ENHANCING PETROLEUM HYDROCARBON REMOVAL EFFICIENCY IN SOIL WASHING PROCESS BY ADDING BIOSURFACTANT

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การเพิ่มประสิทธิภาพในการบำบัดปิโตรเลียมไฮโดรคาร์บอนในดินโดยกระบวนการล้างด้วยการเติมสารลดแรงตึงผิวชีวภาพ

นางสาวบุญญิสา สุขสมบูรณ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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บุญญิสา สุขสมบูรณ์ : การเพิ่มประสิทธิภาพในการบำบัดปิโตรเลียมไฮโดรคาร์บอนในดินโดยกระบวนการล้างด้วยการเติมสารลดแรงตึงผิวชีวภาพ (ENHANCING PETROLEUM HYDROCARBON REMOVAL EFFICIENCY IN SOIL WASHING PROCESS BY ADDING BIOSURFACTANT) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. จันทรา ทองคำเภา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. เอกวัล ลือพร้อมชัย, 108 หน้า.

การปนเปื้อนปิโตรเลียมไฮโดรคาร์บอนในดินอาจทำให้เกิดผลกระทบทั้งทางด้านสิ่งแวดล้อมและสุขภาพของมนุษย์ การศึกษานี้จึงนำหลักการ Hydrophilic-Lipophilic Deviation (HLD) และไมโครอิมัลชันมาใช้สร้างสูตรสารชะล้างเพื่อบำบัดดินปนเปื้อนปิโตรเลียมไฮโดรคาร์บอน เมื่อกำหนดค่า HLD =0 จะสามารถคำนวณค่าองค์ประกอบต่างๆ จากสมการให้สอดคล้องกับค่า EACN ของไฮโดรคาร์บอน และสร้างระบบที่มีความสมดุลระหว่างวัฏภาคน้ำมันและวัฏภาคน้ำได้ สารชะล้างดังกล่าวประกอบด้วย สารลดแรงตึงผิวชีวภาพชนิดลิโพเปปไทด์ที่ผลิตจากแบคทีเรีย *Bacillus* sp. GY19, สารลดแรงตึงผิวสังเคราะห์ ได้แก่ AMA หรือ AOT และโซเดียมคลอไรด์ ไฮโดรคาร์บอนที่ใช้ในการศึกษานี้ คือ เฮกเซน เดกเคนและเฮกซะเดกเคน (EACN=6, 10, 16 ตามลำดับ) เพื่อครอบคลุมกลุ่มไฮโดรคาร์บอนที่พบได้ในสิ่งแวดล้อม ในการศึกษานี้ใช้ลิโพเปปไทด์ที่ผ่านกระบวนการแยกด้วยเมมเบรนเพื่อเพิ่มความบริสุทธิ์ ซึ่งพบว่ามีความสามารถในการกระจายน้ำมันกับไฮโดรคาร์บอนชนิดต่างๆ มีความเข้มข้นที่ทำให้เกิดไมเซลล์ต่ำและมีความไม่ชอบน้ำสูง จึงนำมาผสมกับสารลดแรงตึงผิวสังเคราะห์ที่มีความชอบน้ำมากกว่าเพื่อปรับให้เกิดความสมดุลของระบบ พบว่าสูตรที่มีสารลดแรงตึงผิวผสมสามารถเกิดไมโครอิมัลชันชนิดที่ 3 ได้กับไฮโดรคาร์บอนหลายชนิด และสามารถลดแรงตึงระหว่างผิวประจันได้ดีกว่าสูตรที่มีสารลดแรงตึงผิวชนิดเดี่ยว นอกจากนั้น การเติมลิโพเปปไทด์ในสูตรสารชะล้างสามารถเพิ่มประสิทธิภาพการกำจัดปิโตรเลียมไฮโดรคาร์บอนในดิน โดยสามารถชะล้างน้ำมัน 70-85 เปอร์เซ็นต์ออกจากดินที่มีความเข้มข้นปิโตรเลียมไฮโดรคาร์บอนเริ่มต้น 5-15 เปอร์เซ็นต์โดยน้ำหนัก แต่ประสิทธิภาพของสารชะล้างที่มีเพียงสารลดแรงตึงผิวสังเคราะห์จะอยู่ที่ 60 เปอร์เซ็นต์ ดังนั้นหลักการ HLD และไมโครอิมัลชัน สามารถนำมาใช้สร้างสูตรสารชะล้างสำหรับไฮโดรคาร์บอนที่มี EACN ต่างๆ ได้

สาขาวิชา	การจัดการสารอันตรายและสิ่งแวดล้อม	ลายมือชื่อนิสิต	....
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BOONYISA SUKSOMBOON: ENHANCING PETROLEUM HYDROCARBON REMOVAL EFFICIENCY IN SOIL WASHING PROCESS BY ADDING BIOSURFACTANT. ADVISOR: ASST. PROF. CHANTRA TONGCUMPOU, Ph.D., CO-ADVISOR: ASSOC. PROF. EKAWAN LUEPROMCHAI, Ph.D., 108 pp.

Soil contamination with petroleum hydrocarbons can cause environmental and health problems. This work aimed to combine Hydrophilic-Lipophilic Deviation (HLD) and microemulsion to formulate washing solution for petroleum removal from soil. When HLD value was fixed at zero, the composition of washing solution were calculated based on EACN and creates an optimum condition between oil and aqueous phases. The washing solutions contained lipopeptide biosurfactant produced from *Bacillus* sp. GY19, synthetic surfactant i.e. AMA or AOT, and NaCl. Hexane, decane, and hexadecane (EACN=6, 10, 16, respectively) were used to cover hydrocarbons found in the environment. Lipopeptide increased its purity by membrane filtration showed the potential in oil displacement, low CMC, and high hydrophobicity. It was mixed with hydrophilic surfactants to balance the system. The mixed surfactant systems formed microemulsion type III with various hydrocarbons and reduced IFT better than the single surfactant systems. The addition of lipopeptide in the formulations could enhance petroleum removal efficiency (70%-85%) from 5%-15% (w/w) petroleum hydrocarbon contaminated soil but around 60% came from the single synthetic surfactants. Consequently, microemulsion and HLD concept could create efficient soil washing solutions for different EACN hydrocarbons.

Field of Study:	Hazardous Substance and Environmental Management	Student's Signature ....
		Advisor's Signature ....
		Co-Advisor's Signature ....
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CHAPTER 1

INTRODUCTION

1.1.Statement of problem

Soil contamination has become a global issue and can be a problem for sustainable development. It causes imbalance of the ecosystem, increasing economic loss, and human health damage. Petroleum exploration and application are major sources of soil contamination, for example aboveground oil spills, storage tanks leakage, and other accidents (Han et al., 2009). Accidental discharge of fuels can be considered as a common case. Fuels are obtained from petroleum distillation. They include ethane and other short-chain alkanes, diesel fuel, fuel oils, gasoline, jet fuel, kerosene, and liquefied petroleum gas (LPG). However, the hydrocarbons may be mixed with other compounds resulting in other end products such as alkenes, lubricants, motor oils, greases, wax, sulfur or sulfuric acid, petroleum coke, and paraffin wax (Mosaed et al., 2015). Petroleum hydrocarbons are hydrophobic compounds which have low water solubility, and can adsorb into hydrophobic soil particles and soil organic matters. Petroleum and its products have been a major concern because of their hazardous properties which may affect the ecosystem and water reservoirs for human supply (Mena Ramirez et al., 2015).

Although several approaches are provided to control environmental damage by contaminated soil, for example landfill disposal, in-situ bioremediation, re-injection, thermal desorption, stabilization, and combustion, these techniques may be unsustainable because of their limitations such as slow process, high energy consumption, potential high cost, and low removal efficiency of contaminants (Al-Ansary and Al-Tabbaa, 2007). Surfactant enhanced washing has become a remarkable treatment to remediate soils and sands contaminated with crude oils (Urum and Pekdemir, 2004). In case of an ex-situ soil washing, the contaminated soil is excavated from the site and brought to surfactant washing steps. After washing, the surfactant solutions can be separated and regenerated for the next round. Other advantages are provided, for example various types and concentrations of contaminants could be treated, and the clean coarse fractions of soils may be returned to the site at a relatively low cost. Furthermore, the soil washing is one of the few treatment techniques which can intensely separate heavy metals, organics, and radionuclides from contaminated soils (Mao et al., 2015).

However, traditional synthetic surfactants can cause environmental problems because of their toxicity and low biodegradability. As a result, biosurfactants which can be produced by various microorganisms have become an interesting alternative. Furthermore, biosurfactants are easily produced from renewable resources, less toxicity, good biodegradability, great environmental compatibility, and high activity at extreme environmental conditions (Yan et al., 2011). Generally, biosurfactants can be divided into four main types such as glycolipids, phospholipids, lipopeptides and lipoproteins, and polymeric (Healy et al., 1996). In this study, lipopeptide biosurfactant produced by *Bacillus* sp. strain GY19 which showed high potential in surface activity (Khondee et al., 2015), was used as a main composition in washing solution for cleaning the petroleum contaminated soil.

The optimization of washing solution containing lipopeptide biosurfactant is interesting. The reason is mixing lipopeptide biosurfactant with synthetic surfactant, and electrolyte could produce the lower IFT against several light non-aqueous-phase liquids (LNAPLs) than the IFT from the individual biosurfactant (Sabatini et al., 2006). In addition, the mixed surfactant system has been used for synergism effect, when the properties of the mixture are better than those achievable with the individual surfactants. For example, the mixtures of anionic and nonionic surfactants are widely used for detergency because of their excellent cleaning properties which have high volume of surfactant. However, most laundry detergents are a mixture of two anionic surfactants because the nonionic surfactants make the surfactant system less sensitive to water hardness (Cowan-Ellsberry et al., 2014). According to previous researches, the mixtures may be anionic-nonionic, anionic-cationic, or anionic-anionic surfactants depended on the objective of that work.

Microemulsion formation has been considered as an effective approach to enhance oil solubilization and reduce the oil-water interfacial tension (IFT) in many industrial applications, for example subsurface remediation, drug delivery, and detergency. However, different applications require specific degrees of solubilization and IFT reduction. Microemulsion can be divided into three main types described by Winsor. Winsor type I contains two phases which are the lower microemulsion phase in equilibrium (oil/water, o/w) with the upper excess oil. Winsor type III consists of three phases which are middle microemulsion phase in equilibrium (o/w plus w/o, called bicontinuous) with upper excess oil and lower excess water. Winsor type II includes two phases which are the upper microemulsion phase in equilibrium (water/oil, w/o) with excess water (Paul and Moulik, 2001).

Hydrophilic-Lipophilic Deviation (HLD) was proposed by Salager et al. (1979) as a dimensionless equation. The equation contains the parameters that can be quantified to describe microemulsion systems. Therefore, the equation is adapted to use in several approaches. Recently, the HLD concept was suggested that it is useful for predicting the microemulsion phase behavior of oil/surfactant system because the phase behaviors predicted from the equation corresponds to the experimental phase behaviors of the anionic mixtures with the vegetable oils, for example at low NaCl, microemulsion type I occurred and and HLD value calculated into negative value; both scenarios are related to show microemulsion type I (Do et al., 2014). In the present study, the original HLD equation was derived for describing the mixed surfactant system created without alcohol additives and at room temperature. Molar fractions of each surfactant were fixed and sodium chloride concentration was calculated to achieve HLD*=0, where microemulsion type III might occur. Microemulsion type III was determined because it could give the lowest IFT and could increase oil removal from soil.

Therefore, this study aims to apply microemulsion formation and HLD concept to determine phase behavior of mixed surfactant system with different petroleum hydrocarbons. Three hydrocarbons (hexane, decane, hexadecane) which have different equivalent alkane carbon numbers (EACNs) were selected to represent a wide range of petroleum pollutants in the environment. The mixed surfactant system was divided into two groups. The system for low EACN hydrocarbons such as hexane and decane contained lipopeptide biosurfactant as a hydrophobic surfactant and dihexyl sulfocuccinate, sodium salt (AMA) as a hydrophilic surfactant. Another system for high EACN hydrocarbons such as hexadecane consisted of lipopeptide biosurfactant as a hydrophobic surfactant and dioctyl sulfosuccinate, sodium salt (AOT) as a hydrophilic surfactant. The selection of synthetic surfactants was based on their characteristic curvature (Cc) values, which show hydrophilic-lipophilic nature of surfactants. It was compared to lipopeptide biosurfactant which has Cc=4.93 (Rongsayamanont, 2015). The more positive Cc values indicate that the more hydrophobic surfactant (Witthayapanyanon et al., 2008). Consequently, AMA which has Cc=-0.92 was selected for the low EACN system and AOT which has Cc=2.66 was selected for the high EACN system. In addition, electrolyte was added to increase solubility of organic compounds because it might reduce repulsive forces between each surfactant monomer and increase volume of micelles (Peter and Singh, 2014). Then, some petroleum oils were tested with the mixed surfactant system and the results were compared to these hydrocarbons. The formulations were later used for washing petroleum contaminated soil with varying oil concentrations in a batch experiment. The petroleum hydrocarbon removal efficiency of each formulation was expected to correspond with the microemulsion phase observation and type of petroleum hydrocarbons.

1.2.Hypotheses

1. Mixed surfactant system containing lipopeptide biosurfactant, synthetic surfactant, and electrolyte can reduce IFT of petroleum hydrocarbons lower than a single surfactant system.
2. HLD concept can be used to predict phase behavior of microemulsion containing water, petroleum hydrocarbons, and mixed surfactant system.
3. Addition of biosurfactant in washing solution can enhance petroleum hydrocarbon removal efficiency in soil washing.

1.3.Objectives

The overall objectives of this study are to formulate washing solution by using phase behavior studies and to use the solution for petroleum hydrocarbon removal from soil.

1. To compare the ability of IFT reduction between the mixed surfactant and the single surfactant systems.
2. To describe the phase behavior of microemulsion systems by using HLD concept.
3. To demonstrate a potential of the washing solution for petroleum hydrocarbon removal from soil in batch experiments.

1.4. Scope of Study

1. This study optimized the mixed surfactant systems for using as washing solution by microemulsion formation. The systems contained surfactants, lipopeptide biosurfactant (hydrophobic property), AMA and AOT (hydrophilic property), and NaCl (electrolyte).
2. Surfactant properties such as critical micelle concentration (CMC) were used as the basic information to formulate the mixtures.
3. Three hydrocarbons (hexane, decane, and hexadecane) represented a wide range of petroleum in the environment because of their different EACNs.
4. Soil was collected from a non-contaminated area in Suphanburi province at 0.5 m depth to represent a contaminated site affected by accidental spills from vehicles or leaks in storage tank (Hernández-Espriú et al., 2012).
5. Soil washing procedure was divided into two parts. First, the soil was artificially contaminated with 5% (w/w) petroleum hydrocarbons which were decane, hexadecane, Bongkot crude oil, ARL/AXL crude oil, and diesel. Second, the petroleum concentrations were increased to 10% (w/w) and 15% (w/w) to study the effect of oil concentrations in soil and Bongkot crude oil was selected.
6. The physical conditions for soil washing were modified from the previous study (Urum and Pekdemir, 2004) that were liquid:solid ratio at 2:1, shaking speed at 200 rpm, and shaking time at 30 minutes.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEWS

2.1.Petroleum hydrocarbons

2.1.1. General information

Petroleum and the equivalent term crude oil consist of a wide range of materials (Table 2.1) including mixtures of hydrocarbons and other substances such as sulfur, nitrogen, and oxygen, which may lead to differences in volatility, specific gravity, and viscosity. Because of the varying constituents and proportions of petroleum, its physical properties also vary and the color may be different from colorless to black (Speight, 2007).

The origin of petroleum is from the deposition of died aquatic plants and animals mixed with mud and sand that was geologically transformed into sedimentary rock, but the process took over hundreds of millions of years ago. Then, the organic matter decomposed and eventually formed petroleum or a related precursor which may travel from the origin to more porous and permeable rocks such as sandstone and siltstone where it became entrapped. The place where the entrapped petroleum is accumulated is called reservoir. Petroleum could be found underground at various pressures depending on the depth. Petroleum underground is much more fluid than it is on the surface, for example natural gas in solution; therefore, it could mobile under reservoir conditions because the elevated temperatures in subsurface formations reduce the viscosity (Speight, 2007).

The major components of petroleum are hydrocarbons, compounds of hydrogen and carbon that show great variation in their molecular structure. Firstly, paraffins are considered as the simplest hydrocarbons which are a large group of chain-shaped molecules. This series extend from methane which creates natural gas to crystalline waxes by refining liquids into gasoline. Secondly, a group of ring-shaped hydrocarbons is divided into two categories: (1) naphthenes consist of a range from volatile liquids such as naphtha to high molecular weight substances isolated as the asphaltene fraction. (2) aromatics contain benzene as the dominant compound which is a popular raw material for making petrochemicals (Speight, 2007). On the other hand, nonhydrocarbon components of petroleum include organic derivatives of nitrogen, oxygen, sulfur, and the metals mickel and vanadium. Most of these impurities are removed during refining (Speight, 2007).

Table 2.1 Subdivision of petroleum and similar materials into various subgroups (Speight, 2007)

Natural materials	**Derived materials**	**Manufactured materials**
Natural gas	Saturates	Synthetic crude oil
Petroleum	Aromatics	Distillates
Heavy oil	Resins	Lubricating oils
Bitumen[a]	Asphaltenes	Wax
Asphaltite	Carbenes[b]	Residium
Asphaltoid	Carboids[b]	Asphalt
Ozocerite (natural wax)		Coke
Kerogen		Tar
Coal		Pitch

[a] Bitumen from tar sand deposits.

[b] Usually thermal products from petroleum processing.

According to the complex structure of petroleum hydrocarbons containing organic components with different molecular weights, they are categorized as one of hydrophobic organic compounds (HOCs) which have the potential toxicity, mutagenicity, carcinogenicity and ability to be accumulated in the food chain. Moreover, HOCs characteristics contain low solubility in water, high octanol/water partition coefficient (K_{ow}), a high organic carbon/water partition coefficient (K_{oc}), and leading to their accumulation in soils by sorption mechanisms with soil organic matter (SOM) (Trellu et al., 2015).

Petroleum products such as gasoline, diesel fuel, and other related products consist of high number of different petroleum hydrocarbons. Due to the process of distillation, individual petroleum products are made depend on refinery process performed to give the desired characteristics of each product. For example, gasoline is created by blending different products of distillation with various additives in order to create a product that meets engine performance criteria (Blaisdell and Smallwood, 1993).

2.1.2. Crude oil

Crude oil could be obtained from underground by injecting water or steam into the reservoir to artificially increase the pressure or by injecting other substances such as carbon dioxide, polymers, and solvents to decrease crude oil viscosity. Crude oil is transported to refineries by pipelines or by ocean-going tankers. Distillation is a fundamental refinery process which separates the crude oil into fractions of different volatility. After distillation, other physical methods are applied to isolate the mixtures such as absorption, adsorption, solvent extraction, and crystallization. Then, light and heavy naphtha, kerosene, and light and heavy gas oils, selected petroleum fractions may be sent to conversion processes, for instance thermal cracking and catalytic cracking. The cracking breaks the large molecules of heavier gas oils into smaller molecules that form the lighter, more valuable naphtha fractions. In addition, reforming is used to change the structure of straight-chain paraffin molecules into branched-chain iso-paraffins and ring-shaped aromatics. This procedure could raise the octane number of gasoline obtained by distillation of paraffinic crude oils (Speight, 2007).

Due to their complex structures of crude oils, they are normally characterized by hydrocarbon type analysis. The SARA-separation is one of hydrocarbon type analysis that divides the crude oils into four main classes based on their differences in solubility and polarity. The four fractions are saturates (S), aromatics (A), resins (R), and asphaltenes (A).

First, the saturates or aliphatics are non-polar hydrocarbons which is straight-chain and branched alkanes including cycloalkanes which have one or more rings and a lot of alkyl side chains. In addition, the saturates normally are the lightest fraction of the crude oil. Wax is also considered in the saturate group because it has straight-chain alkanes mainly ranging from C_{20} to C_{30}. However, wax precipitation into a particulate solid could occur at low temperatures, and is realized as emulsion stability properties of crude oil systems (Aske, 2002).

