Adsorption and Adsolubilization of Organic Solutes Using Rhamnolipid Biosurfactant-modified Surface

Davone Keomany[1], and Emma Asnachinda[1*]

[1]*Department of Chemical Engineering, Faculty of Engineering,*
Burapha University, Chonburi, Thailand

Abstract

At present, biosurfactant, a biological-based surface active agent synthesized by various types of microorganisms has been drawing the attention of the scientific community, due to the superior benefits over conventional surfactants. Rhamnolipid is characterized as a biological based surfactant which has lower toxicity, higher biodegradability and better environmental compatibility than coventional ones. It has been used as an alternative choice for environmental applications. In this work, rhamnolipid biosurfactant-modified adsorbent was developed to remove two different types of organic solutes: (styrene and phenanthrene) through adsolubilization process. Results showed that the rhamnolipid surfactant-modified surface has the ability to remove both, styrene and phenanthrene from an aqueous solution in a specific amount. Findings also demonstrated that both styrene and phenanthrene were mainly partitioned into the palisade layer where it was considered as a slightly polar region in the admicelle structure. Typically, styrene was known as an intermediate polarity organic compound while phenanthrene was considered as a smaller polar solute compare to styrene. As a result, phenanthrene tended to separate itself from the strongly polar phase in an aqueous solution to the lesser polar region in the palisade layer of the admicelle and having greater adsolubilization capacity than styrene.

Keywords: Adsorption/ Adsolubilization/ Styrene/ Phenantrene/ Rhamnolipid/ Biosurfactant

1. Introduction

Environmental problems caused by petroleum hydrocarbons released to the environment such as leaks and accidental spills have been attracting worldwide attention. In addition, petroleum hydrocarbons do not easily degrade in nature and some are known to be carsinogenic. In general, petroleum hydrocarbons in soil and ground water can be reduced by physical and chemical methods such asprecipitation, sorption and oxidation – reduction (T. F. Guerin, S. Horner, T. McGovern, and B. Davey,2002). In recent years , soil and groundwater clean up techniques called surfactant-enhanced aquifer remediation (SEAR) have been suggested as an acceptable technique rather than classic treatments such as soil flushing, pump and treat method, because they are relatively expensive,time consuming and not so environmentally friendly. (J. S. Clifford, M. A. Ioannidis, and R. L. Legge,2007;Y. Li, S. Tian, H. Mo, and P. Ning,2011). However, a surfactant can be toxic in itself and slowy to degrade, due to the fact that it is generally synthesised from chemical substances in which the synthesis process itself results in environmental pollution (M. Henkel, M. M. Müller, J. H. Kügler, R. B. Lovaglio, J. Contiero, C. Syldatk, and R. Hausmann,2012). Recently, the biocompatible surfactants have been synthesized from microorganisms and report to be an alternative replacement to the chemical-based ones. The benefits of a biosurfactant over a synthetic surfactant has been reported in literature such as: lower toxicity, higher biodegradability and more environmentally friendly(C. N. Mulligan,2005). Rhamnolipid are a group of anionic microbial glycolipids, that are composed of L-(+)-rhamnose and β-hydroxyalkanoicacid units produced by Pseudomonas aeruginosa (C. N. Mulligan,2007). In addition, previous studies have reported that rhannolipid was useful for the removal of crude oil, oil spills, PAH and organic contaminants by reduction of interfacial tension (C. N. Mulligan,2007;K.-H. Shin and K.-W. Kim,2004). Petroleum hydrocarbons are classified in many compounds such as alkanes, cycloalkanes, aromatics, PAHs, asphaltenes and resins. all of which are commonly known as toxins that can be pollutants, if they are exposed to the environment (B.Z.Fathepure,2014). Many studies have successfully used surfactant-modified material to remove some organic solutes, but none of them have mentioned biosurfactants yet (N. Arpornpong, 2008; C. Attaphong, E. Asnachinda, A. Charoensaeng, D. A,2010). Thus, this study aims to develop rhamnolipid-modified adsorbents to remove styrene and phenanthrene, common solvents applied to many applications through the adsolubilization process.

