogram of fuel A compositions measured by TLC analysis
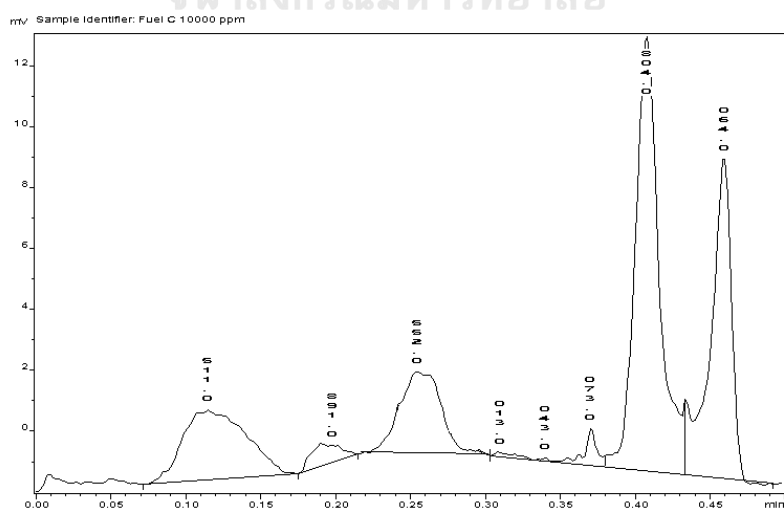
Figure C.2 Chromatogram of fuel C compositions measured by TLC analysis

APPENDIX D

SUPPLEMENTARY DATA OF CHAPTER IV

Dehydol LS7TH

$y = -7.681\ln(x) + 60.693$
$R^2 = 0.9864$

$y = -0.723\ln(x) + 34.453$
$R^2 = 0.8318$

$-7.681\ln(x) + 60.693 = -0.723\ln(x) + 34.453$

$X = 43$ mg/L
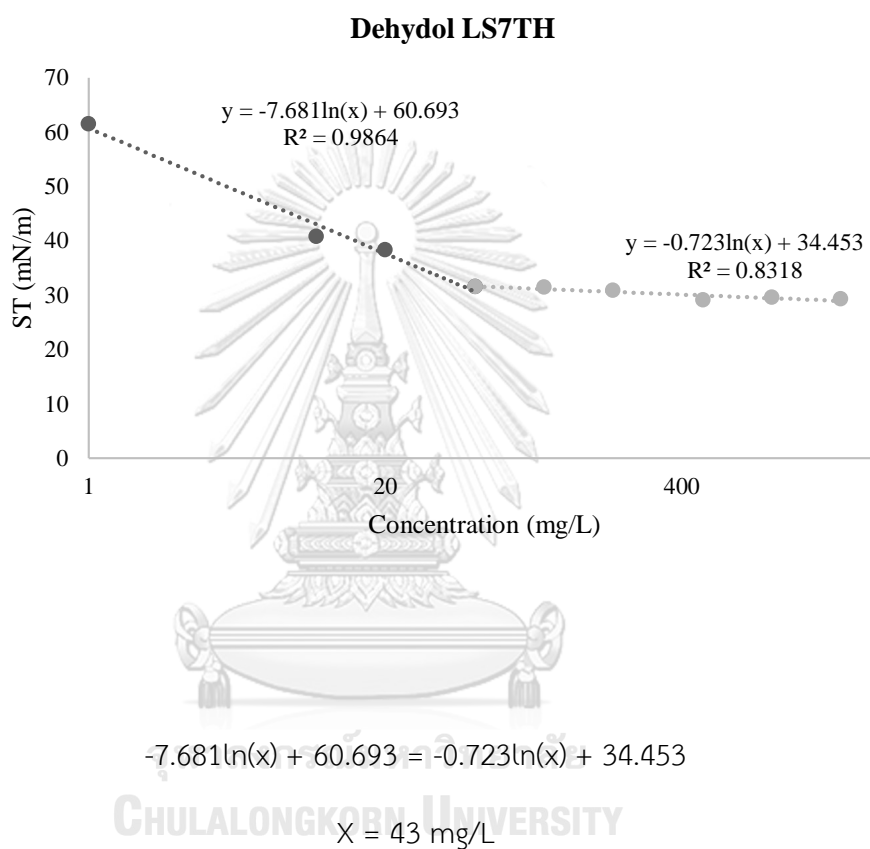
Figure D.1 CMC of dehydol LS7TH measured by tensiometer
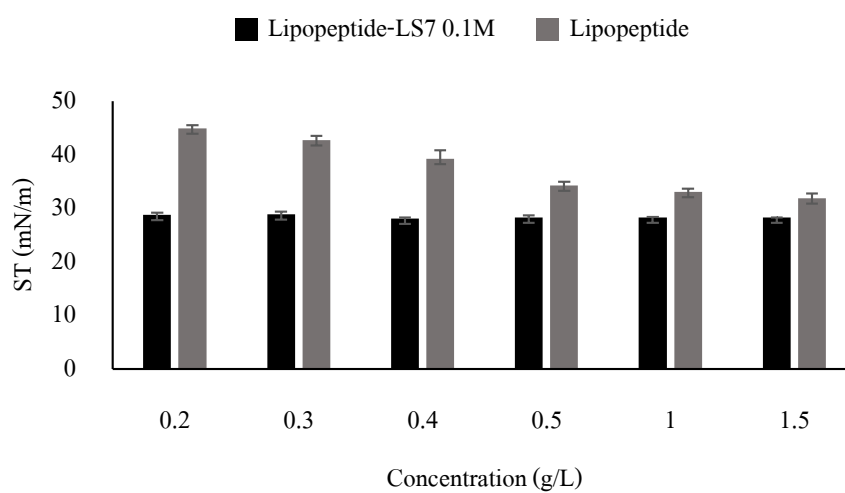
Figure D.2 Surface tension of lipopeptide-dehydol LS7TH formulation total
concentration 0.1 M compared with lipopeptide alone