Second, the aromatics are considered as benzene and its derivatives. Aromatics, which have alkyl chains and cycloalkane rings including additional aromatic rings, are general composition of all petroleum. Nevertheless, polar and higher molecular weight aromatics may fall in the resin or asphaltene fraction (Aske, 2002).

Third, the resins are polar molecules usually comprising heteroatoms such as nitrogen, oxygen, or sulfur. Moreover, naphthenic acids are commonly characterized as a part of the resin fraction. The resin fraction could be defined as the soluble fraction in light alkanes such as pentane and heptane, but it is not soluble in liquid propane (Figure 2.1). Due to its solubility, it is expected that the resin may overlap to the aromatic and the asphaltene fraction. However, resins are identified that their structure may be similar to asphaltenes but the molecular weight (< 1000 g/mole) is smaller than asphaltene (Aske, 2002).

Fourth, the asphaltenes are the fraction that could not solubilize in light alkanes such as pentane, hexane, or heptane (Figure 2.1), but it could be soluble in aromatic solvents such as toluene and benzene. The asphaltene fraction consists of the highest percentage of heteroatoms (O, S, N) and organometallic constituents (Ni, V, Fe) in the crude oil. The molecular weight of asphaltenes has been difficult to measure due to its self-aggregate, but the molecular weight is believed in the range 500-2000 g/mole (Aske, 2002).

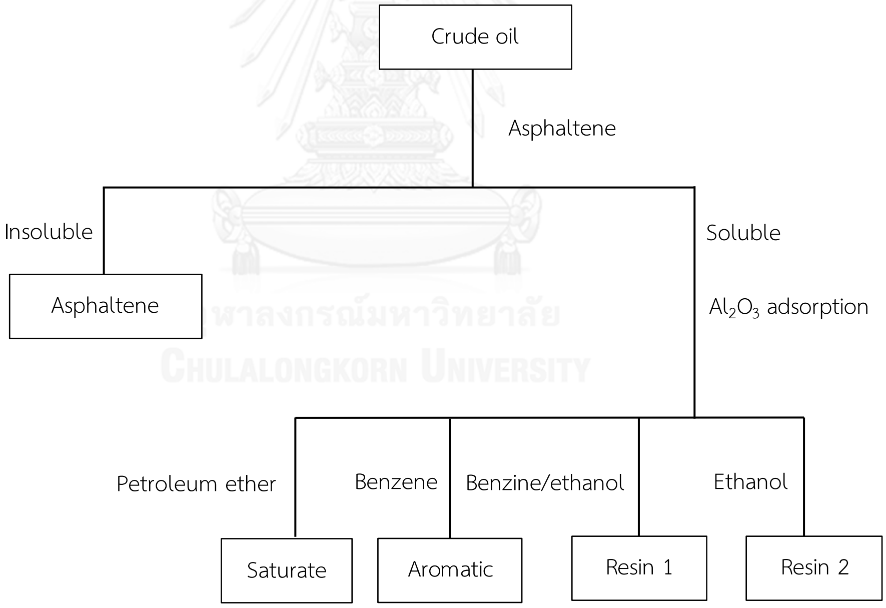


Figure 2.1 Separation of crude oil fraction (Guo et al., 2006)

2.1.3. Diesel fuel

Diesel fuel is widely used in the world; consequently, it has been a permanent source of soil and water pollution. The diesel fuel produced by a refinery is a blend of all the appropriate available streams: straight-run product, fluid catalytic cracking light cycle oil, and hydrocracked gas oil. The straight-run diesel may be acceptable as may need small upgrading for use in diesel fuel prepared for off-road use. To meet the 15 ppm sulfur limit, all the streams used to prepare diesel fuel need hydrotreating to lower the sulfur concentration.

Diesel is from the crude oil distillation and contains more than 2000 compounds which cannot be all separated by chromatography; however, it is composed of 60% of saturated hydrocarbons (n-alkanes and naphtenes) and 40% of aromatics (Khalladi et al., 2009).

Khalladi et al. (2009) investigated a composition of diesel used in their study. They found that 19 hydrocarbons presented in a GC chromatogram and the hydrocarbons ranged from C_8 to $C_{26.}$ In addition, they also determined the fractions of diesel in effluent after soil washing using SDS solution. They found that diesel fractions in the effluent could be divided into three different classes, for instance major fraction (C_{15}-C_{20}), minor fraction (C_{12}-C_{14}), and the small amount in diesel fuel which was C_{21}-C_{26}.

2.2.Petroleum contaminated soil

2.2.1. Source of petroleum contaminated soil

Crude oil could be refined into several products. The main purpose of oil utilization is as an energy source in traffic, heating and electricity production. Furthermore, crude oil is used as a raw material in various man-made products such as plastics, paints and solvents. Crude oil is the most exploited non-renewable natural resource and oil demand has been increased. As a result, derivative compounds from crude oil are the most common polluters of the environment and it is generally that most fuel oil ends up in its intended place of use. The most common type of petroleum contamination in the environment is from sources of smaller volumes such as leaking heating oil containers, gasoline station tanks and lines, improper handling of waste and small accidental spills comprise most of the oil pollution in soil, surface water, and groundwater.(Valentín et al., 2013)

Weathering processes of crude oil involve adsorption of hydrocarbons to soil particles, volatilization of hydrocarbons, and low water solubility of hydrocarbons. Due to their structures, alkanes and alkenes tend to be more volatile than aromatics. Therefore, the lower molecular weight aliphatics will be the highest loss from the crude oil; then aliphatics may be the main pollutants in the atmosphere near the spill sites (Williams et al., 2006). However, the persistence of petroleum hydrocarbons in soils causes a serious hazard to human health, produces organic pollution of ground water, causes environmental problems, and reduces the soil qualities (Thapa et al., 2012).

2.2.2. Current remediation technology

Soil contamination in many cases, for example leaking of underground storage tank is an environmental concern and may lead to the more serious problem of ecosystem including groundwater. There are several cleanup methods for soil treatment, but each method provides both benefits and drawbacks (Scheel, 2011).

1) In-situ soil treatment

This method has been used such as vapor extraction and biological treatment. In general, in-situ treatment can be expensive but it may be more cost effective in the case of large amounts of contamination or difficult removal.

Therefore, in-situ methods are used as primary treatments in complex cleanup projects.

2) Above ground biological treatment

This method is suitable for gasoline contamination and works with diesel, waste oil, and other heavy hydrocarbon contamination which does not have aeration. It results in destruction of the contaminants and reduces long term liability. However, bioremediation is a relatively high cost for dealing with a large soil contamination.

3) Landfill disposal

Generally, this method moves contaminated soil from the source to landfill disposal; therefore, it requires cleanup treatment of landfill to prevent further problems. Moreover, landfill cost may increase because landfill area becomes limited. However, it may be the most cost effective option for some projects.

4) Soil aeration

This method may be less expensive for cleaning contaminated soil. In addition, aeration technique works well with gasoline contaminated soil, but it shows less effective with diesel and heavier hydrocarbons. As it involves the volatilization of hydrocarbons, it may cause air pollution such as photochemical smog.

5) Soil washing

Soil washing is a mechanical process. It requires liquids or aqueous solutions to separate pollutants from soil. This is one of the few methods that can totally remove heavy metals, organics, and radionuclides from contaminated soil (Mao et al., 2015).

2.2.3. Related regulations of soil and petroleum hydrocarbons

Agency for Toxic Substances and Disease Registry (ATSDR) presents that some total petroleum hydrocarbons (TPH) compounds can damage their nervous system such as headaches and dizziness. TPH has been categorized at least 23 of the total 1,467 National Priorities List sites identified by EPA (ATSDR, 1999).

Some recommendations are proposed from the Occupational Safety and Health Administration (OSHA). For example, exposure limit is set at 500 ppm of petroleum distillates in air for an 8-hour workday, 40-hour workweek (ATSDR, 1999).

World Health Organization (WHO) determined toxicity and risk assessment of petroleum products in drinking water. The main sources are spills, penetration of distribution system, or treatment works. However, a small proportion of the petroleum hydrocarbon compositions are significantly soluble in water. Aliphatic fractions are one example. No-observed-adverse-effect levels (NOAELs) of commercial n-hexane were ranging from 1840-5520 mg/m^3 in air due to its high volatility and low solubility in water. Furthermore, Reference dose (RfD) from oral exposure of aliphatics (C9-C12) and (C10-C13) was determined as 0.1 mg/kg of bodyweight/day.

According to Canada-wide standards for petroleum hydrocarbons in soil from Canadian Council of Ministers of the Environment, it provides summary (Table 2.2) of hydrocarbon fraction levels for different land uses which is called Tier 1.

Table 2.2 Summary of Tier 1 levels (mg/kg) for surface soil

Land use	Soil texture	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Agricultural	Coarse-grained soil	30	150	300	2800
	Fine-grained soil	210	150	1300	5600
Residential	Coarse-grained soil	30	150	300	2800
	Fine-grained soil	210	150	1300	5600
Commercial	Coarse-grained soil	320	260	1700	3300
	Fine-grained soil	320	260	2500	6600
Industrial	Coarse-grained soil	320	260	1700	3300
	Fine-grained soil	320	260	2500	6600

* Fraction refers to the equivalent normal straight-chain hydrocarbon (nC)

Fraction 1= nC6 to nC10

Fraction 2= >nC10 to nC16

Fraction 3= >nC16 to nC34

Fraction 4= ≥nC35

2.3.Surfactant technology and application

2.3.1. Surfactant

Surfactants are amphiphilic molecules that contain a hydrophilic head and a hydrophobic tail. They are surface active compounds that could be used in soaps (Figure 2.2) and detergents because of their ability to attach at the air-water interface and could be used to remove oily materials from a media. Surfactants are classified as ionic and non-ionic with different chemical structures according to their hydrophilic group (Urum and Pekdemir, 2004). Ionic surfactant classification is based on the carrying charge when dissociated in water at neutral pH; therefore, the classifications are namely anionic, cationic, and zwitterionic or amphoteric (Salager, 2002).

1) Nonionic surfactants

They do not ionize in aqueous solution because of their non-dissociable hydrophilic group such as alcohol, phenol, ether, ester, or amide. In addition, a large amount of nonionic surfactants, which are made to be more hydrophilic by adding a polyethylene glycol chain came from the polycondensation of ethylene oxide, are called polyethoxylated nonionics.

2) Anionic surfactants

They are dissociated in water into an amphiphilic anion and a cation which is generally an alkaline metal (Na^+, K^+) or a quaternary ammonium. This group is commonly used in various purposes such as alkylbenzene sulfonates (detergents), fatty acid (soaps), lauryl sulfate (foaming agent), di-alkyl sulfocuccinate (wetting agent), lignosulfonates (dispersants).

3) Cationic surfactants

They are dissociated in water into an amphiphilic cation and an anion mostly is the halogen type. A huge proportion of this class relates to nitrogen compounds, for instance fatty amine salts and quaternary ammoniums with some alkyl chain coming from natural fatty acids. Cationic surfactants are normally expensive than anionics due to a high pressure hydrogenation reaction in synthetic process. Therefore, they are only used in two cases for example (1) as bactericide, (2) as positively charged substance to adsorb on negatively charged substances to create antistatic and hydrophobant effect, often found in corrosion inhibition.

4) Zwitterionic or amphoteric surfactants

The single surfactant molecule demonstrates both anionic and cationic dissociation. Synthetic products are namely betaines or sulfobetaines, while natural compounds are aminoacids and phospholipids. These substances are expensive; as a result, their use is limited to some special applications such as cosmetics which require high biological compatibility and low toxicity.

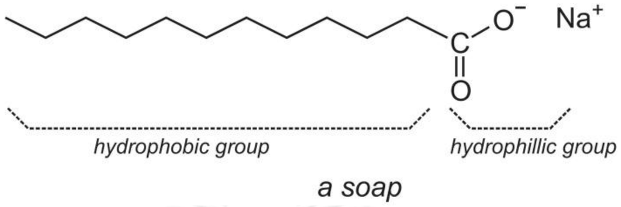


Figure 2.2 Example structure of anionic surfactants

Surfactants increase the aqueous solubility of non-aqueous phase liquids (NAPLs) by reducing their surface or interfacial tension (IFT) at air-water and water-oil interfaces. Reduced IFT and increased surfactant concentration make surfactant monomers forming into micelle (Figure 2.3). The concentration that first micelle begins to form is known as the critical micelle concentration (CMC) which shows the lowest surface tension. Several physical properties used to characterize surfactants depend on the CMC such as emulsion formation, oil solubilization, foaming and detergency, IFT and surface tensions. In addition, these properties could be used to evaluate suitability of surfactants to apply in environmental remediation such as soil washing (Urum and Pekdemir, 2004).

Surfactants have been used as extracting agents in soil washing. According to their structures, they are amphiphilic molecules consisted of the hydrophobic tail group and the hydrophilic head group. Generally, chemical structure, hydrophilic-lipophilic balance, and CMC are used to characterize them. Relationship between CMC and surface tension is showed (Figure 2.3); the surface tension changes strongly with the surfactant concentration at below CMC but it changes much lower at above

CMC. However, solubility hydrophobic organic compounds is strongly enhanced at above CMC (Trellu et al., 2015).

Nowadays, surfactants could be divided into two main types. Surfactants produced from chemically based materials are known as synthetic surfactants; in contrast, those produced from biologically based materials are biosurfactants (Urum and Pekdemir, 2004).

Marin et al. (2015) reviewed that CMCs of synthetic surfactants such as Triton N-101, Tween 20 and Tween 80 are in the range of 63-157 mg/L. Moreover, CMCs of Tergitol TMN-10, sodium dodecyl sulfate (SDS) and Citrikleen are in the range of 1200-2000 mg/L. In this study, they also evaluated the effectiveness to apply the liquor obtained from sisal (*Agave sisalana*) pulp hydrolysis as substrate for biosurfactant production by *Bacillus subtilis* ATCC 21332. They found that the surfactin produced from acid hydrolysate showed a surface tension of 29.8 mN/m, IFT of 5.7 mN/m, and CMC of 1394 mg/L. Moreover, the surfactin produced from enzymatic hydrolysate showed a surface tension of 28.7 mN/m, IFT of 3.8 mN/m, and CMC of 64 mg/L.

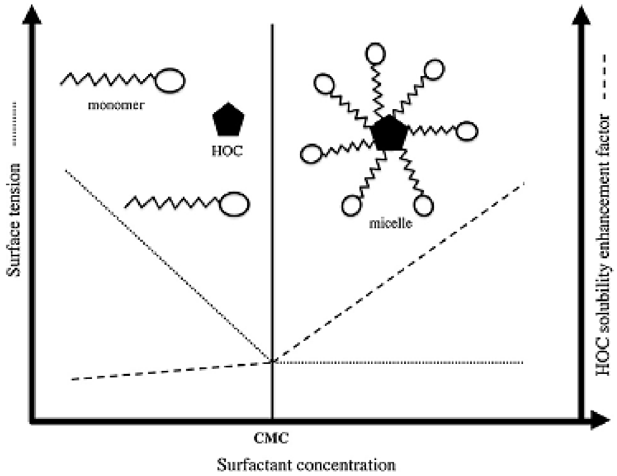


Figure 2.3 Relationship between surfactant concentration and two parameters: surface tension and hydrophobic organic compounds (HOCs) solubility which are related to soil washing (Trellu et al., 2015)

2.3.2. Biosurfactant

Biosurfactants have been interested for recent years. In general, biosurfactants are produced by microorganisms in aerobic conditions from a carbon source feedstock such as carbohydrates, hydrocarbons, oils and fats or mixtures. The emulsifiers are excreted into the culture medium during the microorganism growth. Then, the emulsifiers assist in the transport and translocation of the insoluble substrates across cell membranes. Biosurfactants can be nonionic or anionic types. There are no literature reports of cationic structures. Generally, biosurfactants consist of one or several lipophilic and hydrophilic properties. Lipophilic part can be a protein or a peptide with a high proportion of hydrophobic side chains which is usually the hydrocarbon chain of a fatty acid with 10-18 carbon atoms. Hydrophilic part can be an ester, an hydroxy, a phosphate or carboxylate group or a carbohydrate (Bognolo, 1999).

Biosurfactants can be divided into four main types (Table 2.3). First, glycolipids are compounds of a carbohydrate and a lipid including ether or ester linkage, for example rhamnolipids, mycolates of mono, -di, and -trisaccharides, and sophorolipids. Second, phospholipids contain a lipid and a phosphate connected the alcohol groups by the esters. Third, lipopeptides and lipoproteins consist of a lipid attached to a polypeptide chain. Fourth, polymeric biosurfactants are composed of saccharide units and fatty acid residues (Healy et al., 1996).

Biosurfactants are needed because of the benefits of easy production from renewable resources and possible reuse by regeneration, high specificity, less toxicity, and biodegradability.

Table 2.3 Microbial biosurfactants (Deleu and Paquot, 2004)

Biosurfactant types	**Example**	**Microorganisms**
Glycolipids	Trehalose lipid	*Rhodococcus erithropolis*
	Sophorose lipid	*Torulopsis magnoliae,* *Candida bombicola*
	Rhamnose lipid	*Pseudomonas aeruginosa*
	Mannosylerithritol lipid	*Shizonella melanogramma,* *Pseudozyma Antarctica*
Phospholipids		*Corynebacterium lepus, Aspergillus*
Lipopeptides	Surfactin	*Bacillus subtilis*
	Viscosine	*Pseudomonas fluorescens*
Liposaccharides	Emulsane	*Acinetobacter calcoaceticus*
	Alasan	*Acinetobacter radioresistens*
Fatty acids and neutral lipids	Corynomycolic acid	*Corynebacterium insidibasseosum*

Shakerifard et al. (2009) found that lipopeptide (surfactins) biosurfactant could reduce the surface tension of water from 72 mN/m to 25-30 mN/m. It is related to the amphiphilic property composing of the long chain fatty acid and some lipophilic amino acids as a hydrophobic part, and two acidic amino acid residues as a hydrophilic part.

Michael J. McInerney (2009) concluded that CMCs of lipopeptide biosurfactant vary from 8-50 mg/L, and are lower than synthetic surfactants orders of magnitude. As a result, lipopeptides are more effective at low concentration compared to synthetic surfactants.

2.3.3. Microemulsion

Microemulsions are thermodynamically stable systems containing water, oil, and surfactant. According to microemulsions properties, equal volume of oil and water can be produced by using low surfactant concentrations; in addition, extremely low interfacial tension (IFT) (Figure 2.4) between oil and water phases can be achieved (Tongcumpou et al., 2003). Microemulsion formation has been considered as an effective approach to enhance oil solubilization and reduce the oil-water IFT in many industrial applications, for example subsurface remediation, drug delivery, and detergency. However, different applications require specific degrees of solubilization and IFT reduction. An efficient microemulsion system can be formed by making equal interactions between surfactant-oil and surfactant-water; therefore, a well understanding of molecular interactions and thermodynamic principles is needed to produce microemulsions (Witthayapanyanon et al., 2008).

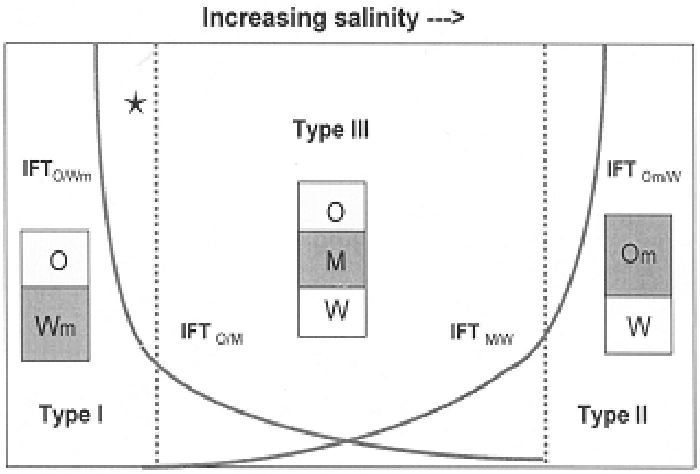


Figure 2.4 Phase behavior and interfacial tension (IFT) of oil/water/surfactant system with increasing salinity modified from Tongcumpou et al. (2003).

Different structures of microemulsions are classified by Winsor (Baran, 2001). Winsor type I (o/w), Winsor type II (w/o), and Winsor type III (bicontinuous or microemulsion) systems are created by changing the curvature of interface. Many factors are related to alter the curvature, for example salinity, and temperature.