*Corresponding author:
E-mail: emma@eng.buu.ac.th

2.Methodology

2.1 CMC measurements

Rhamnolipid critical micelle concentration (CMC) measurement was performed to investigate the concentration that the first micelle is formed in the system. In this work, the anionic rhamnolipid biosurfactant was produced by *Pseudomonas aeruginosa* as a mixture of 50% w/v monorhamnolipid and 50% w/v dirhamnolipid as shown in Figure1.

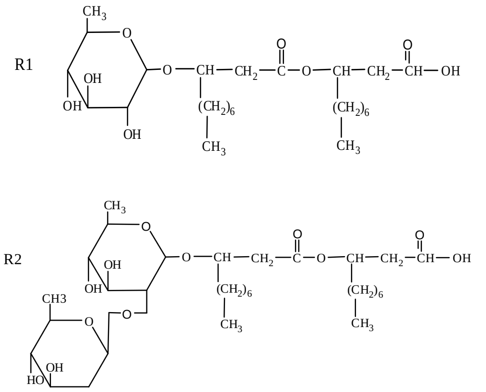


Figure1.Struture of the rhamnolipids:R1 monorhamnolipid and R2 dirhamnolipid

The experiment was conducted using LAUDA tensiometer with platinum plate at room temperature (28±2°C) in the presence and absence of electrolyte concentration of 1 mM NaCl. The break point was plotted between the surface tension versus surfactant concentration is the CMC of the surfactant system.

2.2 Adsorption Study

The processes of surfactant adsorption begins when, surfactant molecules migrate from the bulk solution to the surface. The adsorption at the solid-liquid interface is most commonly characterized by the adsorption isotherm in a typical S-curve. The amount of the adsorbed surfactant is plotted versus an equilibrium surfactant concentration, and the adsorption isotherm can be divided into four regions (R. Zhang and P. Somasundaran,2006) as shown in Figure 2.

In this work, a rhamnolipid biosurfactant adsorption isotherm onto the positively charged surface of aluminum oxide was studied to evaluate the rhamnolipid biosurfactant adsorption behavior at a solid/liquid interface. The point of zero charge (PZC) PH of aluminium oxide was reported as 9.1 and the specific surface area of aluminium oxide used in this work is $155m^2$/g.(P. Asvapathanagul, P. Malakul, and J. O'Haver,2005).

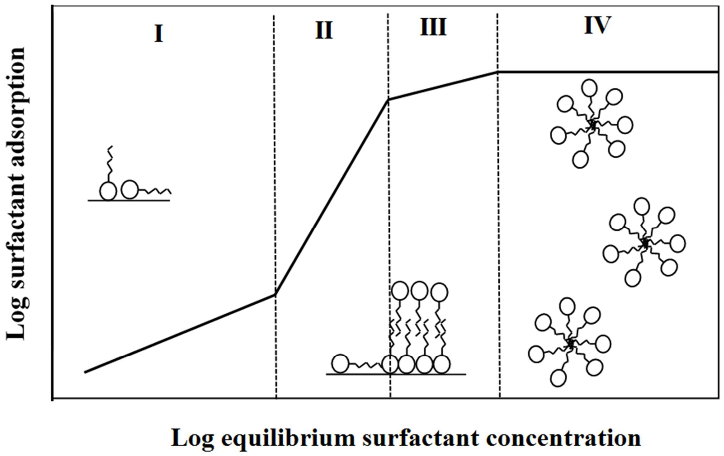


***Figure*2**. Schematic presentation of a typical surfactant adsorption isotherm.

The experiment was conducted at room temperature (28±2ºC) with varies rhamnolipid concentration from $3x10^{-5}$- $8x10^{-3}$M in a presented of 1 mM NaCl. Experiment pH was controlled between 8.0-8.5, which were periodically adjusted by hydrochloric acid (HCl) and sodium hydroxide (NaOH) solution.

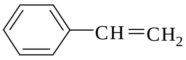
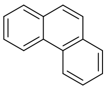
Solution samples were controlled to be constant as 40 mL with 0.01g of aluminium oxide absorbent. The well-prepared sample solution was shaken at 150 rpm for 48 hours. All of the experiments were carried out in triplicate and the average values were used for further calculations.Then, solid particles were separated from aqueous solution by centrifugation, and the supernatant of rhamnolipid concentration was then analyzed by a High Performance Liquid Chromatography (HPLC). It was assumed that the adsorption of water and salt was negligible and the adsorption of rhamnolipid had no effect on solution density.