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

BKC_std

y = 0.2119x + 0.0312
R² = 0.999

y = 0.1183x + 0.0122
R² = 0.9994

y = 0.0779x + 0.0073
R² = 0.999

ARL_std

y = 1.334x + 0.4678
R² = 0.9835

y = 0.7208x + 0.2049
R² = 0.9839

y = 0.4332x + 0.1228
R² = 0.9838
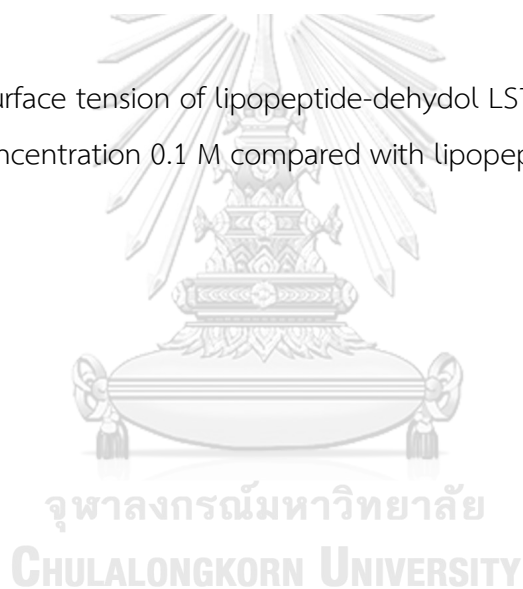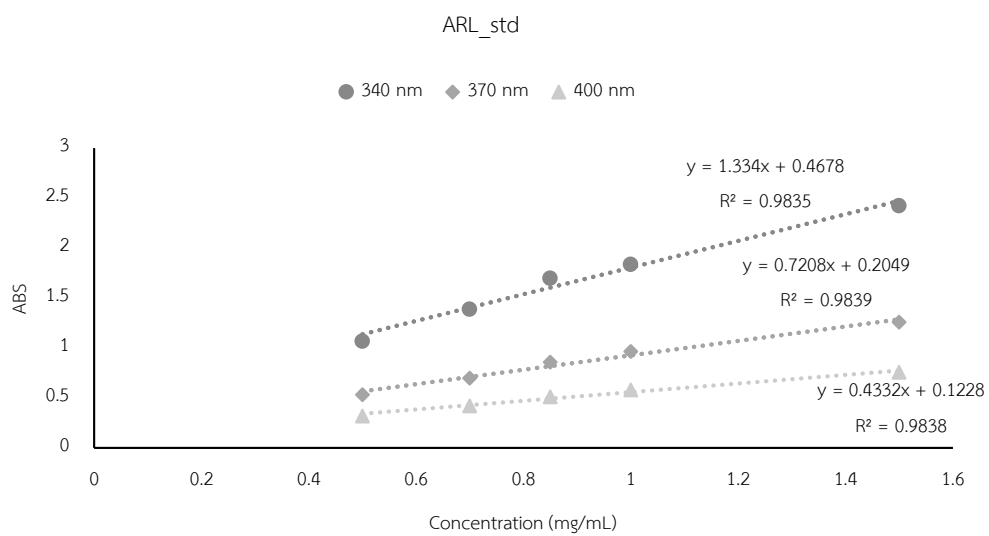