Generally, microemulsion is well-defined phase regions of multicomponent systems containing water, oil, surfactant, and electrolyte. Tongcumpou et al. (2003) evaluated phase behavior of hexadecane and motor oil by mixing surfactants and other components such as electrolyte. They found that microemulsion phase transition could be determined for both systems, and the microemulsion based formulation could give an ultralow interfacial tension (IFT) and modest solubilization of very hydrophobic oils which could be used in detergency application. Tanthakit et al. (2010) investigated laundry detergency of palm oil on a polyester/cotton blend under conditions of ultralow oil/water IFT microemulsion formation. The results showed that the oil removal was higher than 90%.

Sabatini et al. (2006) determined the lipopeptide biosurfactant mixtures for light non-aqueous-phase liquid (LNAPL) mobilization. Lipopeptide biosurfactants were obtained from different biosurfactant-producing *Bacillus* species. The surfactants used in the study could be ordered from the most hydrophilic to the most hydrophobic as follows: rhamnolipid>lipopeptides>C12, C13-8PO-SO_4Na. For toluene (EACN=1), they found that the system of 20% lipopeptide (strain T89-42) produced with valine addition mixed with 80% rhamnolipid could produce IFT at 0.02 mN/m. They concluded that mixing lipopeptide with the more hydrophilic rhamnolipid was an effective way to achieve low IFT against low EACN hydrocarbons. For hexane (EACN=6) and decane (EACN=10), the hydrophobicity of the system should be increased for higher EACN hydrocarbons. They found that the mixture of lipopeptide (strain T89-3), 5% NaCl and 25% C12, C13-8PO-SO_4Na could give IFT at 0.011 mN/m against hexane. Furthermore, the mixture of lipopeptide (strain ROGG-2), 5% NaCl and 50% C12, C13-8PO-SO_4Na could give IFT at 0.013 mN/m against decane.

Nguyen et al. (2008) investigated the efficiency of the mixed surfactant system between rhamnolipid biosurfactant and synthetic surfactant to improve the IFT of the system with various LNAPLs. In the study, rhamnolipid (anionic surfactant) was considered as hydrophilic surfactant (HLB=22-24) and the synthetic surfactants (anionic surfactants) were considered as hydrophobic surfactants (HLB=36-40) including alkyl propoxylated (PO) and alkyl propoxyl ethoxylated (EO) sulfate surfactants. They used toluene, hexane, decane, and hexadecane as the representatives of organic contaminants in the environment. They could formulate the different mixed surfactant systems containing rhamnolipid/synthetic surfactant/electrolyte for each LNAPL. For toluene, rhamnolipid 0.08%wt mixed with C12,13–8PO sulfate (the lowest hydrophobic used in the study) 0.02%wt and NaCl 4%wt. For hexane, rhamnolipid 0.05%wt mixed with C16–10.7PO sulfate 0.05%wt and NaCl 6%wt. For decane and hexadecane, rhamnolipid 0.03%wt mixed with C16–18PO–2EO sulfate 0.07%wt for both, and NaCl 5%wt and 8%wt, respectively. In addition, IFT values <0.1 mN/m for all systems were achieved.

In addition, alcohol- free biosurfactant-based microemulsions were investigated (Nguyen and Sabatini, 2008). They aimed to formulate microemulsions with high EACN oils such as limonene (EACN around 6) and diesel oil (EACN 12-14); therefore, rhamnolipid which was very hydrophilic relative to toluene should be mixed with more hydrophobic surfactant such as sodium bis(2-ethyl) dihexyl sulfosuccinate (SBDHS). They found that 0.05 M at equal molar mixture of SBDHS and rhamnolipid biosurfactant could produce microemulsion type I, III, and II under varying NaCl concentrations and give ultralow IFT (<0.1 mN/m) for both limonene and diesel oil. It could be synergistic effect between these two surfactants.

Furthermore, rhamnolipid was determined as a hydrophilic cosurfactant because it could increase the solubilization capacity of the systems.

Nguyen et al. (2010) determined alcohol free lecithin-based biocompatible microemulsions with rhamnolipid and sophorolipid biosurfactants for a range of oils. First, they found that sophorolipid are more hydrophobic than SBDHS, and then sophorolipid (SPL-O has unsaturated C18 in the tail) was the hydrophobic component in lecithin/rhamnolipid/sophorolipid mixtures. Microemulsion formation was done by fixing the ratio of lecithin to SPL-O at 1:1 by weight, and the electrolyte concentration at 0.9% w/v. On the other hand, the rhamnolipid (JBR) concentration was varied to determine phase transition between different microemulsion types. Their results showed that lecithin/JBR/SPL mixtures could produce microemulsions with different types of oils, for example polar oils (limonene, isopropyl myristate) and nonpolar oils (decane, hexadecane); in addition, these oils also have varying EACN from 5.9 for limonene to 16 for hexadecane. Ultralow IFT (<0.1 mN/m) was achieved for microemulsions of all oils. The low IFT values are desirable for several applications such as cosmetics, hard surface cleaners, pharmaceuticals, and detergents.

2.3.4. Hydrophilic-Lipophilic Deviation (HLD)

Hydrophilic-Lipophilic Deviation (HLD) was proposed by Salager et al. (1979) as a dimensionless equation to describe microemulsion systems. The HLD value is similar to the Winsor R-ratio because it measures the departure from the optimum formulation, but the parameters are easier to quantify. Negative, zero, or positive HLD values suggest the formation of Winsor Type I, Type III or Type II microemulsions, respectively (Witthayapanyanon et al., 2008). The general HLD equation (Eq.1) described as follows is useful to evaluate the performance of anionic surfactants and to predict the phase behavior of the microemulsion systems (Mejia, 2015).

$$HLD = \ln(S) - K(EACN) - f(a) + \sigma - a_T(\Delta T) \qquad \text{(Eq.1)}$$

In this equation, HLD is determined by the parameters that are S, represents the electrolyte concentration in aqueous phase, K, is an empirical constant characterized by the head group of the surfactant, EACN is the equivalent alkane carbon number which shows the hydrophobicity of the oil phase, f(a) represents the alcohol type and concentration, σ is defined as characteristic curvature or Cc of surfactant, a_T is the empirical temperature constant related to the type of microemulsion system, and ΔT is the temperature difference measured from the reference temperature (T_{ref} = 25 °C) (Mejia, 2015; Witthayapanyanon et al., 2008).

Mostly, HLD concept is used to determine surfactant characteristics such as Cc value, and K value for several surfactants. Witthayapanyanon et al. (2008) evaluated the Cc and Cc/K parameters from the HLD equation to compare hydrophilic-lipophilic nature of conventional and extended surfactants. At the optimum condition, microemulsion type III, the minimum IFT, and the maximum oil solubilization occurred. Therefore, HLD value equals to zero at the optimum salinity was useful for determining the parameters in the original equation (HLD = ln(S) - K(EACN) - f(a) + σ - aT (ΔT)). For single anionic surfactant, AMA surfactant was used to form microemulsion with a broad range of oils without other additives and phase transition was observed by increasing NaCl concentrations. The optimum salinity which showed the lowest IFT for each oil was obtained. The linear regression equation (y = ax+b) from the plot between ln(S*) and EACN could be compared to the original HLD equation: ln(S*) = K(EACN)-Cc then the slope (a) and intercept (b) of the linear regression equation are K and Cc values of AMA. For mixed anionic surfactant, comparison between unknown-characteristic (K and Cc) surfactant and known-characteristic surfactant was done. After the optimum salinity was obtained from microemulsion phase studies, the HLD equation for mixed surfactant was modified to be Slope = $[(K_2-K_1)(EACN)+(Cc_1-Cc_2)]$. The plot between ln(S*) and mole fraction of unknown surfactant was carried out. Finally, all known parameters were substituted in the equation and Cc value of unknown surfactant was achieved. In conclusion, they found that HLD concept could be used as alternative method to determine hydrophilic-lipophilic nature of conventional and extended surfactants. Furthermore, the results showed that Cc value of extended surfactants suggests their behaviors better than HLB, and could be used in HLD concept for guiding the optimum formulation.

Moreover, Nguyen and Sabatini (2011) also used HLD concept (HLD = 0) to evaluate the characterization of rhamnolipid biosurfactant comparing to the conventional surfactant which is AMA (Cc value = -0.92). They found that Cc value of rhamnolipid was -1.41. The negative Cc value indicates that the surfactant is hydrophilic and rhamnolipid is more hydrophilic than AMA because of its larger negative Cc value. The results from the HLD concept were corresponded to experimental observation. Therefore, the Cc values of biosurfactants are useful to compare hydrophilic-lipophilic nature with synthetic surfactants and can be used to select the similar biosurfactans to replace the synthetic surfactants in microemulsion formation.

Do et al. (2014) determined the mixed surfactant system containing C10–18PO–2EO–$NaSO_4$, sodium dioctyl sulfocuccinate (SDOSS) and NaCl for cold temperature detergency of vegetable oils and semi-solid fats from fabric. They demonstrated that the optimum salinity for varying C10–18PO–2EO–$NaSO_4$ mole fractions in the mixture could be predicted by the HLD method ($HLD^*_{mix}=0$) at 25°C. Detergency performance was tested for the different mixed surfactant systems with canola oil compared to single surfactant system. The results showed that the surfactant mixtures had more than 90% canola oil detergency efficiency compared to 68% of SDOSS alone or 85% of C10–18PO–2EO–$NaSO_4$ alone. They suggested that the anionic surfactant mixtures showed synergism in detergency performance compared to using the individual surfactant. Moreover, they determined phase behaviors of coconut and palm kernel oils by fixing the mole fractions of each surfactant to demonstrate correlation between the phase behaviors of varying NaCl concentrations predicted from the HLD concept and the experimental phase behaviors. They found that the phase behaviors from the HLD concept and the experiments were correlated.

2.3.5. Surfactant enhanced soil washing

Surfactant application in environmental remediation is growing interested, for example in soil washing. Many studies have conducted soil washing to remove single and double components of petroleum hydrocarbons using surfactant solutions and concluded the contaminated soil characteristics must be investigated such as soil particle size distribution, organic and inorganic materials contents (Barathi and Vasudevan, 2001; Bhandari et al., 2000).

Two main steps are proposed as mechanisms behind surfactant enhanced oil removal from soil: mobilization and solubilization. The mobilization mechanism occurs at surfactant concentration below CMC. Some phenomena related with this step are reduction of IFT and surface tension, reduction of capillary force, wettability and reduction of contact angle. The reduced IFT and surface tension between air/water, oil/water, and soil/water systems occurs at concentration below CMC leading to the increased contact angle of soil/oil system and the reduced capillary force holding oil and soil together. However, surfactant adsorption on soils may result in the loss and reduction of surfactant concentration making less efficient soil treatment. Therefore, the mobilization depends on the surfactants’ ionic charge. On the other hand, surfactant concentration above CMC, the solubility of oil dramatically increases because of aggregation of surfactant micelles. The hydrophobic part of surfactant combine together inside the micelle structure with the hydrophilic part exposed to the aqueous phase at the external structure. As a result, the interior structure of micelles is a compatible environment for hydrophobic organic molecules and incorporation of these molecules into a micelle is known as solubilization (Urum and Pekdemir, 2004).

Other study also proposed mechanisms to remove oil droplet from the solid surface (Figure 2.5), for example solubilization, snap-off, and roll-up. The solubilization is occurred because the oil is dissolved in the hydrophobic core of micelles that are spontaneously formed when surfactant concentration is above the CMC. The snap-off mechanism is related to the mechanical agitation and the work of cohesion ($W_C=2\gamma_{O/W}$) of the droplet. When the mechanical agitation is stronger than the work of cohesion, the droplet is removed but some oil film is still attached to the solid surface. In the roll-up mechanism, the work of adhesion of the droplet ($W_A=\gamma_{O/W}(\cos\theta+1)$) to the surface is zero or negative ($\theta>90°$) that makes it easier for the mechanical forces to completely detach the oil droplet from the solid surface (Sabatini et al., 2001).

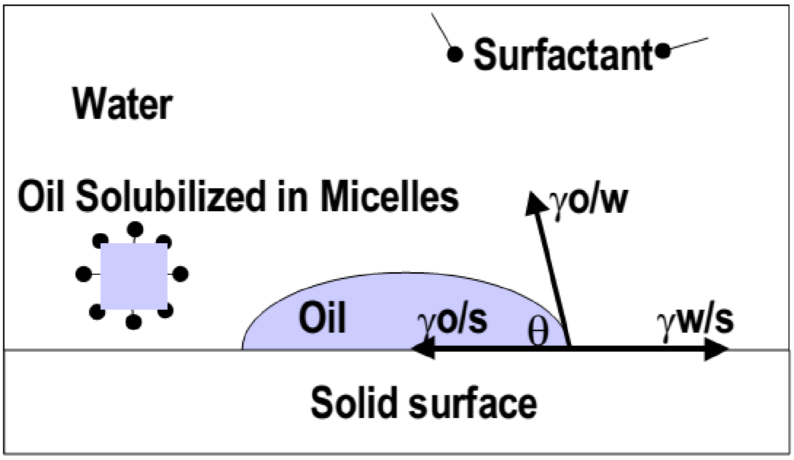


Figure 2.5 Oil on a solid surface (Sabatini et al., 2001)

Soil washing is an ex situ process which means the contaminated soil are excavated before the treatment. It is operated batch system at a certain solid/liquid ratio within the range 1-100% and 5-40% of extracting agents are applied to the system to remove the contaminants attached to soil. This process enhances the contact between extracting agents and soil contaminants; therefore, better treatment efficiency and shorter contact time are provided comparing to the soil flushing process (Trellu et al., 2015).

Several studies related to surfactant enhanced soil washing have been conducted. Urum and Pekdemir (2004) determined a possibility to apply various aqueous biosurfactant solutions in contaminated soil remediation comparing to sodium dodecyl sulphate (SDS) which is a well-known conventional surfactant. The selection of biosurfactant was based on their different origins for instance aescin are produced from seeds of the horse chestnut tree: *Aesculus hippocastanum* L. (Hippocatanacea), lecithin is derived from soybean, and rhamnolipid is a blend of $C_{26}H_{48}O_9$ and $C_{32}H_{58}O_{13}$. The surfactant concentrations were varied from 0.0001-0.5 mass-% to cover CMC of all surfactants used. The results demonstrated that crude oil in contaminated soil could be significantly removed by the biosurfactants, especially rhamnolipid which showed oil removal efficiency up to 80% similar to SDS. On the other hand, the oil removal efficiencies were reduced for aescin, lecithin, saponin, and tannin when the concentrations of them above CMC because their bulk structures may lead to micelle instability and reduction of detergency. However, high solubility of crude oil and ultralow IFT could not be achieved in the study; as a result, mobilization of oil was the main mechanism of the study.

In addition, Urum et al. (2006) studied surfactants from different origins for soil washing. All surfactant solution at 0.1 mass % showed the oil removal less than 50% because the more volatile components of the oil were lost due to evaporation, and the complex compounds existed after weathering process. They found that SDS, rhamnolipid, and saponin could remove crude oil from soils, but the surfactants showed different preferences of crude oil component removal; in other words, rhamnolipid and SDS removed more of the aliphatics than aromatic hydrocarbons while saponin removed higher amount of the aromatic hydrocarbons.

Khalladi et al. (2009) investigated soil column washing by SDS which showed ability to eliminate polycyclic aromatic hydrocarbons (PAHs). They found that 97% of diesel was eliminated after a soil washing by 8 mM SDS solution (CMC) with a rate 3.2 mL/min. When 5 days difference of contamination age was tested, there was no significant effect on the removal n-alkane from soil. Consequently, they suggested that the maximum tolerable time interval should be evaluated before doing remediation process.

Singh and Cameotra (2013) demonstrated that lipopeptide biosurfactant, consisting of surfactin and fengycin obtained from *Bacillus subtilis* A21 could be used as washing solution. The lipopeptide biosurfactant used in the study was prepared at different concentrations for example 0, 0.5×CMC, CMC, 10×CMC, and 50×CMC. The solubility of petroleum hydrocarbon was higher when the biosurfactant concentration was increased. The results showed that the 50×CMC biosurfactant removed significant amount of petroleum hydrocarbon (64.5 %) and metals namely cadmium (44.2 %), cobalt (35.4 %), lead (40.3 %), nickel (32.2 %), copper (26.2 %) and zinc (32.07 %) from contaminated soil. However, the chromatographic profile of the extracted total petroleum hydrocarbon (TPH) left in the soil after soil washing showed that the biosurfactant from *Bacillus subtilis* A21 could remove all fraction of TPH with equal efficiency. Therefore, the mixture of synthetic surfactants is suggested to increase the solubility of PAHs, and improve the performance of surfactant-enhanced soil washing (Zhu and Feng, 2003).

Influence of operating parameters in soil washing was studied. Yan et al. (2011) investigated the optimal conditions which gave the highest organics removal efficiency from contaminated drill cuttings; they found that the optimal conditions were L:S ratio, 3:1; washing time, 20 minutes; stirring speed, 200 rpm; rhamnolipid concentration, 360 mg/L; temperature, 60 °C. In addition, (Peng et al. (2011)) determined the effect of six influencing factors on the PAHs removal from a contaminated soil by using TW80 and TX100 surfactant washing. The conditions that could provide the removal efficiency 79% and 83% for TW80 and TX100, respectively were stirring speed, 250 rpm; surfactant concentration, 5 g/L; L/S, 10:1 for both TW80 and TX100; washing time, 60 and 30 min for TW80 and TX100, respectively.

2.4. Literature reviews

Application of surfactant technology in soil washing to remove petroleum hydrocarbons has been studied. The experiments were determined by using various kinds and concentrations of surfactant. Some of the studies were summarized in Table 2.4

Table 2.4 Summary of surfactant enhanced soil washing studies

Soil properties	Soil washing Condition	Contaminants	Surfactant and use	Effectiveness (%Removal)/ Findings	Ref.
Sand 54% Silt 33% Clay 13%	- 5 g soil with 100 mL surfactant solution were mixed in 250 mL glass vials at 21°C - The vials were shaken overnight by shaker.	Diesel 10,000 mg/kg	- 10^{-6} to 10^{-3} M Tergitol NP-10	Surfactant solutions below effective CMC can remove 20% diesel from soil in one step by mobilization mechanism.	Vreysen and Maes (2005)
Silt 6%, Sand 72% Gravel 22% pH 7.43	- 20°C - Shaken with 20 cm^3 of solutions at 200 strokes/min - 20 min.	Initial Ekofisk crude oil=92 mg/g soil After weathering process=87 mg/g	0.1% wt Rhamnolipid 0.1% wt Saponin 0.1%wt SDS	44% 27% 46%	Urum et al. (2006)

Table 2.5 Summary of surfactant enhanced soil washing studies (Cont.)

Soil properties	Soil washing Condition	Contaminants	Surfactant and use	Effectiveness (%Removal)/ Findings	Ref.
1. Sandy loam pH 6.9 Organic matter 0.9% 2. Silty clay pH 7.1 Organic matter 4.8%	- 5 g soil was added into conical flasks with 50 mL of solution, yielding a 0.1 (w/v) soil/solution ratio - The flasks were placed a rotary shaker at 100 rpm for 12 hr.	Uncontaminated soil	$5x10^{-5}$ to $1x10^{-2}$ M SDS $5x10^{-5}$ to $5x10^{-3}$ M AOT	Anionic surfactants can be weakly adsorbed onto negatively charged soil surfaces through interactions either OM or Ca^{2+} ions.	Hagenhoff et al. (2009)
1. Low TPH soil : 3000 mg/kg dry soil Sandy loam 2. High TPH soil : 9000 mg/kg dry soil Sandy loam	- 50 g contaminated soil were added into 500 mL flask with 100 mL surfactant solution - The flask was shaken in shaker at 25°C, agitation speed of 50 rpm, 24 hr. or 168 hr.	1. Low TPH soil 3000 mg/kg dry soil 2. High TPH soil 9000 mg/kg dry soil	0.05 %wt Surfactin	1. Low TPH soil 7.9% 2. High TPH soil 27.1%	Lai et al. (2009)

Table 2.6 Summary of surfactant enhanced soil washing studies (Cont.)