2.3 Absolubilization Study

Adsolubilization process refers to the partitioning of organic solute from aqueous phase into the two-dimensional of surfactants arrangement called admicelles that is already adsorbed by the absorbent.(A. Charoensaeng, D. A. Sabatini, and S. Khaodhiar,2009). In this work, adsolubilization of styrene and phenanthrene were conducted at room temperature (28±2ºC) with 1 mM of electrolyte (NaCl) and solution of pH of 8.0 - 8.5.

The equilibrium surfactant concentration was selected at 90 percent lower of the CMC where the alteration of the plot slope was observed. This is to confirm none of micelle will be formed in the solution. Properties of organic solutes are shown in Table 1.

Table 1. Properties of the organic solutes (N. Arpornpong, 2008).

Organic	Molecular formula		MW	Water	Density	Log	Dipole
solutes	Formula	Structure		solubility (mg/l)	25°C (g/ml)	K_{ow}	moment
Styrene	C_8H_8		104.15	310	0.9059	2.95	0.13
Phenanthrene	$C_{14}H_{10}$		178.234	1.6	1.063	4.57	0.00

In styrene adsolubilization, various amounts of styrene were added in a pre-equilibrated absorbed surfactant sample. All prepared solutions were shaken for 48 hours at 150 rpm to achieve equilibrium followed by centrifugation to remove aluminum oxide. The concentration of rhamnolipid and styrene were analyzed by HPLC with an evaporative light scattering detector (ELSD) detector for rhamnolipid whereas the equilibrium concentration of styrene was analyzed by diode array detector at 247 nm.

Another batch of the experiment was repeated for phenanthrene adsolubilization and the phenanthrene equilibrium concentration was analyzed using UV/VIS spectrophotometer at 254nm wave length.

3. Results

3.1 *Measurement of Critical micelle concentration*

The critical micelle concentration of rhamnolipid biosurfactant in the presence and absence of electrolyte were determined by the breakpoint in the curve of the surface tension versus the logarithm of the total concentration of surfactant as shown in Figure 3. The change of the plotted slope indicated the alteration of surfactant forming in the solution where the first micelle was formed. Results showed that the CMC value of rhamnolipid with the presence of electrolyte (0.3 mM) was lower than rhamnolipid alone (0.6 mM).

Table 1 shows the summary data of rhamnolipid adsorption at liquid-air interface. Surface excess concentration results for both of rhamnolipid and rhamnolipid with electrolyte were reported as 4.29 and 2.97 mmol/1000m^2, respectively. The effective area per molecule was calculated from the relation of Avogardo's number and surface excess concentration. Results showed that the effective area per molecule of rhamnolipid alone (38.72 A^{o2}/molecule) was lower than rhamnolipid with the presence of an electrolyte (55.90 A^{o2}/molecule).

Table 2 Proximate analysis of bagasse and AC

	Liquid-air interface				Solid- liquid interface			
Surfactant	CMC (mM)	Average plateau[b] (mN/m)	Surface excess concentration (Γ) (mmol/1000m^2)	Effective areas per molecule (A^{o2}/molecule)	CMC (mM)	Maximum adsorption (q_{max}) mmole/g	 Molecule/nm^2	 A^{o2}/molecule
Rhamnolipid	0.6	27.23	4.29	38.72	N/A	N/A	N/A	N/A
Rhamnolipid +electrolyte	0.3	27.14	2.97	55.90	1.18	0.87	3.39	29.50