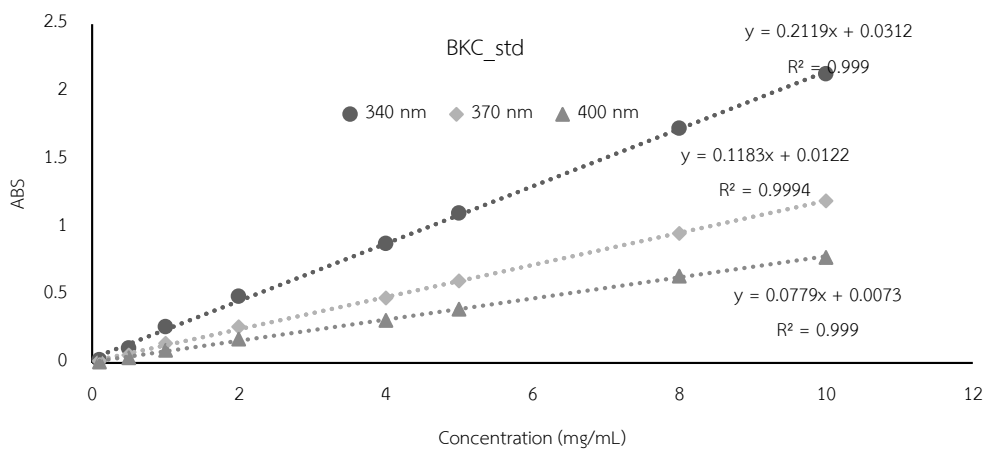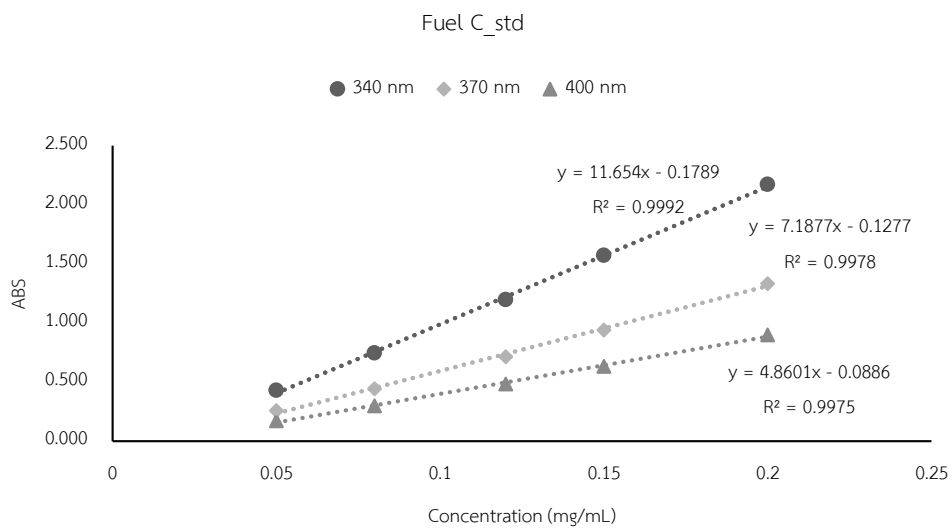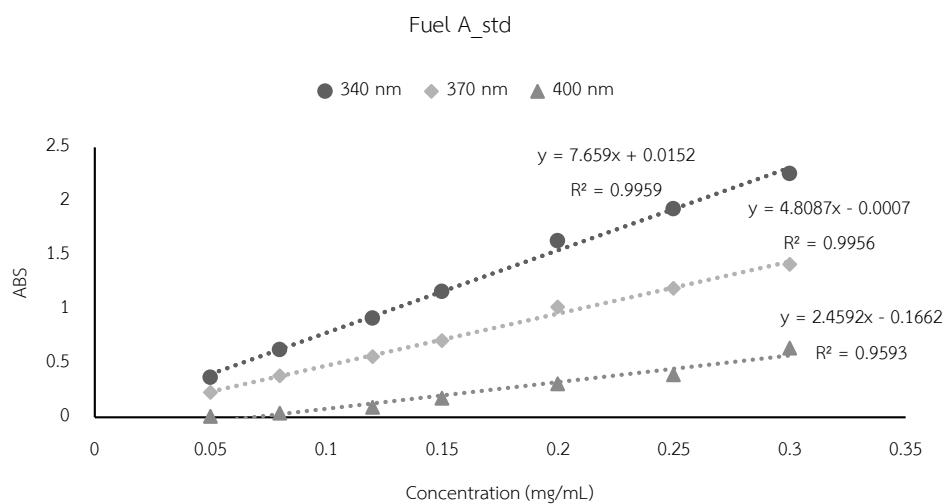
Figure D.3 Standard curves of petroleum oils (a) BKC (b) ARL (c) fuel A and (d) fuel C used in this study at different wavelength (340, 370 and 400 nm) for calculating response factors (Rf) values for baffle flask test.