Soil properties	Soil washing Condition	Contaminants	Surfactant and use	Effectiveness (%Removal)/ Findings	Ref.
Gravel 13% Sand 67% Fine fraction 20%	- 6 g of contaminated soil was put into vials with 30 mL - flask was kept at 28ºC during 24 hr in rotational agitation shaker at 150 rpm	TPH-diesel 32,100 mg/kg	- 5 g/L Maranil LAB - 5 g/L Texapon 40 - 5 g/L SDS - 5 g/L Surfacpol G	- 78.51% - 71.27% - 60.13% - 48.19%	Hernández-Espriú et al. (2012)
Sandy loam (Industrial soil) pH 5.8	- 1 g soil mixed with 25 mL of solution - Agitation speed 200 rpm	Total petroleum hydrocarbon (TPH)=1886.2 mg/kg	50xCMC Lipopeptide	64.5%±6.2	Singh and Cameotra (2013)
1. Soil 1: Loam 2. Soil 2: Sand	- 1.5 g of contaminated soil and 50 mL washing solution were added into 100 mL conical flask with stopper - The content in the flask was shaken at 200 rpm for 24 hr at room temperature	crude oil 6g was dissolved in hexane and added into 150 g of unpolluted soil	0.01-0.1%wt rhamnolipid	n-C10, n-C12, n-C14, n-C16, n-C18 and n-C20 (i.e. 87.9%, 89.3%, 81.2%, 77.1%, 75.7%, 75.5% and 64.5%, respectively)	Zhang (2015)

CHAPTER 3

METHODOLOGY

3.1.Research overview

The research was divided into two phases including Phase I (part I): the determination of lipopeptide biosurfactant properties, Phase I (part II): the mixed surfactant formulations containing lipopeptide biosurfactant, AMA or AOT, and NaCl for hexane (EACN =6), decane (EACN =10), and hexadecane (EACN =16) which represent wide range of organic pollutants found in the environment (Nguyen et al., 2008). In this section, Hydrophilic-Lipophilic Deviation (HLD) concept and microemulsion formation were applied to optimize the surfactant systems. Phase II: .the evaluation of the surfactant formulations from the first phase as washing solutions to remove petroleum hydrocarbons in soil washing process. In soil washing, two stages were performed; 5% (w/w) artificially contaminated soil and 10%, 15% (w/w) artificially contaminated soil representing the high oil concentration from intentionally contamination. Flowchart of the research was illustrated in Figure 3.1.

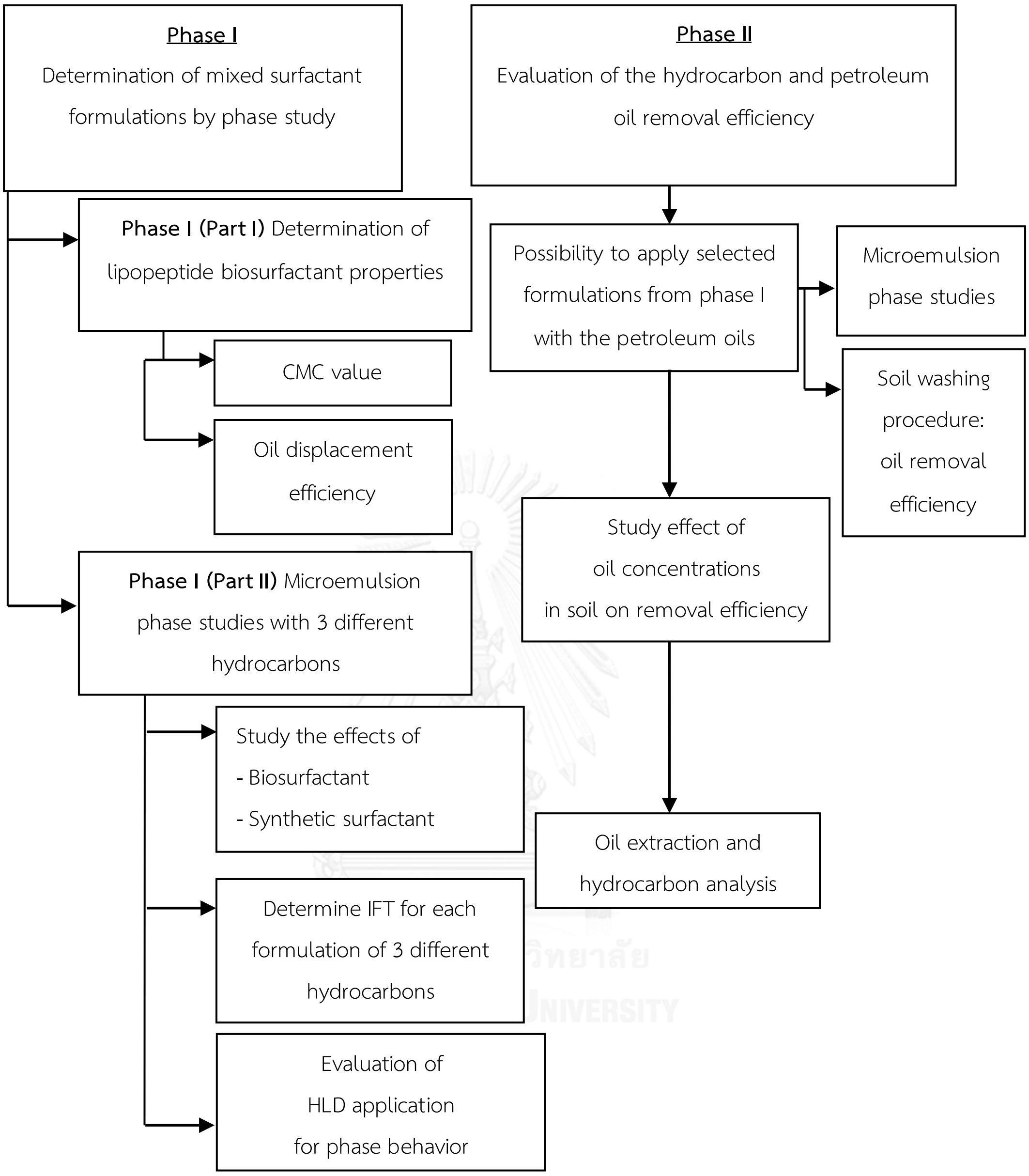


Figure 3.1 Experimental framework

3.2.Materials

3.2.1. Surfactants

3.2.1.1. Biosurfactant

Lipopeptide biosurfactant (Table 3.1, Figure 3.2a) is produced from *Bacillus* sp. GY19 and increased the purity by membrane filtration technique, Department of Microbiology, Faculty of Science, Chulalongkorn University. The biosurfactant is in form of aqueous solution (Figure 3.2b) that has biosurfactant concentration 7.6 g/L.

Table 3.1 Lipopeptide biosurfactant characteristics

Biosurfactant	Type	MW (g/mol)	K	Cc
Lipopeptide	anionic biosurfactant	1049	0.17(1)	4.93(2)

Note (1) Acosta and Bhakta (2008)

(2) Rongsayamanont (2015)

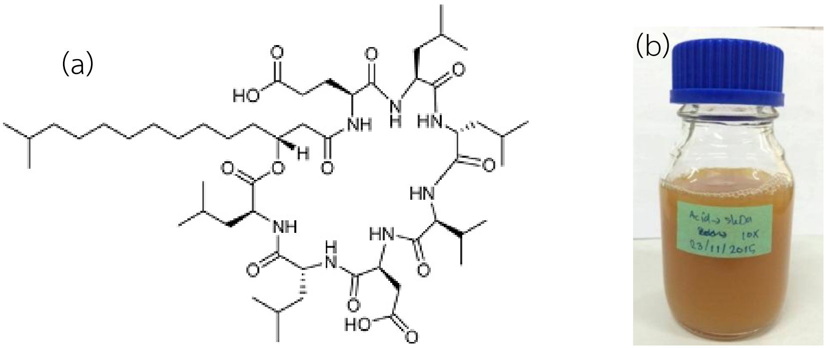


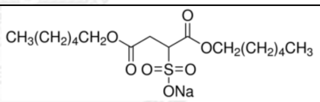
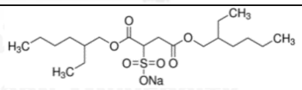
Figure 3.2 (a) Structure of ipopeptide or surfactin (b) Lipopeptide biosurfactant produced from *Bacillus* sp. GY19

3.2.1.2. Synthetic surfactants

The synthetic anionic surfactants (Table 3.2), dihexyl sulfocuccinate, sodium salt (AMA, 80% in water) and dioctyl sulfosuccinate, sodium salt (AOT, 96%, anhydrous) were purchased from Alfa Aesar (USA).

AMA and AOT have been used in various industrial applications such as emulsifiers, and environmental remediation such as soil washing. AMA has low toxicity and it is suitable for in situ remediation because its low affinity to soil and low degree of mineralization (Franzetti et al., 2006). In addition, AOT is not toxic to living organisms in soil and public health (Ramamurthy et al., 2015).

Table 3.2 Synthetic surfactant characteristics

Synthetic surfactant	Type	MW (g/mol)	Molecular structure	K	Cc
AMA	anionic	388.45		0.17(1)	-0.92(2)
AOT	anionic	444.56		0.17(1)	2.66(3)

Note (1) Acosta and Bhakta (2008)

(2) Kiran et al. (2009)

(3) Witthayapanyanon et al. (2008)

3.2.2. Petroleum hydrocarbons

Three hydrocarbons used as model oils in this study; hexane, decane, and hexadecane (see their properties in Table 3.3) were purchased from Alfa Aesar (USA). Generally, petroleum hydrocarbons have alkane carbon number (ACN) or equivalent alkane carbon number (EACN) of 7-8 and motor oil has EACN of 19; therefore, these three compounds represent a wide range of organic contaminants (Nguyen et al., 2008). Crude oils which are Bongkot crude oil, ARL/AXL crude oil and diesel oil were obtained from PTT Public Company Limited (Thailand). Their properties were shown in Table 3.4.

Table 3.3 Hydrocarbon properties (Nguyen et al., 2008)

Hydrocarbons	EACN	Density (g/mL)	Viscosity (cPs)	Aqueous solubility (%)	Vapor pressure (mmHg)
Hexane	6	0.664	0.32	0.014	124
Decane	10	0.728	0.92	Insoluble	1.3
Hexadecane	16	0.776	3.3	Insoluble	1 (at 105 °C)

Table 3.4 Crude oil properties characterized by TLC-FID

Crude oil	Hydrocarbon fraction (%)			
	Saturated	Aromatic	Resin	Asphaltenes
Bongkot light (BKC)	100	-	-	-
Arab light/ Arab extra light (ARL/AXL)	29	31	22	18
Diesel	70	14	5	12

3.2.3. Soil sample

Soil was collected from a non-contaminated area in Suphanburi province at 0.5 m. The soil properties were analyzed by Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand (Table 3.5). The soil sample was clay with very low organic matter, neutral pH, not saline, and high amount of calcium and magnesium. The samples were air-dried, homogenized, and sieved to obtain soil particle size 0.08-0.2 mm (Figure 3.3) for soil washing.

Artificially contaminated soil was prepared by thoroughly mixed the soil with the petroleum hydrocarbons to achieve the initial concentration at 5% (w/w) to cover the highest acceptable limit of an average total petroleum hydrocarbon (TPH) in soil. According to EPA, soil with an average TPH > 5% (w/w) is not acceptable for disposal at any Sanitation Districts' landfill. The spiked soil was allowed to stabilize for 2 days at room temperature prior to washing experiments (Zheng et al., 2012).

Furthermore, effect of higher oil concentration at 10% and 15% (w/w) was studied. The spiked soil was also allowed to stabilize for 2 days at room temperature prior to washing experiments (Zheng et al., 2012).

Figure 3.3 Soil sample used to generate artificially contaminated soil

Table 3.5 Soil properties

Parameter	Value
Soil texture	
Sand	13%
Silt	38%
Clay	49%
pH	7.1
Organic matter	0.54%
Electrical conductivity (25°C)	1.53 dS/m
Calcium	2034 mg/kg
Magnesium	730 mg/kg

3.2.4. Chemicals

All chemicals used as received are analytical reagent grade, for example, choloform and sodium chlorides. All solutions was prepared with deionized water with a resistance of 18 MΩ cm.

3.3.Methods

3.3.1. Determination of surfactant formulations

3.3.1.1. Biosurfactant properties

3.3.1.1.1 Oil displacement efficiency

Lipopeptide from membrane filtration was determined for oil displacement efficiency against several hydrocarbons and some petroleum oils. The experiment was done by adding 20 mL of distilled water to petri dish, followed by adding 20 µL of hydrocarbon to the surface of the water (Figure 3.4a). Ten µL of lipopeptide from membrane was dropped onto the surface of the petroleum oil layer (Figure 3.4b). The diameter of the clear zone was measured to calculate oil displacement efficiency compared with the diameter of the surface of water using the equation as follow:

Oil displacement (%) = (Diameter of clear zone/water surface) × 100

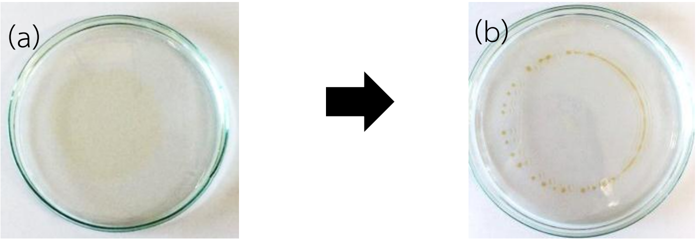


Figure 3.4 Oil displacement experiment of Bongkot crude oil

(a) Before dropping biosurfactant (b) After dropping biosurfactant

3.3.1.1.2 Critical Micelle Concentration (CMC)

Lipopeptide was diluted into different dilution factor. Then, the surface tension was measured for each dilution by a digital tensiometer (Kruss K10ST, Germany) at 25 °C using the plate method. CMD values were obtained from the cross section of the plot between surface tension and serial dilution of samples of the lipopeptide from membrane filtration. Finally, CMD was converted to CMC by multiplying CMD value with initial biosurfactant concentration.

3.3.1.2. Microemulsion formation by HLD concept

Microemulsion formation was created by using HLD concept. In this study, all parameters in HLD concept were considered in the mixed surfactant system, for example NaCl concentration, the surfactant characteristics such as K and Cc (Tables 3.1, and 3.2), hydrophobicity of the oil phase as EACN value (Table 3.3), and molar fraction of each surfactant in the system. HLD equation for anionic-anionic surfactant mixtures is provided:

$$HLD_{mix} = \ln(S) - (K \times EACN) + X_1Cc_1 + X_2Cc_2$$

whereas:

S represents salinity concentration (g/100mL)

K represents the K value of surfactant showing the hydrophilicity

EACN is Equivalent Alkane Carbon Number of the oil phase

X_1, X_2 represent the molar fraction of each surfactant

Cc_1, Cc_2 represent the Cc values of each surfactant showing the hydrophobicity

Known values of K, Cc, EACN, and molar fraction of surfactants were added into the equation. Then, the optimum salinity or S* of NaCl (g/100 mL) for each mixture was calculated from HLD_{mix} =0 (Tables 3.6, 3.7, 3.8) which is considered as the optimum condition where the system transfer to be microemulsion type III and yield the lowest interfacial tension (IFT).

Furthermore, microemulsion phase behavior was evaluated for each mixture to determine whether microemulsion type III occurs or not. Phase studies were conducted by adding equal volumes of the aqueous and oil phases (0.5 mL of each phase) in 1 mL glass tubes (diameter of 6 mm) with caps (Figure 3.5). The tubes was gently hand-shaken for 1 minute once a day for the first 3 days and left for reaching equilibrium conditions for 2 weeks. Phase studies were conducted for three hydrocarbons (hexane, decane, and hexadecane) with different EACN (6, 10, and 16) and also tested with three petroleum oils (Bongkot crude oil, ARL/AXL crude oil, and diesel).

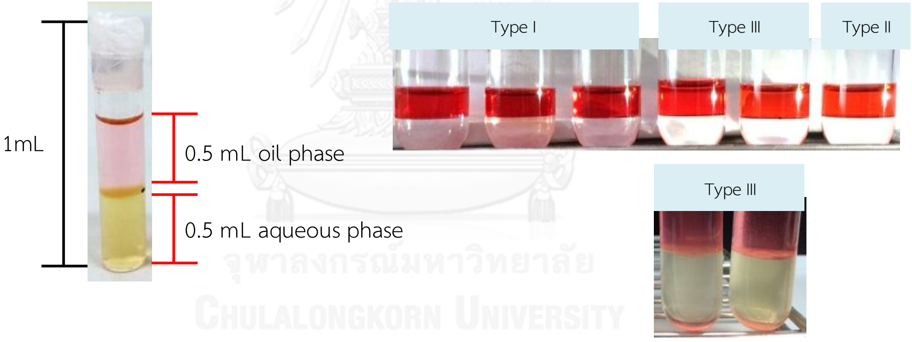


Figure 3.5 Phase behavior study

Table 3.6 Different surfactant formulations for hexane (EACN = 6) at total surfactant concentration 0.1 M

EACN	Biosurfactant concentration		AMA concentration	Molar fraction		Optimum NaCl
	xCMC	mM	mM	Biosurfactant	AMA	(g/100 mL)
6	0	0.00	100	0.0000	1.0000	6.96
6	10	0.55	99.45	0.0055	0.9945	6.74
6	50	2.75	97.25	0.0275	0.9725	5.92
6	90	4.95	95.05	0.0495	0.9505	5.21

Table 3.7 Different surfactant formulations for decane (EACN = 10) at total surfactant concentration 0.1 M

EACN	Biosurfactant concentration		AMA concentration	Molar fraction		Optimum NaCl
	xCMC	mM	mM	Biosurfactant	AMA	(g/100 mL)
10	0	0.00	100	0.0000	1.0000	13.74
10	10	0.55	99.45	0.0055	0.9945	13.30
10	50	2.75	97.25	0.0275	0.9725	11.69
10	70	3.85	96.15	0.0385	0.9615	10.28

Table 3.8 Different surfactant formulations for hexadecane (EACN = 16) at total surfactant concentration 0.1 M

EACN	Biosurfactant concentration		AOT concentration	Molar fraction		Optimum NaCl
	xCMC	mM	mM	Biosurfactant	AOT	(g/100 mL)
16	0	0.00	100	0.0000	1.0000	1.06
16	10	0.55	99.45	0.0055	0.9945	1.05
16	50	2.75	97.25	0.0275	0.9725	1.00
16	90	4.95	95.05	0.0495	0.9505	0.95

3.3.1.3. Interfacial tension (IFT) measurement

The IFT between the aqueous surfactant solution and the oil phase was measured using a spinning drop tensiometer (Dataphysics, model SVT20) (Figure 3.6b). A small amount of petroleum hydrocarbon (light phase) was injected into the tube filled with the surfactant solution (heavy phase). The spinning speed was set at 6000 rpm. All the measurements were done in triplicate at 25±1 °C.

For equilibrium IFT, the aqueous surfactant solution was mixed with the hydrocarbon and left to equilibrate for two weeks (Figure 3.6a) before the IFT between the excess aqueous phase and the excess oil phase was measured.

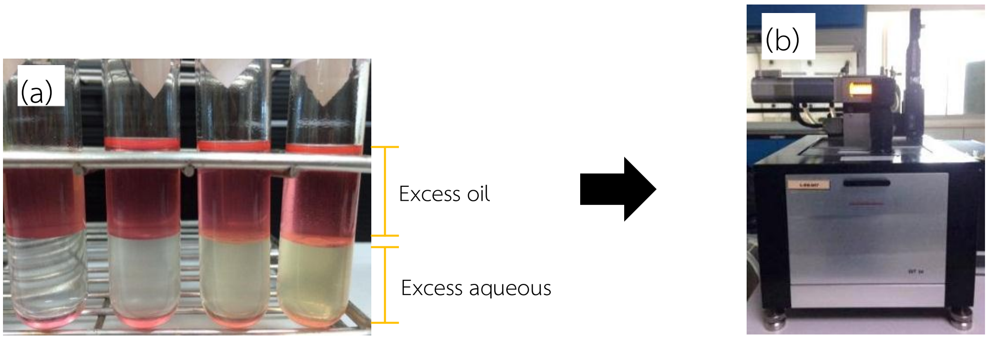


Figure 3.6 (a) Equilibrium phase studies and (b) spinning drop tensiometer

3.3.1.4. HLD evaluation for single and mixed surfactant systems

Due to HLD equation, it is used to predict microemulsion type formation (Witthayapanyanon et al., 2008). Therefore, it is interesting to evaluate its efficiency in both single and mixed surfactant systems.