[a] from figure 1

[b] at minimum surface tension

N/A Not available

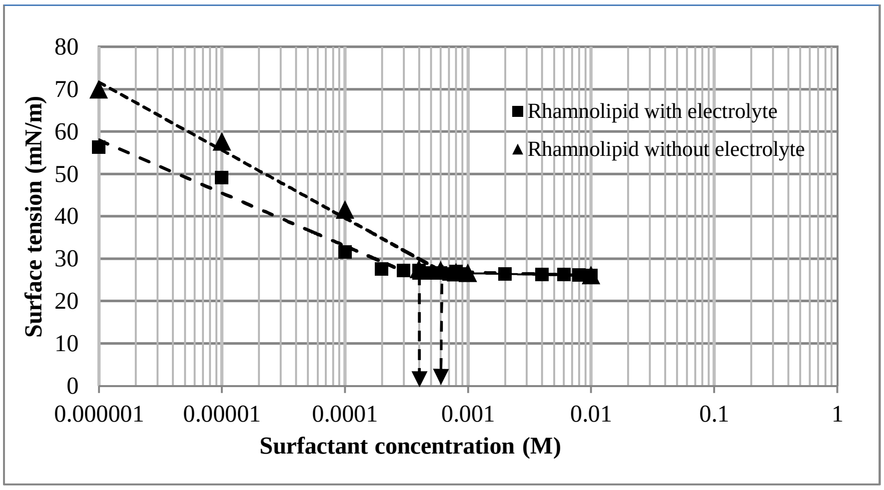


Figure 3. Relationship between surface tension of rhamnolipid surfactant in a presence and absence of 1.0 mM NaCl at equilibrium pH of 8.0-8.5, and temperature of 28±2°C.

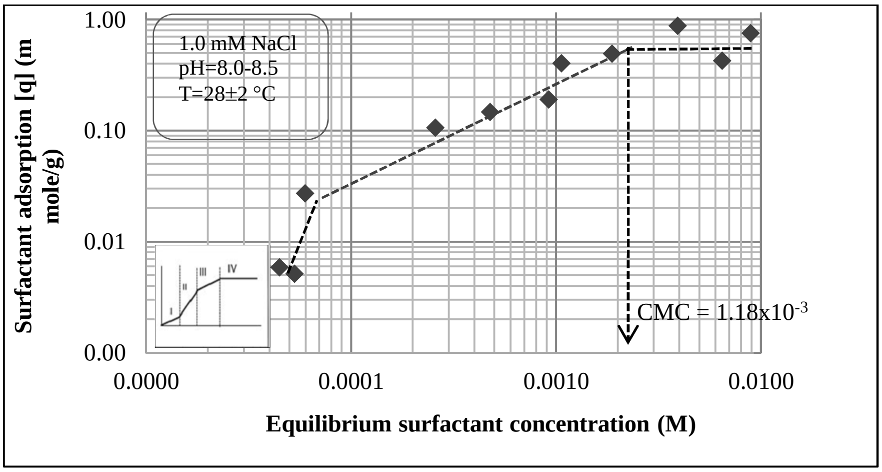


Figure 4*:* Adsorption isotherm of the rhamnolipid biosurfactant system onto aluminum oxide at 1.0 mM NaCl, equilibrium pH of 8.0-8.5 and temperature of 28±2°C.

3.2 Surfactant adsorption study

The study of adsorption isotherm of rhamnolipid surfactant is shown in Figure 4. Results demonstrated that the adsorption isotherm of rhamnolipid was elongated as a classical S-shaped curve which is divided into four regions. However, surfactant concentrations in Region I were not able to be analyzed. The result also showed that the adsorption of rhamnolipid increased as a function of equilibrium concentration. Data from Table 2 showed the maximum adsorption capacity of rhamnolipid which was 0.87mmol/g.

The CMC from the isotherm was reported as 1.18 mM, which was higher than that obtained from the surface tension (0.3mM). In addition, the effective area per molecule of rhamnolipid from adsorption isotherm (29.50 A$^{\circ 2}$/molecule) was lower than the effective area per molecule from surface tension (55.90 A$^{\circ 2}$/molecule).

3.3 Absolublization Study

The partition of each organic solutes into the admicelle was calculated using the admicellar partition coefficient (K_{adm}) shown in equation1,where the X_{adm} and X_{aq} are the mole fractions of the organic solute in the admicelle and the aqueous phase, respectively. (P. Asvapathanagul, P. Malakul, and J. O'Haver, 2005).

$$K_{adm} = \frac{X_{adm}}{X_{eq}} \quad \textbf{(1)}$$

3.3.1 Styrene adsolubilization

Adsolubilization isotherm was plotted between mole fractions of styrene in admicelles (X_{adm}) and in the aqueous phase (X_{aq}) as shown in Figure 5. Results demonstrated that as the increasing of equilibrium concentration of styrene, the amount of adsolubilizaiton styrene in admicelles of rhamnolipid biosurfactant also increased. As expected, the styrene adsolubilization reaches its maximum amount when water solubility of styrene is reached.