Table D.1 Response factors (Rf) values at different wavelength of petroleum oils used in this study for calculating oil concentration in baffle flask test follow US. EPA. standard method (40 CFR Appendix C to Part 300).

Petroleum	Rf_x		
	340	370	400
BKC	4.3987	7.9620	12.2360
ARL	0.5271	1.0360	1.7259
Fuel A	0.1290	0.2097	0.3110
Fuel C	0.1040	0.1737	0.2587

The Rf values were calculated from the absorbance of standard oils at three analytical wavelengths i.e. 340, 370 and 400 nm following equation:

$$RF_x = C/A_x$$

where:

RF_x = Response factor at wavelength x (x = 340,370, or 400 nm)

C = Oil concentration, in mg of oil/ml of DCM in standard solution

A_x = Spectrophotometric absorbance of wavelength x

Table D.2 Dispersion effectiveness of lipopeptide-dehydol LS7TH formulations with Bongkot light crude oil (BKC) at seawater condition (Salinity=34 ppt)

Sample	%EFFD	SD
E8_ion_0.1	23.0	3.2
E8_ion_0.3	43.3	8.5
E8_non_0.1	15.3	3.1
E10_ion_0.3	48.9	3.1
E10_non_0.1	57.0	0.9
E12_ion_0.1	24.2	0.9
E12_ion_0.3	45.1	2.0
E12_non_0.1	30.0	2.4
Slickgone NS	41.0	5.8
Superdispersant-25	27.6	3.3
control	5.3	1.8

Remark: Dispersant to oil ratio is 1:25

Table D.3 Dispersion effectiveness of lipopeptide-dehydol LS7TH formulations with fuel A at seawater condition (Salinity=34 ppt)