Phase studies were done for single surfactant system by fixing total concentration of AMA at 0.1 M, and varying NaCl concentration (%). On the other hand, phase studies were done for mixed surfactant system by fixing total concentration of AMA and lipopeptide at 0.1 M, and varying NaCl concentration (%). Phase behavior was observed. IFT of each surfactant system was determined to demonstrate relationship between microemulsion formation and IFT changes.

3.3.2. Application of the surfactant formulations in soil washing

The possibility to apply the formulations from previous steps with petroleum oils was investigated by comparing the results of microemulsion phase studies between two groups: hydrocarbons (hexane, decane, and hexadecane), and petroleum oils (crude oils, and diesel oil). The suitable formulations for petroleum oils were selected based on microemulsion phase observation. Microemulsion type III was selected as the optimum condition between oil and aqueous phases and it was expected to give the lowest IFT. Then, the selected formulations were prepared as washing solutions applied in soil washing experiments.

3.3.2.1. Phase behavior of petroleum oils

Phase studies were conducted by adding equal volumes of the aqueous and oil phases (0.5 mL of each phase) in 1 mL glass tubes (diameter of 6 mm) with caps. The tubes were gently hand-shaken for 1 minute once a day for the first 3 days and left for reaching equilibrium conditions for 2 weeks.

3.3.2.2. Soil washing

Soil washing was performed in laboratory scale. The contaminated soil at 10 g and 20 mL of washing solution were added into a glass bottle. A control test was performed by mixing 10 g of soil with 20 mL of distilled water. The bottles were placed on a shaker. Then, these following factors were set: shaking speed 200 rpm, and shaking time 30 minutes (Urum and Pekdemir, 2004). After washing, the contents of the bottle were settled by centrifugation at 3600 rpm over 15 minutes (Figure 3.7). The washing solution was separated from the bottle. The soil was rinsed with distilled water for two times to remove the remaining oil, washing solution, and to prevent an emulsion formation when extracting with solvent.

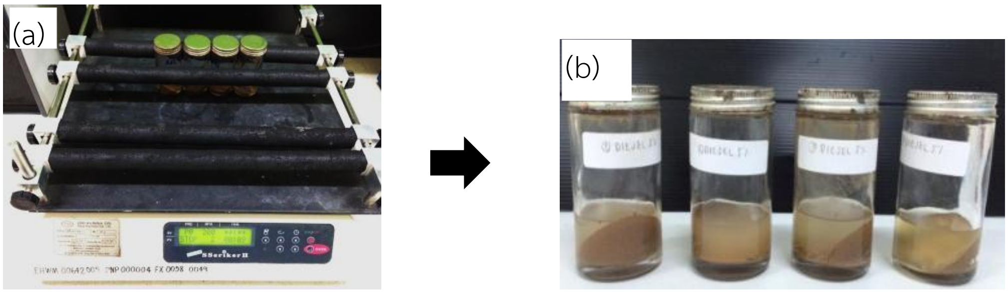


Figure 3.7 Soil washing experiment (a) washing in laboratory scale (b) settlement of soil after washing

3.3.2.3. Oil extraction and hydrocarbon analysis

Petroleum hydrocarbons in the soil samples were extracted by chloroform at ratio 1:1 (w/v) and stearyl alcohol (12.5 mg/mL) was added into extraction solvent as internal standard. Then, the solvent was evaporated. The amount of petroleum oil was analyzed by TLC-FID. The analytical conditions were done followed Khondee (2013). The peak areas of all presented fractions were combined to represent the amount of total petroleum hydrocarbons (TPH). Removal efficiency of each formulation was determined by determination of TPH left in soil after washing compared to initial TPH in soil.

%Removal efficiency = [(initial TPH -TPH left in soil after washing)/initial TPH] x 100

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Lipopeptide biosurfactant properties

Lipopeptides are most commonly produced and characterized biosurfactants. They show remarkable surface activities and biological activities such as crude oil recovery, removal of petroleum hydrocarbon and heavy metals from contaminated soils (Bezza and Chirwa, 2015). In this part, surface properties of lipopeptide biosurfactant produced from *Bacillus* sp. GY19 were determined, for example oil displacement efficiency and critical micelle concentration (CMC) to investigate its potential properties for further application.

4.1.1. Oil displacement efficiency

Oil displacement efficiency was determined as a primary surface activity of surfactant. The diameter of the clear zone on the oil surface was measured and related to the activity and concentration of biosurfactant (Bezza and Nkhalambayausi Chirwa, 2015). Lipopeptide biosurfactant, partially purified by membrane filtration technique showed 76%, 65%, 67%, and 75% oil displacement efficiencies with decane, dodecane, tetradecane, and hexadecane, respectively (Figure 4.1). Consequently, lipopeptide was effective and may be used to remediate hydrocarbon contaminated environment. In addition, it showed 61% and 25% oil displacement efficiencies with Bongkot crude oil and ARL/AXL crude oil, respectively. Oil displacement efficiency of lipopeptide from membrane with Bongkot and ARL/AXL which are light crude oil was lower than the efficiency of foamate with

Arabian light crude oil (76%) reported by Khondee et al. (2015). The different oil displacement efficiencies could be caused by the different properties of the tested oil, for example density and complexity. However, the addition of other components such as electrolyte or co-surfactant can improve the surface activity of biosurfactant (Khondee et al., 2015)

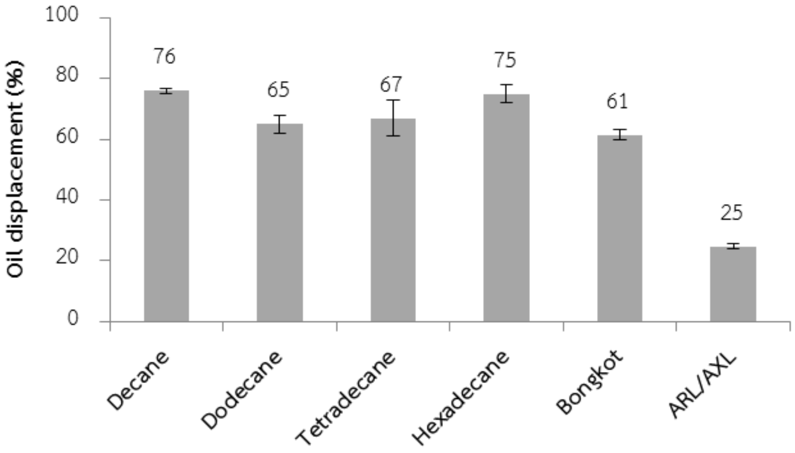


Figure 4.1 Oil displacement efficiencies of lipopeptide biosurfactant were determined with various hydrocarbons.

4.1.2. Critical Micelle Concentration (CMC)

CMC is the concentration at that the surface is fully occupied with surfactant molecules and a cluster of surfactant or a micelle is initially formed in solution (Dalili et al., 2015). CMC value of lipopeptide in this study was 0.058 g/L with the surface tension of 32 mN/m (Table 4.1). The result corresponds to the previous studies that lipopeptide (surfactins) could reduce the surface tension of water from 72 mN/m to 25-30 mN/m (Shakerifard et al., 2009; Singh and Cameotra, 2013). It is related to the amphiphilic property composing of the long chain fatty acid and some lipophilic amino acids as a hydrophobic part, and two acidic amino acid residues as a hydrophilic part (Shakerifard et al., 2009). Lipopeptide in this study showed lower CMC than lipopeptide produced from *Bacillus subtilis* ATCC 21332 (Marin et al., 2015). However, CMCs of lipopeptide in several studies might be different due to microorganism strain, culture conditions, substrates, structural components e.g. the fatty acid chain length, and contents of the surface active compound (Liu et al., 2015b; Marin et al., 2015; Shakerifard et al., 2009; Singh and Cameotra, 2013). In addition, CMD, which is the dilution necessary to reach CMC of lipopeptide in this study (131x CMD) was 7 times greater than the lipopeptide in cell free both from *Paenibacillus dendritiformis* CN5 (Bezza and Nkhalambayausi Chirwa, 2015) and higher than foamate which had 23x CMD (Khondee et al., 2015). The differences of biosurfactant activity may be from biosurfactant production such as optimal addition of media components and selection of the optimal culture conditions. Furthermore, purification procedure is an important factor. Membrane ultrafiltration has been recognized that it provides a high degree of surfactant purity (Chen et al., 2015; Ines and Dhouha, 2015).

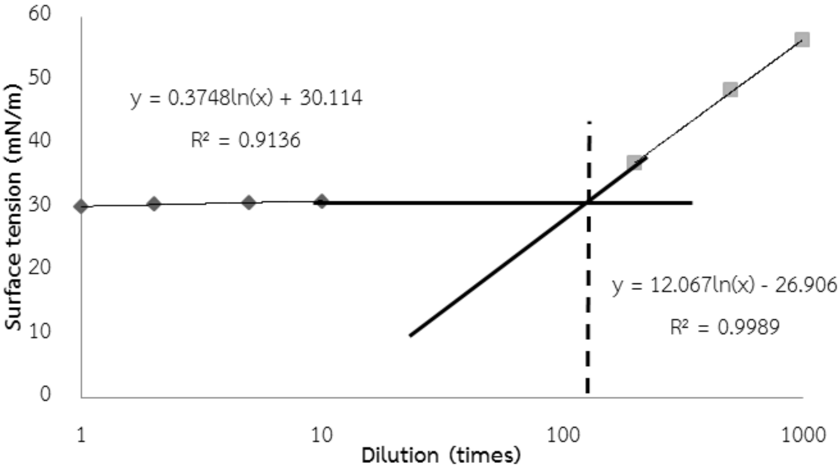


Figure 4.2 Critical Micelle Dilution (CMD) of lipopeptide biosurfactant

Table 4.1 Lipopeptide produced from *Bacillus* sp. GY19 properties

Biosurfactant concentration (g/L)	CMD in water system (dilution times)	CMC in water system (g/L)	Surface tension (mN/m)
7.6	131x	0.058	32

These results (oil displacement efficiency and CMC) demonstrate that lipopeptide from membrane filtration technique showed the surface activity with various hydrocarbons, while its activity with the complex petroleum oils such as ARL/AXL and diesel should be improved by mixing with other components. Due to the high purity of lipopeptide, it provided the low CMC indicating that less surfactant is required to saturate interfaces and form micelles. Therefore, lipopeptide could be applied to produce surfactant formulations for different petroleum hydrocarbons.

4.2. Surfactant formulations of microemulsion by HLD concept

Lipopeptide biosurfactant has a surface active property and can be used to remove petroleum hydrocarbons from contaminated soils. However, mixing lipopeptide with a synthetic surfactant is expected to provide the proper hydrophobic-hydrophilic condition of surfactant system leading to low interfacial tension (IFT) against non-aqueous phase liquids (NAPLs) (Sabatini et al., 2006). Two synthetic anionic surfactants with known characteristic curvature or Cc value; AMA (Cc = -0.92) and AOT (Cc = 2.66) were selected for this study. Compared to lipopeptide biosurfactant which has Cc value of 4.93 (Rongsayamanont, 2015), both AMA and AOT are considered more hydrophilic than lipopeptide. Electrolyte is also an important factor to increase solubility of organic compounds

(Peter and Singh, 2014) and NaCl is chosen. Microemulsion is an approach that could generate the optimum condition between oil and aqueous phases. Phase behavior is determined to observe hydrophilicity and hydrophobicity of the surfactant system with target oils. Microemulsion approach is introduced since it can provide low IFT which is an important parameter govern the performance of the oil removal from contaminated soil.

4.2.1. Preparation of middle phase (type III) microemulsion by HLD concept for hydrocarbon with EACN=6

In this experiment, known values of parameters namely K, EACN, molar fraction and Cc values of each surfactant were substituted into the HLD equation. Then, optimum NaCl for each formulation was calculated from HLD =0. Lipopeptide concentrations were varied into 10, 50, 90 times of its CMC. Total surfactant concentration was 0.1 M due to the limited amount of biosurfactant produced. It can be observed that the requirement of NaCl in the system decreased as the lipopeptide concentration increased (Table 4.2). The reason is lipopeptide added is hydrophobic similar to the addition of NaCl which also increase hydrophobicity of the systems. Electrolytes are essential in microemulsion formation using anionic surfactants because it reduces the CMC and increases the micellar aggregate size (Liu et al., 2015a).

According to a previous research of Rongsayamanont (2015), it was found that phase behavior of lipopeptide mixtures with Bongkot crude oil (BKC) was similar to that of hexane. Therefore, it may be assumed that BKC has EACN ranging from 6 to 9. BKC was used to demonstrate possibility to apply formulations from model hydrocarbon to other complex petroleum oils which have similar EACN.

Table 4.2 Concentrations of lipopeptide biosurfactant, AMA, and optimum NaCl calculated from HLD =0 for hexane (EACN=6)

No.	Lipopeptide conc. (xCMC)	Lipopeptide conc. (mM)	AMA conc. (mM)	S* (NaCl) (g/100mL)
FH-S	0	0	100	6.96
FH-M1	10	0.55	99.45	6.74
FH-M2	50	2.75	97.25	5.92
FH-M3	90	4.95	95.05	5.21

Note: FH =*F*ormulations calculated from *H*exane (EACN =6)

S =Single surfactant

M1 =Mixed surfactant containing 10xCMC lipopeptide

M2 =Mixed surfactant containing 50xCMC lipopeptide

M3 =Mixed surfactant containing 90xCMC lipopeptide

To prove that microemulsion type III could occur at the HLD =0, phase studies of the formulations were performed with hexane and Bongkot crude oil. Phase behaviors showed that all formulations formed the micoemulsion type III with both hexane and Bongkot crude oil (Table 4.3, Figure 4.3). These results indicate that the surfactant systems are suitable for short chain hydrocarbons. In addition, the IFT measurement was done to investigate the ability of surfactant formulations in lowering the IFT of hexane and BKC. It was found that the mixed surfactant system (FH-M1, 2, 3) could reduce the IFT better than FH-S (AMA alone) or the single surfactant system (Figure 4.4). Moreover, the mixtures that contained lipopeptide at 50x and 90xCMC could reduce IFT to be lower than 0.1 mN/m, thus high concentration of lipopeptide was required. It was corresponded to Nguyen and Sabatini (2008), who found that the efficiency of the mixed surfactant system between hydrophilic-hydrophobic surfactants could improve the IFT of the systems with various LNAPLs to be lower than 0.1 mN/m.

Table 4.3 Microemulsion formation of each formulation at total concentration of 0.1 M with hexane and Bongkot crude oil

No.	Microemulsion type	
	Hexane	Bongkot crude oil
FH-S	III	III
FH-M1	III	III
FH-M2	III	III
FH-M3	III	III

Note: FH =*F*ormulations calculated from *H*exane (EACN =6)

S =Single surfactant

M1 =Mixed surfactant containing 10xCMC lipopeptide

M2 =Mixed surfactant containing 50xCMC lipopeptide

M3 =Mixed surfactant containing 90xCMC lipopeptide

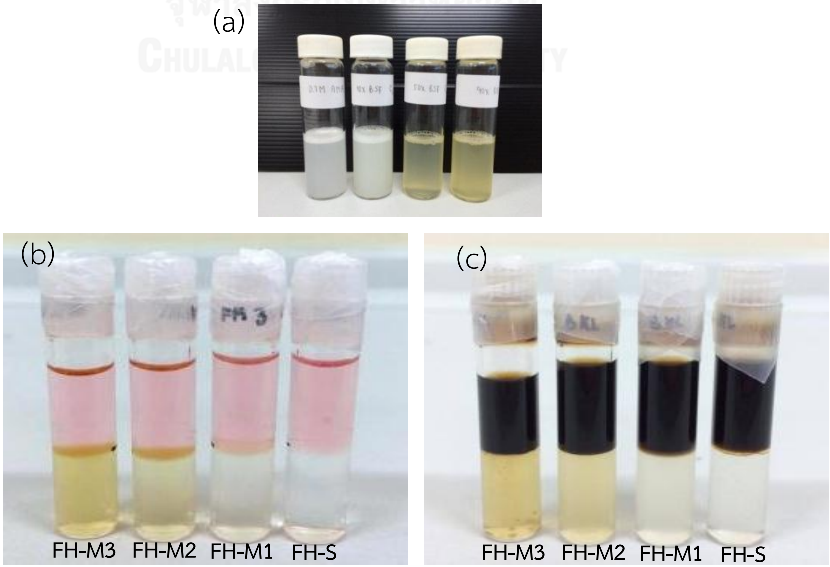


Figure 4.3 (a) Surfactant solutions (b) Phase behavior of hexane at different formulations (c) Phase behavior of Bongkot crude oil at different formulations

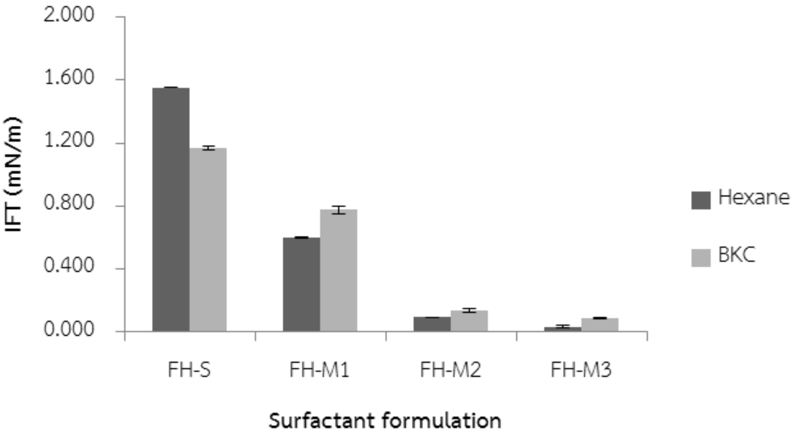


Figure 4.4 The equilibrium IFT measurement of each surfactant formulation at total concentration 0.1 M with hexane and Bongkot crude oil (BKC)

4.2.2. Preparation of middle phase (type III) microemulsion by HLD concept for hydrocarbon with EACN=10

Phase behaviors (Table 4.5, Figure 4.5) showed that the system of single surfactant; AMA (FD-S) could not form microemulsion with both decane and diesel. However, once lipopeptide was mixed with AMA at 0.55 and 2.75 mM (FD-M1, and FD-M2, respectively), microemulsion type III could be formed with both decane and diesel. In addition, FD-M3 which has higher ratio of lipopeptide demonstrated microemulsion type II with both types of oil. In this experiment, the FD-M3 mixed the ratio of lipopeptide up to 70xCMC because at the ratio 90xCMC of lipopeptide the surfactant solution had precipitated. The formation of microemulsion type II of the FD-M3 formulation indicated that the surfactant solution is more hydrophobic than the target oils. In other words, the higher the concentration of lipopeptide, the hydrophobicity of the surfactant solution is higher. Therefore, FD-M1 and FD-M2 were considered as the suitable formulations for decane and diesel. Moreover, the mixed surfactant system (FD-M1 and FD-M2) could decrease the IFT better than FD-S or the single surfactant system (Figure 4.6). The results indicate that the lipopeptide mixtures had ability to form microemulsion with decane which is medium hydrophobic at total concentration 0.1 M, but the AMA system may need higher NaCl concentration to increase ability to solubilize and interact with more hydrophobic hydrocarbon. Nguyen et al. (2008) found that the optimum formulations of surfactant mixtures for decane required more fraction of hydrophobic surfactant to give an appropriate hydrophilic-hydrophobic balance and to achieve ultralow IFT. Moreover, Sabatini et al. (2006) also found that mixing lipopeptide with the more hydrophilic rhamnolipid was an effective way to achieve low IFT against hexane (EACN=6) and decane (EACN=10), and the hydrophobicity of the system should be increased for higher EACN hydrocarbons. They concluded that the mixture of lipopeptide (strain ROGG-2), 5% NaCl and 50% C12, C13-8PO-SO4Na could give IFT at 0.013 mN/m against decane.