Moreover, styrene admicellar partition coefficient (K_{adm}) was decreased as a function of the styrene aqueous mole fraction (X_{aq}) shows in Figure 6. Result indicated that the (K_{adm}) of rhamnolipid biosurfactant decreased as the styrene mole fraction (X_{aq}) increased. In addition, admicellar partitioning coefficient (K_{adm}) of styrene in rhamnolipid biosurfactant onto aluminium oxide surface equal to 16,917.

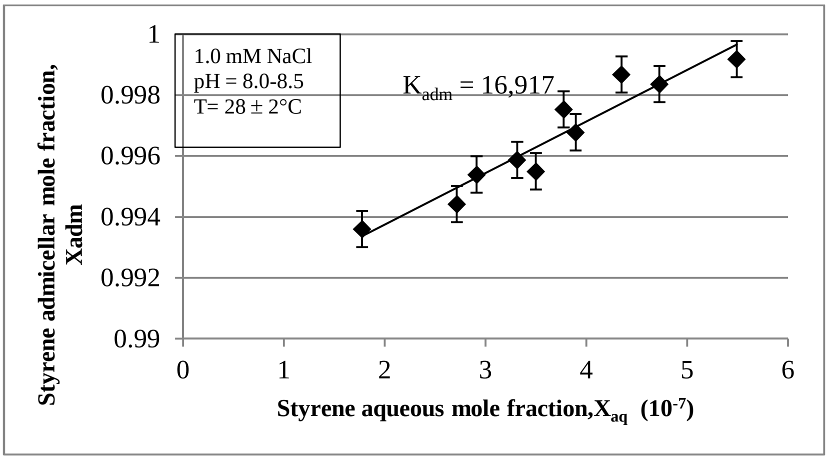


Figure 5. Adsolubilization capacities of styrene by rhamnolipid biosurfactant onto aluminium oxide at 1.0 mM NaCl, an equilibrium pH 8.0-8.5, and 28±2°C.

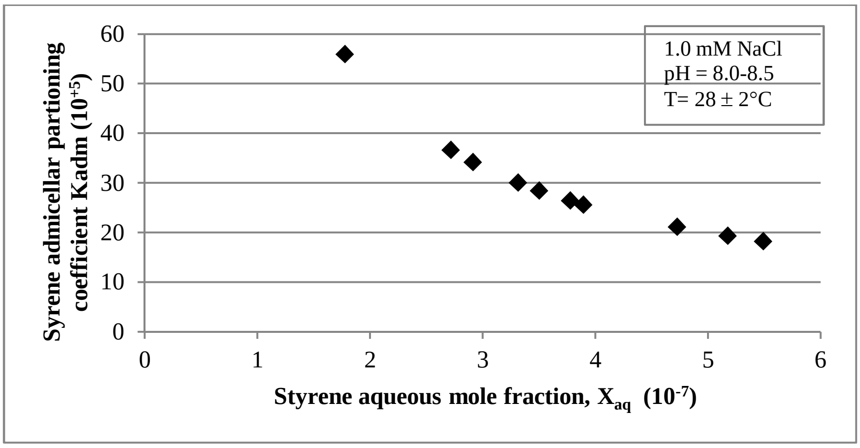


Figure 6. The styrene admicellar partition coefficient (K_{adm}) versus styrene aqueous mole fraction (X_{aq}) of the rhamnolipid biosurfactant onto aluminium oxide, in 1 mM NaCl, an equilibrium pH of 8.0-8.5 and a temperature of 28±2°C.

3.3.2 Phenanthrene adsolubilization

Figure 7 shows the phenanthrene adsolubilization in rhamnolipid biosurfactant admicelles versus the aqueous phenanthrene concentration in the rhamnolipid biosurfactant system. Since, the equilibrium concentration of phenanthrene increased, the amount of phenanthrene adsolubilized also increased.

Phenanthrene adsolubization also attained its maximum level when the concentration of phenanthrene reached its water solubility. This finding showed a similar agreement with styrene as well. The relationship between the phenanthrene admicellar partition coefficient (K_{adm}) and the phenanthrene aqueous mole fraction (X_{aq}) is shown in Figure 6.