Sample	%EFFD	SD
E8_ion_0.1	12.6	1.5
E8_ion_0.3	59.7	2.4
E8_non_0.1	13.2	2.0
E10_ion_0.3	46.7	3.4
E10_non_0.1	8.6	2.3
E12_ion_0.1	21.6	0.5
E12_ion_0.3	33.7	1.2
E12_non_0.1	20.5	2.7
Slickgone NS	76.9	0.9
Superdispersant-25	58.7	7.9
control	10.7	0.3

Remark: Dispersant to oil ratio is 1:25

Table D.4 Dispersion effectiveness of lipopeptide-dehydol LS7TH formulations with fuel C at seawater condition (Salinity=34 ppt)

Sample	%EFFD	SD
E8_ion_0.1	13.7	1.6
E8_ion_0.3	70.5	5.2
E8_non_0.1	9.3	0.4
E10_ion_0.3	52.6	6.3
E10_non_0.1	9.3	2.3
E12_ion_0.1	16.5	0.8
E12_ion_0.3	24.8	2.7
E12_non_0.1	9.9	3.3
Slickgone NS	45.2	5.1
Superdispersant-25	57.5	8.0
control	4.8	0.6

Remark: Dispersant to oil ratio is 1:25

The residual petroleum oil concentration was analyzed using UV-visible spectrophotometer at the wavelength 340, 370 and 400 nm and calculate the dispersion efficiency by this equation below (EPA 40 CFR Appendix C to Part 300)

$$\% \ EFF_D = \%EFF_d - \%EFF_c$$

Where

EFF_D % dispersed oil due to dispersant only

EFF_d % dispersed oil with dispersant added

EFF_c % dispersed oil with no dispersant added

$$\text{EFF (\%)} = (C_{mean}/C_{TOT}) \times 100$$

Where

C_{mean}	Mean value for total mass of dispersed oil by spectrophotometric analysis
C_{TOT}	Total mass of oil initially added to the experimental

Table D.5 The amount of seed germination of green bean after sprayed dispersant testing solutions and cultivated in dark place for 3 days.

Dispersant	Day	Replicate			Average	SD
		1	2	3		
Lipopeptide-dehydol LS7TH formulation	1	9	10	10	9.7	0.6
	2	9	10	10	9.7	0.6
	3	10	9	9	9.3	0.6
Slickgone NS	1	10	9	9	9.3	0.6
	2	10	9	9	9.3	0.6
	3	10	8	9	9	1.0
Superdispersant-25	1	7	7	9	7.7	1.2
	2	7	7	9	7.7	1.2
	3	7	7	9	7.7	1.2
Control	1	9	10	10	9.7	0.6
	2	9	9	10	9.3	0.6
	3	8	8	9	8.3	0.6

Remark: Initial seed amount = 10 seeds

Table D.6 Root length of green bean after sprayed dispersant testing solutions in day 3

Dispersant	Replicates	Root length (cm)										Average
		Day 3										
		1	2	3	4	5	6	7	8	9	10	
Lipopeptide-dehydol LS7TH formulation	1	3.5	5.2	4.9	5.1	5.3	4.1	5.7	2.6	4	3.5	4.14
	2	6.1	3.8	4.5	-	4	3.5	2	6.3	4.5	2.4	
	3	6	3.7	4.2	4.3	3	3.8	2.6	3.4	3.2	5	
Slickgone NS	1	4.4	3.2	4.5	3.1	4	4.3	3.1	5.4	4.3	4.9	3.81
	2	5.1	3.8	3.2	3.6	4.1	4.4	4.6	3.8	2.6	-	
	3	4	4	2.9	2.5	5.2	3.3	-	4.2	2.6	1.9	
Superdispersant-25	1	5.6	2.5	3	2.7	1.9	-	-	4.3	4.9	-	3.94
	2	4.8	4.5	5.1	-	-	-	2.8	5	2.7	2.6	
	3	3.3	5.4	4.6	2.2	4.4	6.1	6.5	4.3	2.3	-	
Control	1	4.9	4	4.6	3.9	-	3.5	-	4.5	5	4.6	4.25
	2	3	3.8	2.2	4.2	5.5	5.1	2.5	4.2	5	-	
	3	4.8	3.8	-	5	6	5	3.1	4.1	3.7	-	

จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

Table D.7 Root length of duckweed cultivated in dispersant testing solutions in day 5

Dispersant	Replicates	Root length (cm)										Average
		Day 5										
		1	2	3	4	5	6	7	8	9	10	
2 µL of Lipopeptide-dehydol LS7TH formulation	1	1.5	2	2	2	2	2	2.1	2.2	1.7	1.4	1.77
	2	1.8	1.3	1.7	1.4	1.3	1	1.5	1.6	2	1.1	
	3	2.5	2.5	1.5	2	1.2	2	1.7	2.1	2	2.1	
11 µL of Lipopeptide-dehydol LS7TH formulation	1	2.2	1.6	2	1.5	1.6	2.3	2	1.6	1.5	1.6	1.54
	2	1	1.9	1.8	1.8	1.9	1.5	1.5	1.6	1.5	1.7	
	3	2	2.2	0.6	0.6	1.3	1.3	1.3	0.7	1.3	0.5	
20 µL of Lipopeptide-dehydol LS7TH formulation	1	1.3	1	1.9	1.5	1.8	1.1	1.1	1.4	0.7	1.6	1.43
Slickgone NS	1	2.3	2	2	2	2.1	1.3	1.3	1.7	2	1.5	1.89
Superdispersant-25	1	2.2	2.5	2.1	1.8	1.6	2.2	2.2	1.3	2.1	1.2	1.91
Control	1	1.6	1.8	2.4	1.3	1.3	2	2	1.3	1.6	1.4	1.72

Table D.8 The growth of *Microbacterium saccharophilum* RK15 in different dilution concentrations of the testing solution

Dispersant	Replicates	Bacterial growth											
		Dilution											
		1	2	3	4	5	6	7	8	9	10	11	12
Lipopeptide-dehydol LS7TH formulation	1	No growth	Growth										
	2												
	3												
Slickgone NS	1	No growth								Growth			
	2												
	3												
Superdispersant-25	1	No growth										Growth	
	2												
	3												
Positive control	1	Growth											
	2												
	3												
Negative control	1	No growth											
	2												
	3												

Remark:

Possitive control = only NaCl and inoculated solution, no tested solution

Negative control = only NaCl solution, no tested solution

Table D.9 The growth of *Gordonia amicalis* JC11 in different dilution concentrations of the testing solution

Dispersant	Replicates	Bacterial growth											
		Dilution											
		1	2	3	4	5	6	7	8	9	10	11	12
Lipopeptide-dehydol LS7TH formulation	1	No growth						Growth					
	2												
	3												
Slickgone NS	1	No growth									Growth		
	2												
	3												
Superdispersant-25	1	No growth									Growth		
	2												
	3												
Positive control	1	Growth											
	2												
	3												
Negative control	1	No growth											
	2												
	3												

Remark:

Possitive control = only NaCl and inoculated solution, no tested solution

Negative control = only NaCl solution, no tested solution

VITA

NAME	Parisarin Nawavimarn
DATE OF BIRTH	19 September 1993
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	2006 – 2011 : Rajini School, Thailand

2012–2015   : Mahidol University, Thailand

Bachelor degree of Environment and Resource Studies

(Science and Environmental Technology)

2016-present : Chulalongkorn University, Thailand

Center of Excellence on Hazardous Substance

Management

HOME ADDRESS	307164 Jarunsanitwong Rd., Bangkhunsri, Bangkoknoi, Bangkok 10700
PUBLICATION	Nawavimarn P., Rongsayamanont W., Luepromchai E. 2018. Formulation of solvent-free dispersant for oil spill remediation by mixing lipopeptide biosurfactant and fatty alcohol ethoxylate. Oral presentation. the 8th IUPAC International Conference on Green Chemistry. Bangkok. 9-14 September, 2018.
AWARD RECEIVED	- An internship student in IERI 2017 Fall Internship Program at Gwangju Institute of Science and Technology in South Korea.
	- Scholarship from the 8th IUPAC International Conference on Green Chemistry. Bangkok 2018.