Diesel is previously reveal to has EACN around 19 (Nguyen et al., 2008) Hence it was tested with the formulations calculated for hexadecane but they showed differences in phase behavior. As a result, diesel was tested with the formulations calculated for decane and the results indicate that diesel had a phase behavior more similar to decane. It may be from compositions of diesel containing different hydrocarbons and some additives. As a result, EACN may be used as a basic parameter for some oil to predict their suitable formulations but phase behavior should be checked for ensuring the results.

Table 4.4 Concentrations of lipopeptide biosurfactant, AMA, and optimum NaCl calculated from HLD =0 for decane (EACN=10)

No.	Lipopeptide conc. (xCMC)	Lipopeptide conc. (mM)	AMA conc. (mM)	S* (NaCl) (g/100mL)
FD-S	0	0	100	13.74
FD-M1	10	0.55	99.45	13.30
FD-M2	50	2.75	97.25	11.69
FD-M3	70	3.85	96.15	10.28

Note: FD =*F*ormulations calculated from *D*ecane (EACN =10)

S =Single surfactant

M1 =Mixed surfactant containing 10xCMC lipopeptide

M2 =Mixed surfactant containing 50xCMC lipopeptide

M3 =Mixed surfactant containing 70xCMC lipopeptide

Table 4.5 Microemulsion formation of each formulation at total concentration 0.1 M with decane, diesel, and ARL/AXL crude oil

No.	Microemulsion type		
	Decane	Diesel	ARL/AXL
FD-S	No ME	No ME	*
FD-M1	III	III	*
FD-M2	III	III	No ME
FD-M3	II	II	No ME

Note: FD =*F*ormulations calculated from *D*ecane (EACN =10), * = droplets

S =Single surfactant

M1 =Mixed surfactant containing 10xCMC lipopeptide

M2 =Mixed surfactant containing 50xCMC lipopeptide

M3 =Mixed surfactant containing 70xCMC lipopeptide

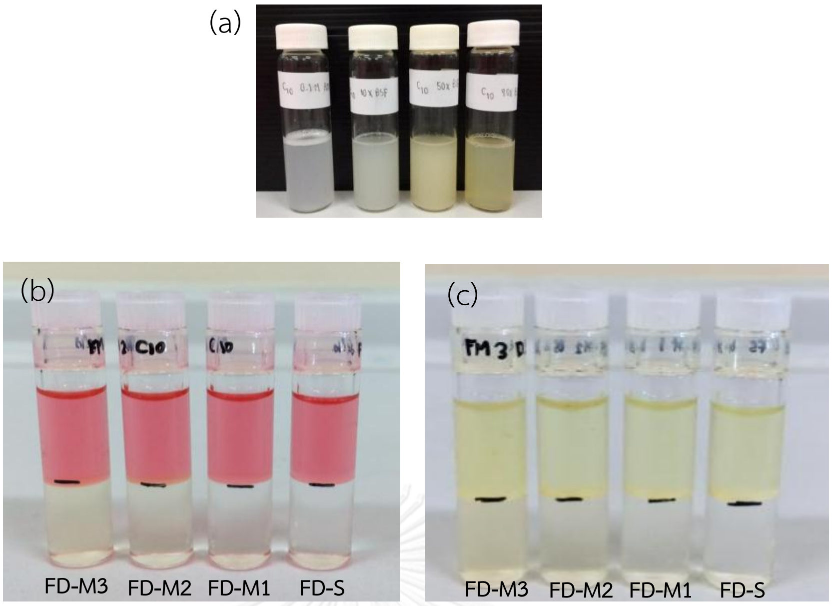


Figure 4.5 (a) Surfactant solutions and (b) Phase behavior of decane at different formulations and (c) Phase behavior of diesel at different formulations

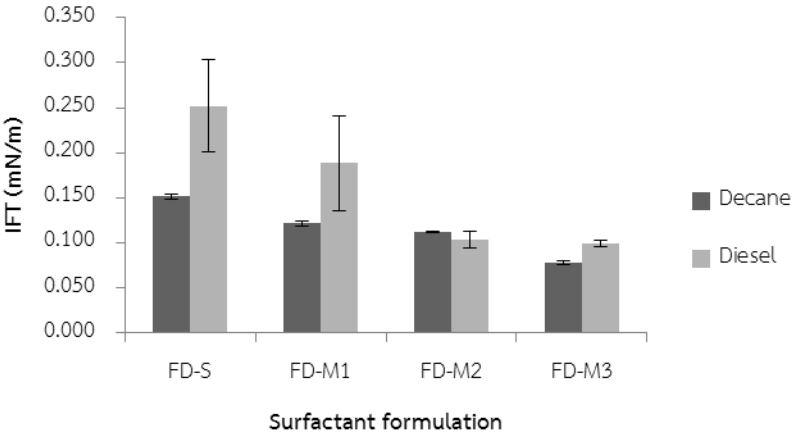


Figure 4.6 The equilibrium IFT measurement of each surfactant formulation at total concentration 0.1 M with decane and diesel

According to Rongsayamanont (2015), the phase behavior of ARL/AXL crude oil was similar to that of decane. Therefore, it may be assumed that ARL/AXL has EACN ranging from 10 to 12. But when phase studies of mixtures (FD-S, FD-M1) were applied with ARL/AXL crude oil, some precipitates or small droplets appeared. It was occurred immediately and remained stable after 24 hours (Figure 4.7). It could be from the compositions of ARL/AXL crude oil which is the complex mixture of hydrocarbons namely saturates, aromatic, resin, and asphaltenes (Table 3.4). Therefore, some fractions such as wax may precipitate into a particulate solid which could occur at low temperatures, and is realized as emulsion stability properties of crude oil systems (Aske, 2002).

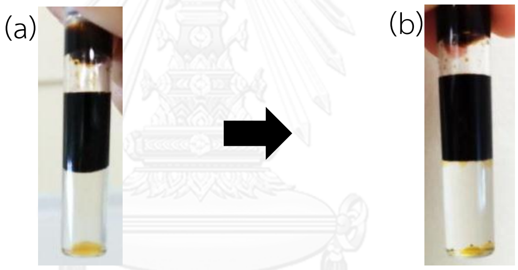


Figure 4.7 Phase study of FD-M1 with ARL/AXL crude oil (a) immediately and (b) after 24 hours

4.2.3. Preparation of middle phase (type III) microemulsion by HLD concept for hydrocarbon with EACN=16

Hexadecane is a high EACN hydrocarbon. Therefore, AOT which is more hydrophobic than AMA was used to mix with lipopeptide in order to decrease to increase hydrophobicity of the mixed surfactant system to reduce NaCl concentration needed for the system to transfer to be microemulsion type III.

The results of phase behavior were shown in Table 4.7. Olefins which contains C9-C21 was selected to test possibility to apply the formulations of hexadecane with other complex hydrocarbons instead of diesel. The results showed that with the formulation of FHD-S and FHD-M1, the systems did not follow the HLD prediction to be microemulsion type III. However, once lipopeptide ratio was increased as in the formulation of FHD-M2 and FHD-M3, microemulsion type III occurred for both hexadecane and olefins. This result indicates that to facilitate oil to from middle phase (microemulsion type III) with a surfactant formulation do not govern only by hydrophobic and hydrophilic balance of the system but the curvature of surfactant is even more important, especially for very hydrophobic oil. However, it was found that middle phases of both hexadecane and olefins were denser than aqueous phase and settled down to the bottom of the tube (Figure 4.8). This denser phase was confirmed to be microemulsion type III by observing the transparent using shooting laser. The light from the shooting laser passed that phase without scattering. The settlement of middle phase may be from some impurity or composition which has high density. According to the previous research, a middle phase could sunk to the bottom of vial because relatively high densities of both surfactant and oil containing in that middle phase (Kiran et al., 2009).

Table 4.6 Concentrations of lipopeptide biosurfactant, AMA, optimum NaCl calculated from HLD =0 for hexane (EACN=16)

No.	Lipopeptide conc. (xCMC)	Lipopeptide conc. (mM)	AOT conc. (mM)	S* (NaCl) (g/100mL)
FHD-S	0	0	100	1.06
FHD-M1	10	0.55	99.45	1.05
FHD-M2	50	2.75	97.25	1.00
FHD-M3	90	4.95	95.05	0.95

Note: FHD =*F*ormulations calculated from *H*exa*D*ecane (EACN =16)

S =Single surfactant

M1 =Mixed surfactant containing 10xCMC lipopeptide

M2 =Mixed surfactant containing 50xCMC lipopeptide

M3 =Mixed surfactant containing 90xCMC lipopeptide

Table 4.7 Microemulsion formation of each formulation at total concentration 0.1 M with hexadecane and olefins

No.	Microemulsion type	
	Hexadecane	Olefins
FHD-S	I	I
FHD-M1	I	I
FHD-M2	III**	III**
FHD-M3	III**	III**

Note: FHD =*F*ormulations calculated from *H*exa*D*ecane (EACN =16)

** = precipitated middle phase, S =Single surfactant

M1 =Mixed surfactant containing 10xCMC lipopeptide

M2 =Mixed surfactant containing 50xCMC lipopeptide

M3 =Mixed surfactant containing 90xCMC lipopeptide

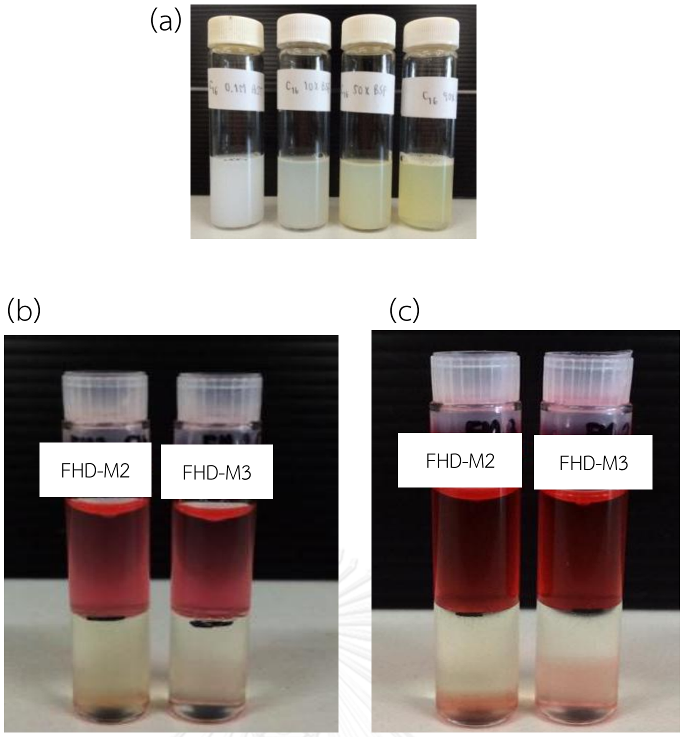


Figure 4.8 (a) Surfactant solutions (b) Phase behavior of hexadecane at different formulations (c) Phase behavior of olefins (C9-C21) at different formulations

4.2.4. Evaluation the middle phase formation of HLD prediction

To formulate surfactant systems for different hydrocarbons, HLD concept requires EACN value, molar fraction of surfactants, surfactant characteristics such as K and Cc value to submit in the equation and then calculate the optimum salinity for each system. The fraction of each surfactant and surfactant nature were important, for example more lipopeptide added leads to lower NaCl needed due to their similar property to increase hydrophobicity of the system. Moreover, the surfactant mixture can produce more desirable surfactant system to target hydrocarbon, for example balancing hydrophilic-hydrophobic to the target hydrocarbon (Nguyen et al., 2008).

According to the previous experiments, four different formulations for each hydrocarbon (one single surfactant system, and three mixtures containing 10x, 50x, and 90xCMC lipopeptide) were evaluated if they could form microemulsion type III with various oil following the HLD prediction. In this part, the optimum surfactant mixtures of hexane and decane providing the lowest IFT and microemulsion type III occurred were selected to compare with the single surfactant of each hydrocarbon (Table 4.8). On the other hand, the optimum formulation of hexadecane was chosen from the formulation giving a bigger middle phase because of its settle down. The results showed that the lipopeptide mixtures could reduce IFT between oil and aqueous phases better than the single surfactant formulations. In addition, the formulation FH-M3 could provide the ultralow IFT (<0.1 mN/m) and microemulsion type III with hexane and Bongkot crude oil. However, the ultralow IFT for higher EACN hydrocarbons such as decane may be received by adjusting the fraction of hydrophobic surfactant to get proper balance between oil and surfactant system (Nguyen et al., 2008). The details of each formulation could be seen in Tables 4.2, 4.4, and 4.6.

Table 4.8 Comparison of single surfactant systems and the optimum surfactant mixtures for different hydrocarbons

Hydrocarbon (HC)	**Petroleum oil**	**Formulation**		**ME type**	**IFT (mN/m)**	
		No.	**S* (g/100mL)**		**HC**	**Petroleum oil**
Hexane	Bongkot	FH-S	6.96	III	1.551	1.171
(EACN =6)	crude oil	FH-M3	5.21	III	0.035	0.089
Decane	diesel	FD-S	13.74	No	0.151	0.252
(EACN =10)		FD-M2	11.69	III	0.139	0.104
Hexadecane	-	FHD-S	1.06	No	-	-
(EACN =16)		FHD-M3	0.95	III	-	-

IFT measurement and phase behavior relationship were determined to evaluate HLD equation in predicting microemulsion type or optimum condition. In this part, 0.1 M AMA was used as the single surfactant system, whereas the mixed surfactant system was total surfactant concentration 0.1 M containing 10xCMC (0.55 mM) and AMA (99.45 mM). NaCl was varied as a factor to change phase behavior. Hexane was selected as oil phase because microemulsion formation was easily observed from the previous experiments.

For both single and mixed surfactant system (Figures 4.9, 4.10), it was found that the minimum IFT could be obtained at the optimum NaCl (7% and 6.7%, respectively) which is calculated from HLD equation. Similarly, microemulsion type III could occur at the optimum NaCl. Therefore, HLD concept could be applied to predict microemulsion formation. However, HLD is just an equation that may have some variations in predicting microemulsion type due to the parameters such as surfactant characters (Cc value and K) which may come from different experimental conditions and materials used between recent and previous studies, for example form of biosurfactant used.

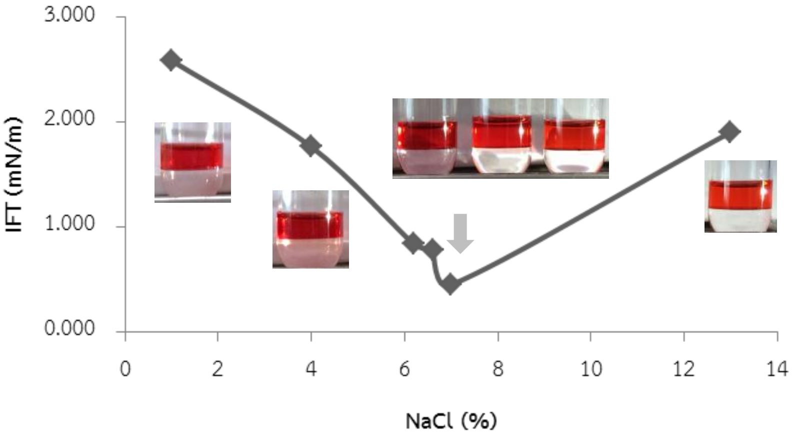


Figure 4.9 The equilibrium IFT measurement for the single surfactant system containing 0.1 M AMA and varying NaCl concentration with hexane.

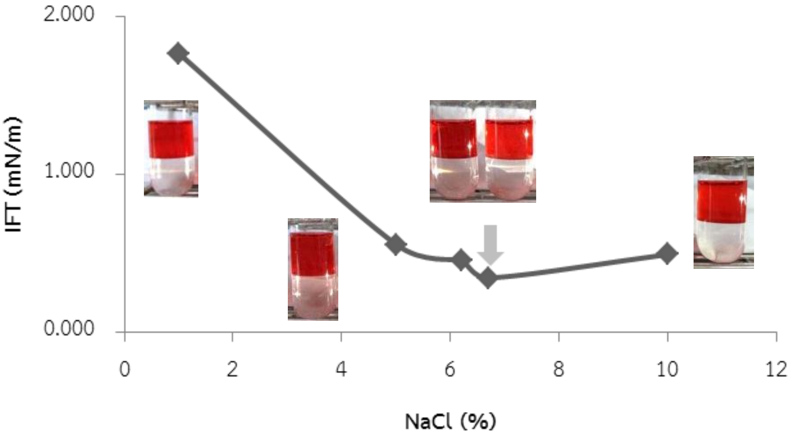


Figure 4.10 The equilibrium IFT measurement for the mixed surfactant system containing 10xCMC lipopeptide and AMA at total concentration 0.1 M and varying NaCl concentration with hexane.

4.3. Evaluation of the surfactant formulations for hydrocarbon and petroleum oil removal from soil

According to the previous section, surfactant formulations for different hydrocarbons (EACN 6, 10, and 16) were obtained. The formulations were determined based on microemulsion formation and IFT measurement between oil and aqueous phases. Some of the formulations could provide microemulsion type III which could reduce IFT related to mobilization and solubilization of oil from contaminated soil (Duffield et al., 2003).

Artificially contaminated soil was created by adding petroleum hydrocarbons into soil at 5% (w/w), and then the soil was stabilized for 2 days before soil washing. Furthermore, the concentrations of Bongkot crude oil were later increased to 10% and 15% (w/w) to represent intentionally contaminated soil such as contamination from petroleum exploration and production. Bongkot crude oil was selected because it demonstrated a good trend of microemulsion formation and IFT reduction from the previous experiment (Table 4.8).

In this part, the formulations, corresponded to each hydrocarbon and petroleum were applied as washing solutions in soil washing process. The washing process in this study was performed in laboratory scale by fixing liquid to solid (L: S) ratio at 2:1 (v/w), shaking speed at 200 rpm, shaking time at 30 minutes. These physical conditions are generally used for soil washing in batch experiments (Urum et al., 2006; Urum and Pekdemir, 2004) and they can represent a similar trend of removal efficiency in scaling up experiments. Then, two times rinsing process with water was done to remove the remaining oil, washing solution, and to prevent an emulsion formation when extracting with solvent. The efficiency of each formulation was evaluated from % removal efficiency of oil from the contaminated soil.

4.3.1. Contaminated soil with 5% (w/w) of petroleum hydrocarbons

4.3.1.1. Hydrocarbon contaminated soil

Hexane contaminated soil was excluded due to its property which easily volatilize. Decane and hexadecane were spiked into the soil to achieve the initial concentration 5% (w/w). After 2 days stabilization to represent short term contamination, they remained in soil 4-5% (w/w). The removal efficiency of petroleum hydrocarbon was determined based on the petroleum hydrocarbon concentration remained in soil after stabilization. After soil washing with various formulations of washing solution; FD-S, FD-M1, FD-M2, FD-M3 and DI (as control) at speed 200 rpm, 30 minutes, and 2:1 of L: S ratio, the results showed that decane and hexadecane were removed almost 100% for all corresponded formulations, whereas about 25% of both decane and hexadecane were eliminated by distilled water washing (Figure 4.11, 4.12). It confirmed that the formulations must be calculated based on the EACN value of hydrocarbons. Therefore, a preference of surfactant to contaminants should be concerned in soil remediation. For example, Zhang (2015) found that rhamnolipid biosurfactant has a positive effect on the removal of saturated hydrocarbons and PAH compounds from both sand and loam soils.