The values of the phenanthrene admicellar partition coefficient (K_{adm}) were obtained at a maximum of the mole fraction of phenanthrene in the aqueous phase (X_{aq}). Figure 8 indicates that the phenanthrene admicellar partition coefficient (K_{adm}) in rhamnolipid decreased as the aqueous molar fraction (X_{aq}) of the phenanthrene increased. Moreover, the admicellar partitioning coefficient (K_{adm}) of phenanthrene in rhamnolipid biosurfactant onto aluminum oxide is equal to $3x10^6$.

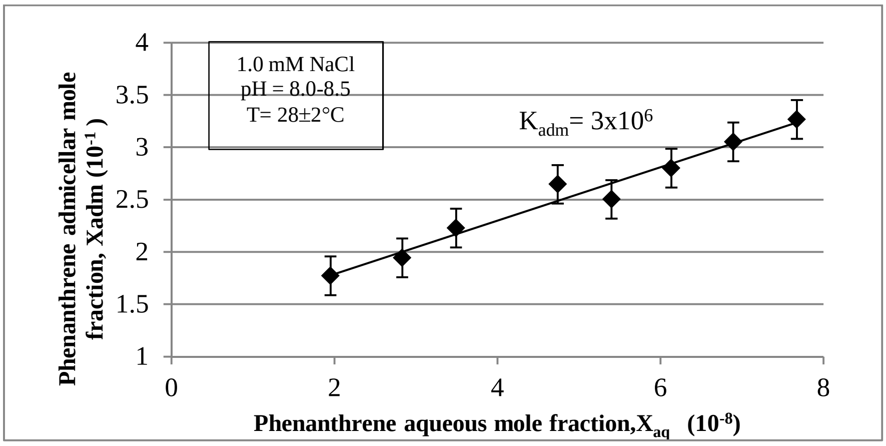


Figure 7. Adsolubilization capacities of phenanthrene by rhamnolipid biosurfactant onto aluminium oxide at 1.0 mM NaCl, an equilibrium pH 8.0-8.5, and 28±2°C.

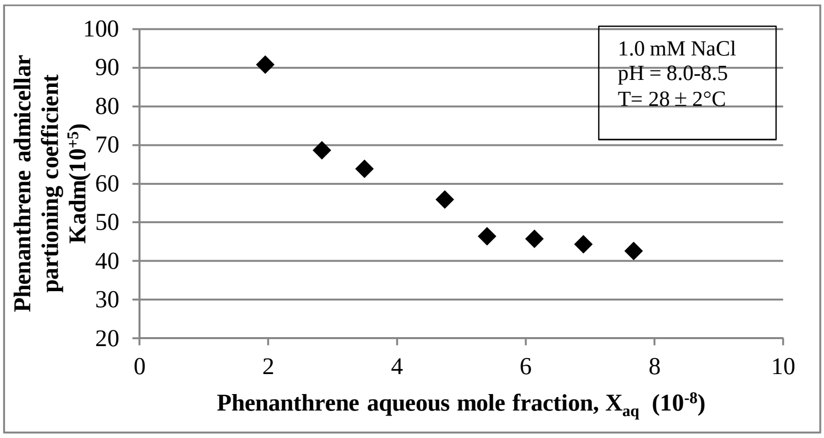


Figure 8. The phenanthrene admicellar partitioning coefficient (k_{adm}) versus phenanthrene aqueous mole fraction (X_{aq}) of the rhamnolipid onto aluminium oxide at 1.0 mM NaCl, an equilibrium pH 8.0-8.5, and 28±2°C.

4.Discussion

4.1Critical micelle concentration of the rhamnolipid

In the presence of an electrolyte, the CMC value of rhamnolipid was reported as 0.3 mM, where as in the absence of electrolyte it was 0.6 mM. This can explain why the electrolyte decreased electrostatic repulsion between adjacent ionic head groups of rhamnolipid and promoted a better arrangement of surfactant molecules at the interface, which is in agreement with previous studies (N.Arpornpong,2008;P.Asvapathanagul, P.Malakul,and J.O'Haver,2005). This finding indicated that rhamnolipid is required in smaller amounts when applied, as compared to conventional and other synthetic surfactants as it can reduce surface tension in the same manner. This results in cost savings when selecting rhamnolipid to use in real circumstances. Results also indicated that the adsorption of rhamnolipid/electrolyte at the liquid-air interface shows a lower surface excess concentration than those in rhamnolipid alone. The effective area per molecule was increased when adding electrolyte to the rhamnolipid surfactant solution. This finding demonstrated that rhamnolipid with an electrolyte required a larger area to form an aggregate at the liquid-air interface than t in a system without. This implies, that less surfactant monomer is needed to fill up at the interface, for a system using rhamnolipid with electrolyte. As a result, it is beneficial in speeding up the arrangement of surfactant forming. In addition, the aggregation numbers of the micelle formation was increased as well.