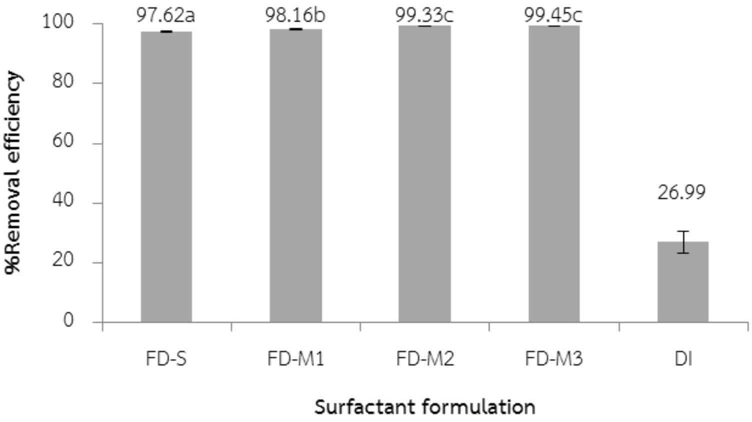


Figure 4.11 Removal efficiency of 5% (w/w) decane contaminated soil of different formulations by soil washing (200 rpm, 30 minutes, 2: 1 of L: S, and two times DI rinsing)

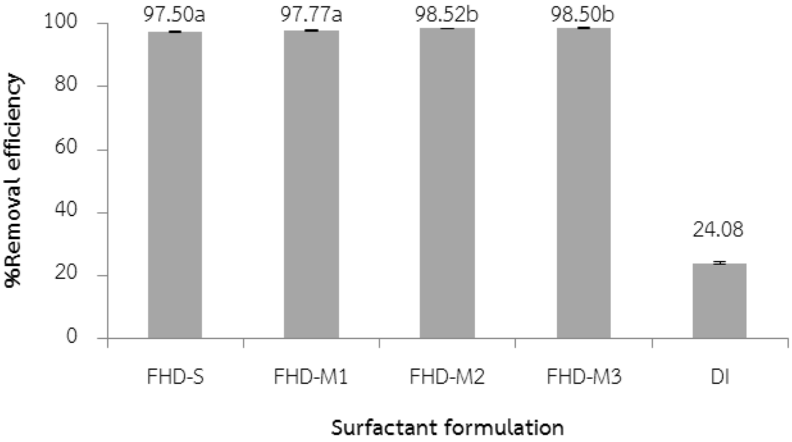


Figure 4.12 Removal efficiency of 5% (w/w) hexadecane contaminated soil of different formulations by soil washing (200 rpm, 30 minutes, 2: 1 of L: S, and two times DI rinsing)

4.3.1.2. Petroleum fraction in soil after stabilization

This result illustrated the group of petroleum hydrocarbon detected by TLC-FID after petroleum oil was spiked into the soil at 5% (w/w) and was stabilized for 2 days. Some of the composition was disappeared, for example resin fraction of ARL/AXL and aromatic of diesel. However, resin may be expected to overlap to the aromatic and the asphaltene fraction due to its solubility in light alkanes (Aske, 2002). In addition, Weathering processes of crude oil involving adsorption of hydrocarbons to soil particles, volatilization of hydrocarbons, and low water solubility of hydrocarbons may affect this result (Williams et al., 2006). On the other hand, Bongkot crude oil still contained only saturated fraction (Figure 4.13). The % weight of each fraction was calculated from total petroleum hydrocarbon left in soil after stabilization.

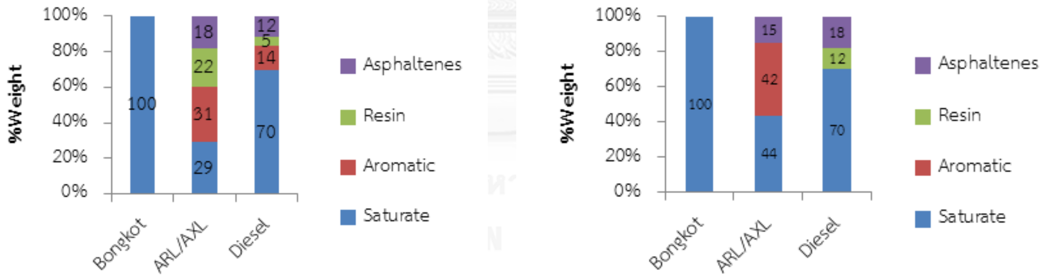


Figure 4.13 Petroleum oil compositions (a) Pure petroleum oil (b) Petroleum in soil after 2 days stabilization

4.3.1.3. Removal efficiency of Bongkot crude oil from soil

The results demonstrated that the formulations from hexane calculation could remove Bongkot crude oil (BKC) higher than 50% (Figure 4.14). Nonetheless, the mixed surfactant formulations (FH-M1, 2, 3) showed greater efficiencies than the single formulation (FH-S). In addition, FH-M3 showed the significantly highest removal efficiency among other formulations.

The control test using distilled water eliminated 20.06% of the TPH-BKC concentration. The single surfactant (AMA only) showed removal rate 55.89%. It was lower than the mixed surfactant systems which were 62.31% (FH-M1), 68.23% (FH-M2), and 73.05% (FH-M3). Similar to Do et al. (2014), they found that mixtures of anionic surfactants and the optimum salinities obtained from HLD =0 presented synergistic effect in detergency performance compared to using a single surfactant.

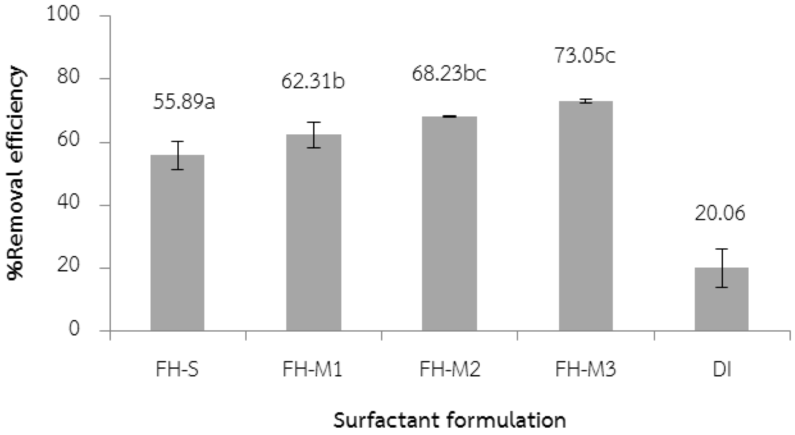


Figure 4.14 Removal efficiency of 5% (w/w) Bongkot crude oil contaminated soil of different formulations by soil washing (200 rpm, 30 minutes, 2: 1 of L: S, and two times DI rinsing)

The formulation obtained from HLD = 0 of the different ratio of anionic surfactant and lipopeptide mixing were used in the previous soil washing experiment (Figure 4.14). Of course, another component of the formulation, NaCl at the optimum NaCl was different for each formulation (Table 4.2). To confirm the effect of lipopeptide concentration on soil washing efficiency, another washing experiment was conducted using the same formulations as in the previous experiment but only NaCl concentration was fixed at 7% for all formulations.

Since all systems used the same total amount of surfactant(s) at 0.1 M and same NaCl concentration, different efficiency for Bongkot crude oil removal should be resulted from the different ratio of anionic surfactant and lipopeptide. The results are shown in Figure 4.15.

It was found that the oil removal efficiency of the single surfactant system was significantly lower than those of mixed surfactant formulation. The results showed that the higher ratio of lipopeptide was in the total surfactant, the higher the oil removal efficiency was obtained (see also Figure 4.16). Therefore, it can be concluded that adding of lipopeptide enhance oil removal efficiency. This may be explained from the result shown in Figure 4.4, once lipopeptide was mixed with AMA, IFT of the mixed surfactant solution was dramatically reduced; hence it led to the detachment of the oil from contaminated soil.

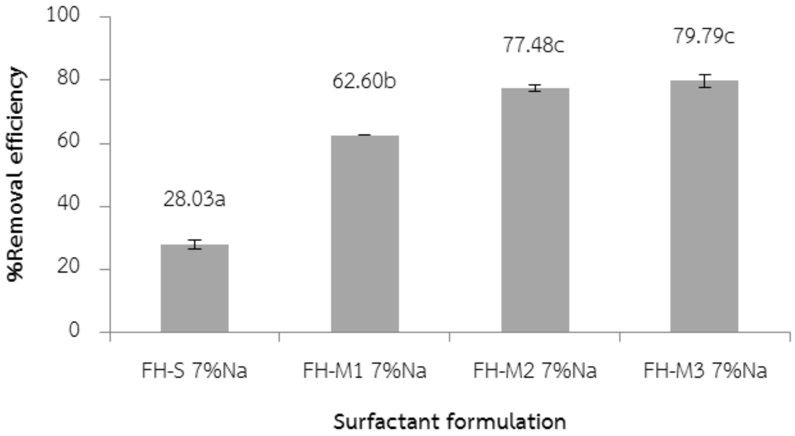


Figure 4.15 Removal efficiency of 5% (w/w) Bongkot crude oil at 7% NaCl for all formulations by soil washing (200 rpm, 30 minutes, 2: 1 of L: S, and two times DI rinsing)

4.3.1.4. Removal efficiency of diesel from soil

Overall, the mixtures of lipopeptide and AMA provided higher diesel removal efficiency than the single surfactant formulation. However, the formulations which gave microemulsion type III (FD-M1, FD-M2) presented higher removal efficiency than the formulation (FD-M3) which gave microemulsion type II and the system was too hydrophobic compared to diesel.

The control test using distilled water removed 34.18 % of the initial TPH-diesel concentration because some compositions such as biodiesel which is amphiphilic compound may be soluble and the diesel could be removed from soil porous by physical conditions, for instance shaking. The single surfactant (AMA only) showed removal rate 63.69 %. It was lower than the mixed surfactant systems which were 77.05 % (FD-M1), 85.66 % (FD-M2), and 77.42 % (FD-M3) (Figure 4.16). Therefore, the higher removal efficiencies suggest that TPH removal is enhanced by the presence of surfactants. This was corresponded to Hernández-Espriú et al. (2012). They reveal that ionic surfactants gave removal efficiencies of diesel from soil above the DI washing for example, removal efficiency of 40%, 60.13 % and 48.19 % by DI, SDS and Surfacpol G, respectively.

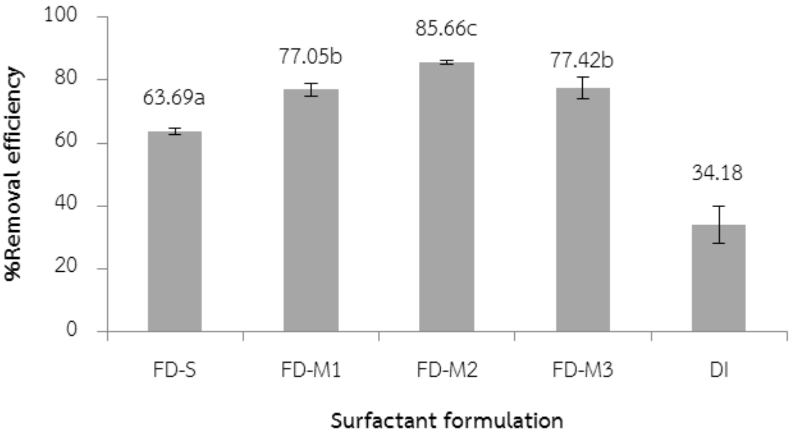


Figure 4.16 Removal efficiency of 5% (w/w) diesel contaminated soil of different formulations by soil washing (200 rpm, 30 minutes, 2: 1 of L: S, and two times DI rinsing)

4.3.1.5. Removal efficiency of ARL/AXL crude oil from soil

As described earlier that decane was selected as a model oil for ARL/AXL crude oil since they have similar EACN (10-12). However, the phase study for microemulsion formation using HLD = 0 for decane could not work with the ARL/AXL crude oil. The formations of both single AMA and mixed with lipopeptide that generated microemulsion type III with decane failed to form microemulsion with the crude oil as described in Tables 4.4 and 4.5. Therefore, AOT which is more hydrophobic surfactant than AMA was introduced to formulate the surfactant systems and calculated the other compositions based on HLD =0 (see Table 4.9). However, microemulsion was still not observed with the ARL/AXL crude oil.

As a consequence, the soil washing experiments for ARL/AXL crude oil were conducted for both formulations using AMA and AOT (see the formulation in Table 4.9) were applied in soil washing experiment to evaluate their efficiencies. The results demonstrated that AOT formulations showed better trend of removal efficiencies of ARL/AXL crude oil compared to that of AMA formulations (Figure 4.17).

The formulations with AOT system showed 68.40% 76.98%, 77.33%, and 77.40% of removal efficiency for FS-AOT, FM1-AOT, FM2-AOT, and FM3-AOT, respectively while the formulations with AMA system showed 23.70%, 60.82%, 51.40%, and 73.24% of removal efficiency for FS-AMA, FM1-AMA, FM2-AMA, and FM3-AMA, respectively (Figure 4.17). Different trends of petroleum hydrocarbon elimination may be from different surfactant structures of AOT and AMA. AOT is more hydrophobic than AMA and hence it may synergize better with lipopeptide to provide lower IFT. This result found to be similar trend reveals by Hagenhoff et al. (2009) that the soil/AOT solution mixtures could reduce surface tension to be lower than AOT solution because a synergistic interaction between the surfactant and soil organic matter transferred from the soil phase to the aqueous phase. This may be a reason that why mixture of AOT and lipopeptide is more suitable to remove ARL/AXL crude oil which has the complicated compositions.

Table 4.9 Concentrations of lipopeptide biosurfactant, AOT or AMA, and optimum NaCl calculated from HLD =0 for decane (EACN=10)

No.	Lipopeptide conc. (xCMC)	Lipopeptide conc. (mM)	AOT or AMA conc. (mM)	S* (NaCl) (g/100mL)
FS-	0	0	100	0.38
FM1	10	0.55	99.45	0.38
FM2	50	2.75	97.25	0.36
FM3	90	3.85	96.15	0.34

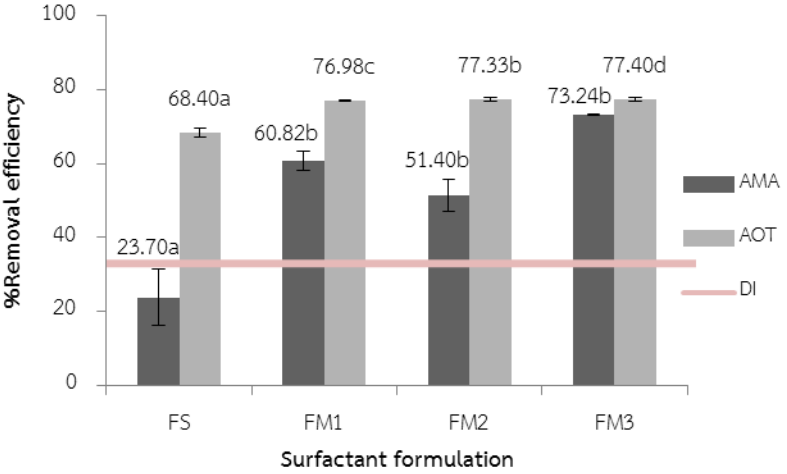


Figure 4.17 Removal efficiency of 5% (w/w) ARL/AXL contaminated soil of different AOT and AMA formulations by soil washing (200 rpm, 30 minutes, 2: 1 of L: S, and two times DI rinsing)

4.3.1.6. Comparison of hydrocarbon and petroleum oil removal efficiency

According to the previous experiments, all formulations for each hydrocarbon (one single surfactant system, and three mixtures containing 10x, 50x, and 90xCMC lipopeptide) were applied in 5% (w/w) soil washing process. In this part, the optimum surfactant mixtures of decane, hexadecane, and petroleum oils providing the highest removal efficiency were selected to compare with the single surfactant of each petroleum hydrocarbon (Table 4.10). The results showed that addition of lipopeptide biosurfactant could enhance petroleum hydrocarbon removal efficiency in soil washing process, particularly in soil washing process of petroleum oils such as Bongkot crude oil, diesel, and ARL/AXL crude oil which have more complex structures.

Each formulation was suitable for each hydrocarbon because it was created based on its EACN value. It was observed by phase behavior studies in the previous sections and Bongkot crude oil was more compatible with its formulation compared to other petroleum oils because microemulsion type III occurred with all formulations calculated from EACN=6 and had total concentration 0.1 M. The removal efficiency of Bongkot crude oil from 5% (w/w) contaminated soil by the lipopeptide mixtures or single surfactant were significantly higher than the DI control test. In addition, the lipopeptide-AMA mixtures also presented great synergistic effect to remove Bongkot crude oil out from the soil (Figure 4.15). On the other hand, some formulations for higher EACN hydrocarbons including some petroleum oils may need to be optimized the hydrophobicity and increase total NaCl concentration for getting microemulsion type III. However, the lipopeptide mixtures still enhanced petroleum hydrocarbon removal efficiency better than the single surfactant.

Table 4.10 Summary of the optimum mixtures providing the highest removal efficiency of petroleum hydrocarbons compared to the single surfactant formulations

Petroleum hydrocarbons	**Formulation**		**Removal efficiency**
	No.	**S* (g/100mL)**	**(%)**
Decane	FD-S	13.74	97.62
(EACN =10)	FD-M2	11.69	99.45
Hexadecane	FHD-S	1.06	97.50
(EACN =16)	FHD-M3	0.95	98.50
Bongkot crude oil	FH-S	6.96	55.89
(EACN 6-9)	FH-M3	5.21	73.05
Diesel	FD-S	13.74	63.69
(EACN 19)	FD-M2	11.69	85.66
ARL/AXL crude oil	FS-AOT	0.38	68.40
(EACN 10-12)	FM3-AOT	0.34	77.40

However, the removal efficiencies of the petroleum oils were not effective compared to those of the saturated hydrocarbons. The reason may be from the petroleum oil compositions containing a wide range of hydrocarbons. They may adsorb with soil particles and may be hardly removed. Furthermore, the formulations were applied with these petroleum oils due to the similar phase behavior with the saturated. The formulations may not be exactly suitable with these petroleum oils.

4.3.2. Contaminated soil with 10% and 15% (w/w) of Bongkot crude oil

The concentrations of petroleum oil in soil were increased to 10 % and 15 % (w/w). They represent the higher oil concentration in soil that could be found in petroleum exploration and production site. Bongkot crude oil was selected because it showed the good trend of microemulsion formation, IFT reduction and removal efficiency from the previous results (Tables 4.8, 4.10).

Overall, the mixtures of lipopeptide biosurfactant and AMA showed higher oil removal efficiency compared to AMA only formulation. The FH-M3 demonstrated significant oil removal efficiency of both 10% and 15% (w/w) which were around 70%. However, the removal rates of 15% contaminated soil were found even better. It may be from the higher amount of oil residues which were on the surface of the soil particles were released out from soil washing process, but some oil is still in soil porous. It could be seen from DI washing test that showed greater removal efficiency compared to the previous 5% (w/w) soil washing. Thus, the surfactant solution could be applied for high oil contaminated in soil and showed higher TPH removal efficiency than using SDS and E-600 surfactants achieving TPH removal efficiencies at 20.4% and 32.9%, respectively for 108,980 mg TPH/kg soil reported by Torres et al. (2005). The reason may be from excess surfactant solution above CMC enhancing oil solubilization in micelles, mixtures of lipopeptide and the optimum salinity created the proper balance to the petroleum hydrocarbons (Microemulsion type III) leading to greater efficiency to extract oil out from the soil particles.

In addition, these surfactant formulations provided higher oil removal efficiency than the DI control test. The total surfactant concentration was used at 0.1 M, which was similar to the experiments of 5% (w/w) soil washing. Therefore, it could be assumed that the surfactant solutions were excess and could be used in the soil washing of the petroleum oil contaminated soil ranging from 5%-15% (w/w). The 90x CMC of lipopeptide formulation could provide the similar removal efficiency of 10% and 15% (w/w) oil contaminated soils. As a result, high fraction of lipopeptide biosurfactant was effective and should be applied into the mixed surfactant formulation.

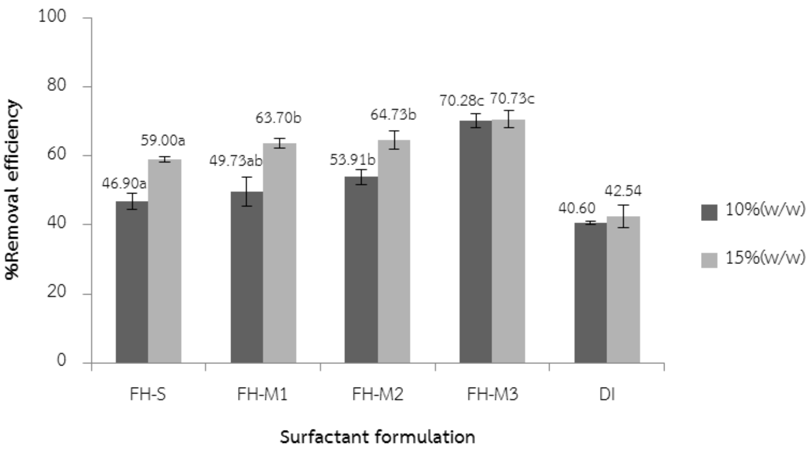


Figure 4.18 Removal efficiency of 10% and 5% (w/w) Bongkot crude oil contaminated soil of different formulations by soil washing (200 rpm, 30 minutes, 2: 1 of L: S, and two times DI rinsing)

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

5.1.Conclusions

Petroleum contamination in soil and subsurface has been remediated by soil washing for a few decades. Most of previous works used commercial synthetic surfactants. The main objective of this study aims to apply biosurfactant to enhance the petroleum removal efficiency from contaminated soil. To obtain the formulation for soil washing solution, Hydrophilic-Lipophilic Deviation (HLD) approach was introduced. HLD value at zero was used for the optimum NaCl calculation of a given surfactant(s) concentrations for different hydrocarbons which were hexane (EACN =6), decane (EACN =10), and hexadecane (EACN =16) in order to obtain microemulsion type III providing the lowest interfacial tension between oil and aqueous phases.

Lipopeptide biosurfactant was produced from *Bacillus* sp. GY19 and its purity was increased by membrane filtration technique. The lipopeptide had high oil displacement activity with several hydrocarbons and low CMC. Due to its Cc value of 4.93, the lipopeptide biosurfactant is considered as a hydrophobic surfactant which was too hydrophobic to the target hydrocarbons. Thus mixing lipopeptide with AMA (for hexane and decane) or AOT (for hexadecane) was expected to provide appropriate property to facilitate the oil to form microemulsion type III. HLD concept (HLD =0) could create the surfactant systems giving microemulsion type III with several petroleum hydrocarbons but some factors may affect the predictions, for example total surfactant concentration, deviation of the equation, and petroleum hydrocarbon compositions. However, the mixed surfactant systems showed the lower IFT with various petroleum hydrocarbons including the saturated hydrocarbons and some petroleum oils (Bongkot crude oil, ARL/AXL crude oil, and diesel) than those of the single surfactant systems. The reason was from the synergistic effects between two surfactants and NaCl addition. Therefore, mixing of hydrophilic and hydrophobic surfactants could increase the surface activity with different hydrocarbons. Moreover, low and high EACN hydrocarbons required the different compositions in the surfactant systems, for example decane needed higher amount of NaCl and hexadecane needed AOT which is more hydrophobic than AMA to increase hydrophobicity of the surfactant systems compared to hexane which has lower EACN value.

The surfactant formulations were applied as soil washing solutions with 5% (w/w) petroleum hydrocarbons. The saturated hydrocarbons (decane and hexadecane) were removed almost 100% for all formulations (both the single and the lipopeptide mixtures) because of the suitable formulations calculated from its EACN for each hydrocarbon and their simple structures easily removed from the soil. On the other hand, the lipopeptide added formulations could enhance the petroleum removal efficiency (Bongkot crude oil, ARL/AXL crude oil, and diesel) better than the single surfactant formulations which was consistent with the microemulsion formation and IFT reduction. The complicated compositions of the petroleum oils may adsorb with soil particles and may be hardly removed leading to the lower removal efficiency compared to the saturated hydrocarbons. Furthermore, the formulations were applied with these petroleum oils due to the similar phase behavior with the saturated; as a result, they may not be exactly suitable with these petroleum oils. However, the removal efficiency of petroleum oils may be increased by adjusting the formulations such as total surfactant concentration, NaCl concentration, and fractions of hydrophilic and hydrophobic surfactants, or changing the physical conditions such as shaking speed and shaking time.

Effect of higher oil concentrations in soil was studied by using the formulations calculated from hexane with 10% and 15% (w/w) Bongkot crude oil contaminated soil. The results showed that the formulations still provided the good trend of petroleum removal; even though, total concentration was used at 0.1 M similar to the experiments of 5% (w/w) soil washing. Therefore, it could be assumed that the surfactant solutions were excess and could be used in the soil washing of the petroleum oil contaminated soil ranging from 5%-15% (w/w). Moreover, the highest lipopeptide added formulation (90x CMC) could provide the similar removal efficiency of 10% and 15% (w/w) oil contaminated soils. Consequently, high fraction of lipopeptide biosurfactant should be applied into the mixed surfactant formulation.

5.2.Management aspects and recommendations for further work

HLD concept and microemulsion formation could be used to determine phase behavior with several petroleum hydrocarbons, but the concepts did not work with some complex petroleum hydrocarbons such as ARL/AXL crude oil. Many factors that should be considered for improving the formulations are listed as follows:

- Higher total surfactant concentration, for example 0.25 M for ARL/AXL crude oil should be determined to see the obvious microemulsion formation.
- Biosurfactant used to create microemulsion by HLD concept should have higher concentration leading to a reduction of NaCl requirement in surfactant formulations because it could increase hydrophobicity of the system as well.

- Characteristic curvature (Cc value) is an important variable in HLD equation but its value in database is still limited; consequently, Cc value of other surfactants such as nonionic surfactants should be investigated.

In this research, one type of soil sample was applied, but soil properties are also important in surfactant-enhanced soil washing. In case of soil contaminants, mixtures of petroleum hydrocarbons may be found in real contamination sites. Therefore, application of the surfactant formulations in soil washing should concern other aspects as follows:

- Soil sample with different soil properties such as soil texture, organic matter, and heavy metals should be used in soil washing.
- The adsorption of petroleum hydrocarbons and surfactants on each soil type should be studied to investigate the interactions between petroleum hydrocarbons and surfactants on each soil type.
- For mixtures of contaminants, EACN of mix pollutants should be determined before submitting to the HLD equation or applying phase study as a primary consideration to define the suitable formulations from this study.

Soil washing in this work was performed in laboratory scale to represent ex-situ soil washing. The proper surfactant formulations with the contaminants are one factor providing the good removal efficiencies, but other considerations to increase the petroleum hydrocarbon removal efficiency including further treatment after soil washing are given below:

- Many physical conditions should be studied in the future, for instance L: S ratio, shaking speed, and shaking time.

- Varying concentration of washing solution should be studied to determine a certain total surfactant concentration above that removal efficiency of petroleum hydrocarbon from soil will not increase.
- Scaling up of soil washing such as jar test is also interesting to determine the efficiency of each formulation in larger scale and some adjustment of physical conditions may be required.
- After soil washing, some remaining petroleum hydrocarbons in soil may be further eliminated by natural attenuation or bioremediation, and the washing solutions may be reused by pumping back into the process

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APPENDICES

APPENDIX A

Table A.1 Oil displacement activity of lipopeptide biosurfactant from membrane filtration technique with various petroleum hydrocarbons

Petroleum hydrocarbon	**Oil displacement (%)**			**Avg**	**SD**
Decane C10	76	76	77	76	1
Dodecane C12	63	68	63	65	3
Tetradecane C14	63	63	74	67	6
Hexadecane C16	74	74	79	75	3
Bongkot crude oil	60	61	63	61	25
ARL/AXL crude oil	25	25	24	25	0

Table A.2 Interfacial Tension (IFT) of each surfactant formulation (HLD =0) with hexane

Formulation	**Rep.**			**Average**	**SD**
	1	**2**	**3**		
FH-S	1.551	1.551	1.552	1.551d	0.001
FH-M1	0.602	0.600	0.600	0.601c	0.001
FH-M2	0.095	0.095	0.093	0.094b	0.001
FH-M3	0.024	0.039	0.042	0.035a	0.010

Table A.3 Interfacial Tension (IFT) of each surfactant formulation (HLD =0) with Bongkot crude oil (BKC)

Formulation	Rep.			Average	SD
	1	2	3		
FH-S	1.156	1.179	1.178	1.171d	0.013
FH-M1	0.789	0.789	0.745	0.775c	0.025
FH-M2	0.153	0.132	0.132	0.139b	0.012
FH-M3	0.082	0.089	0.095	0.089a	0.007

Table A.4 Interfacial Tension (IFT) of each surfactant formulation (HLD =0) with decane

Formulation	Rep.			Average	SD
	1	2	3		
FD-S	0.150	0.154	0.150	0.151d	0.002
FD-M1	0.120	0.120	0.124	0.121c	0.002
FD-M2	0.112	0.113	0.112	0.112b	0.001
FD-M3	0.080	0.075	0.078	0.078a	0.003

Table A.5 Interfacial Tension (IFT) of each surfactant formulation (HLD =0) with diesel

Formulation	Rep.			Average	SD
	1	2	3		
FD-S	0.309	0.237	0.210	0.252b	0.051
FD-M1	0.136	0.189	0.241	0.189b	0.053
FD-M2	0.098	0.098	0.115	0.104a	0.010
FD-M3	0.104	0.097	0.097	0.099a	0.004

Table A.6 Removal Efficiency (%) of 5% (w/w) decane from each surfactant formulation (HLD =0)

Formulation	Rep.		Average	SD
	1	2		
FH-S	97.47	97.78	97.62a	0.21
FH-M1	98.34	97.97	98.16b	0.27
FH-M2	99.33	99.33	99.33c	0.00
FH-M3	99.45	99.45	99.45c	0.01
DI	29.67	24.30	26.99	3.80

Table A.7 Removal Efficiency (%) of 5% (w/w) hexadecane from each surfactant formulation (HLD =0)

Formulation	Rep.		Average	SD
	1	2		
FH-S	97.45	97.55	97.50a	0.07
FH-M1	97.61	97.92	97.77a	0.22
FH-M2	98.45	98.60	98.52b	0.10
FH-M3	98.40	98.61	98.50b	0.15
DI	23.86	24.30	24.08	0.31

Table A.8 Removal Efficiency (%) of 5% (w/w) Bongkot crude oil from each surfactant formulation (HLD =0)

Formulation	Rep.			Average	SD
	1	2	3		
FH-S	50.86	57.06	59.76	55.89a	4.56
FH-M1	64.78	64.50	57.64	62.31b	4.05
FH-M2	68.17	68.29	67.25	68.23bc	0.08
FH-M3	73.23	73.47	72.45	73.05c	0.53
DI	12.93	24.30	22.95	20.06	6.21

Table A.9 Removal Efficiency (%) of 5% (w/w) Bongkot crude oil from each surfactant formulation fixing 7%NaCl

Formulation	Rep.		Average	SD
	1	2		
FH-S	26.96	29.10	28.03a	1.51
FH-M1	62.66	62.53	62.60b	0.09
FH-M2	78.12	76.83	77.48c	0.91
FH-M3	78.42	81.16	79.79c	1.94

Table A.10 Removal Efficiency (%) of 5% (w/w) diesel from each surfactant formulation (HLD =0)

Formulation	Rep.			Average	SD
	1	2	3		
FD-S	63.43	61.75	65.88	63.69a	1.19
FD-M1	73.33	80.16	77.67	77.05b	3.46
FD-M2	86.28	85.54	85.16	85.66c	0.57
FD-M3	78.85	75.99	bottle break	77.42b	2.02
DI	27.62	35.95	38.98	34.18	5.89

Table A.11 Removal Efficiency (%) of 5% (w/w) ARL/AXL crude oil by AOT formulations (HLD =0)

Formulation	Rep.			Average	SD
	1	2	3		
FS	69.25	67.56	68.55	68.40a	1.19
FM1	77.13	77.08	76.71	76.98b	0.23
FM2	76.76	77.88	77.34	77.33b	0.56
FM3	77.94	77.42	76.83	77.40b	0.56
DI	33.82	31.98	-	32.90	1.30

Table A.12 Removal Efficiency (%) of 5% (w/w) ARL/AXL crude oil by AMA formulations (HLD =0)

Formulation	Rep.			Average	SD
	1	2	3		
FS	29.04	18.36	31.98	26.46a	7.55
FM1	61.98	62.66	57.83	60.82c	2.61
FM2	56.42	49.69	48.08	51.40b	4.42
FM3	72.93	73.31	73.48	73.24d	0.28
DI	33.82	31.98	-	32.90	1.30

Table A.13 Removal Efficiency (%) of 10% (w/w) Bongkot crude oil from each surfactant formulation (HLD =0)

Formulation	Rep.			Average	SD
	1	2	3		
FH-S	48.46	48.17	44.06	46.90a	2.46
FH-M1	52.00	52.36	44.83	49.73ab	4.25
FH-M2	50.92	54.01	56.79	53.91b	2.18
FH-M3	69.21	68.95	72.68	70.28c	2.08
DI	40.93	40.26	-	40.60	0.47

Table A.14 Removal Efficiency (%) of 15% (w/w) Bongkot crude oil from each surfactant formulation (HLD =0)

Formulation	Rep.			Average	SD
	1	2	3		
FH-S	58.94	58.23	59.85	59.00a	0.81
FH-M1	62.80	65.40	62.91	63.70b	1.47
FH-M2	62.97	66.76	64.45	64.73b	2.68
FH-M3	73.48	69.58	69.12	70.73c	2.40
DI	44.83	40.26	-	42.54	3.23

Table A.15 Remaining decane (mg/kg) in soil after washing of 5% (w/w) decane contaminated soil from each surfactant formulation (HLD =0)

Formulation	Rep.		Average
	1	2	
FH-S	774	688	731
FH-M1	507	622	565
FH-M2	204	208	206
FH-M3	167	171	169
DI	21532	23412	22472

Table A.16 Remaining hexadecane (mg/kg) in soil after washing of 5% (w/w) hexadecane contaminated soil from each surfactant formulation (HLD =0)

Formulation	**Rep.**		**Average**
	1	**2**	
FH-S	780	759	770
FH-M1	732	636	684
FH-M2	474	434	454
FH-M3	491	430	461
DI	23311	23412	23362

Table A.17 Remaining Bongkot crude oil (mg/kg) in soil after washing of 5% (w/w) Bongkot crude oil contaminated soil from each surfactant formulation (HLD =0)

Formulation	**Rep.**			**Average**
	1	**2**	**3**	
FH-S	15045	13281	12176	13501
FH-M1	10783	10869	12970	11541
FH-M2	9744	9807	9910	9820
FH-M3	8197	8206	8336	8246
DI	26658	23412	23311	24460

Table A.18 Remaining diesel (mg/kg) in soil after washing of 5% (w/w) diesel contaminated soil from each surfactant formulation (HLD =0)

Formulation	Rep.			Average
	1	2	3	
FH-S	13467	13982	13033	13494
FH-M1	9824	7251	8530	8535
FH-M2	5052	5284	5669	5335
FH-M3	7789	8775	8849	8471
DI	21347	20975	21658	21327

Table A.19 Remaining ARL/AXL crude oil (mg/kg) in soil after washing of 5% (w/w) diesel contaminated soil from each AOT surfactant formulation (HLD =0)

Formulation	Rep.			Average
	1	2	3	
F-S	8877	9363	9079	9106
F-M1	6600	6615	6723	6646
F-M2	6709	6384	6540	6544
F-M3	6368	6517	6689	6525
DI	19103	19635	-	19369

Table A.20 Remaining ARL/AXL crude oil (mg/kg) in soil after washing of 5% (w/w) diesel contaminated soil from each AMA surfactant formulation (HLD =0)

Formulation	Rep.			Average
	1	2	3	
F-S	20483	23565	19635	21228
F-M1	10975	10778	12172	11308
F-M2	12580	14522	14986	14029
F-M3	7814	7703	7655	7724
DI	19103	19635	-	19369

Table A.21 Remaining Bongkot crude oil (mg/kg) in soil after washing of 10% (w/w) Bongkot crude oil contaminated soil from each surfactant formulation (HLD =0)

Formulation	Rep.			Average
	1	2	3	
FH-S	37374	37583	40561	38506
FH-M1	34809	34548	40009	36455
FH-M2	35587	33348	31330	33422
FH-M3	22326	22513	19809	21549
DI	44750	50903	42532	46062

Table A.22 Remaining Bongkot crude oil (mg/kg) in soil after washing of 15% (w/w) Bongkot crude oil contaminated soil from each surfactant formulation (HLD =0)

Formulation	Rep.			Average
	1	2	3	
FH-S	59464	59541	59068	59358
FH-M1	54716	50903	54563	53394
FH-M2	54473	48901	52296	51890
FH-M3	39008	44750	45420	43059
DI	81164	87876	85232	84757

APPENDIX B

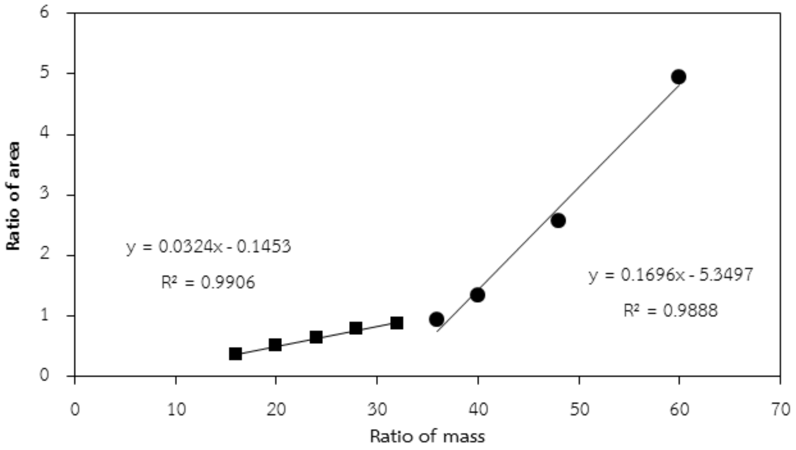

Figure B.1 Standard curve of Bongkot crude oil from TLC-FID. Each data point was averaged from triple spots on chromatorods.

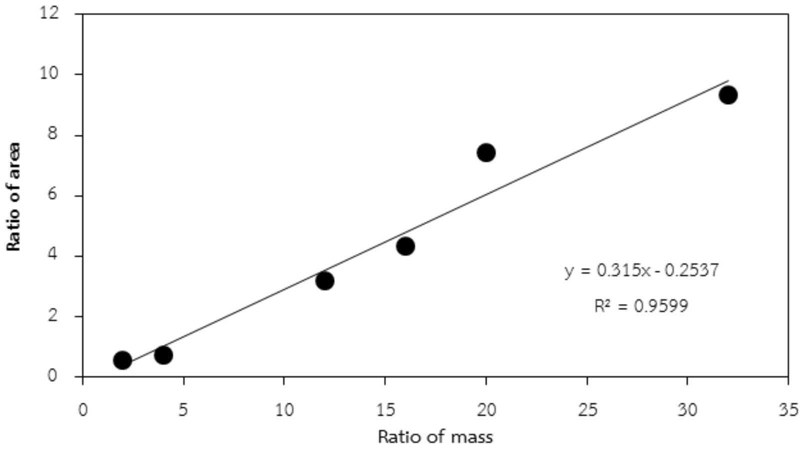

Figure B.2 Standard curve of diesel from TLC-FID. Each data point was averaged from triple spots on chromatorods.

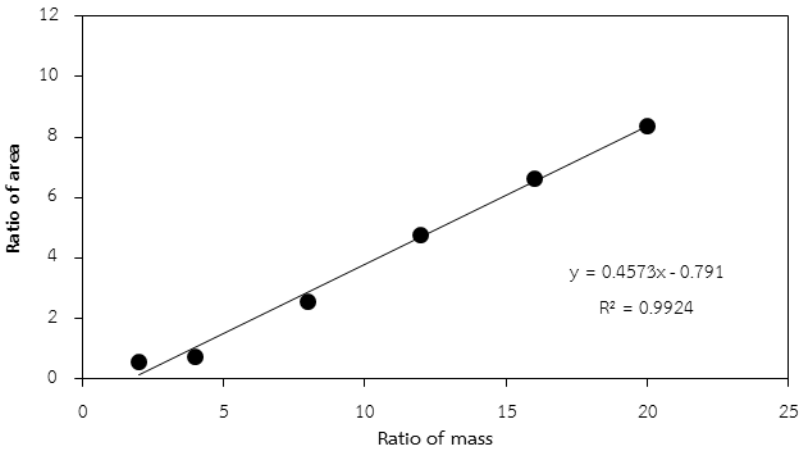

Figure B.3 Standard curve of ARL/AXL crude oil from TLC-FID. Each data point was averaged from triple spots on chromatorods.

VITA

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