4.2 Surfactant adsorption

As shown in the results, the isotherms demonstrated the plot of Regions II, III and IV, while Region I was not be able to be analyzed due to the analytical limits of the equipment. In addition, the value of effective area per molecule of rhamnolipid from adsorption isotherm (29.50 A^{o2}/molecule) was lower than the effective area per molecule from surface tension (55.90

$A^{\circ 2}$/molecule). This finding indicated that the different adsorption site as the air-liquid interface (surface tension result) required a larger area to form surfactant aggregates than those in the solid-liquid interface (adsorption isotherm result) due the target site of air-liquid adsorption being used up more readily. Thus, the CMC from surface tension measurement showed a lower value than that obtained from adsorption isotherm.

4.3 Styrene adsolubilizaiton

Styrene admicellar partition coefficient (K_{adm}) decreased as the styrene mole fraction (X_{aq}) increased due to the intermediate polarity of styrene (dipole moment = 0.13) encouraged its molecule to partitioning well in the palisade. This result was in accord with the results of previous studies. (A.Charoensaeng,D.A.Sabatini, S.khaodhiar,2008;E.Asnachinda,S.Khaodhiar,D.A.Sabatini,2010) Moreover, the admicellar partitioning coefficient (K_{adm}= 16,917) demonstrated that the admicellar capacity of styrene on rhamnolipid was greater than that, in synthesis surfactants due to rhamnolipid increasing the solubility of styrene more than synthesis surfactants.(A.Charoensaeng,D.A.Sabatini,S.khaodhiar,2008;E.Asnachinda,S.Khaodhiar,D.A.Sabatini,2010)

4.4 Phenanthrene adsolubilization

As shown in the results, the phenanthrene 4 admicellar partition coefficient (K_{adm}) in 5 rhamnolipid decreased as the aqueous molar fraction (X_{aq}) of the phenanthrene increased. This finding indicated that phenanthrene preferred partitioning in the palisade layer of rhamnolipid admicelle, rather than in the aqueous phase. The result is consistent with the previous study.(N.Arpornpong,A.Charoensaeng,D.A.Sabatini,and S.Khaodhiar,2010). In addition, the admicellar partitioning coefficient (K_{adm}) of phenanthrene in rhamnolipid biosurfactant onto aluminum oxide was equal to $3x10^6$, which demonstrated that rhamnolipid biosurfactant has a greater phenanthrene adsolulibilzation capacity than extended carboxylate and extended sulfate surfactants(N.Arpornpong,A.Charoensaeng,D.A.Sabatini,and S.Khaodhiar,2010.)

Comparison of admicellar partition coefficient (K_{adm}) between styrene and phenanthrene for rhamnolipid biosurfactant-modified surface showed that K_{adm} of phenanthrene ($3x10^6$) was higher than K_{adm} of styrene (16,917). Due to the water solubility of organic solutes, styrene (which is higher polar organic solute) has a higher solubility in water (310 mg/L) than lower polar phenanthrene (1.6 mg/L). Therefore, styrene tends to solubilize both in the aqueous phase and the biosurfactant admicelles whereas, a lower water solubility, such as phenanthrene tends to separate itself from the aqueous phase and the tends to adsolubilize in biosurfactant admicelles. In addition, comparing styrene and phenanthrene admicellar partition coefficient value (K_{adm}) of rhamnolipid with previous works that focused on adsolubilization of styrene. (C.Attaphong,et al.,2010; P. Asvapathanagul,P. Malakul, and J. O'Haver,2005; E. Asnachinda, S. Khaodhiar, and D. A. Sabatini,2010) and phenanthrene (N. Arpornpong, J. Lewlomphaisan, A. Charoensaeng, D,2013) on an aluminum oxide surface as compared to this study, it could be concluded that the adsolubilization of styrene and phenanthrene on rhamnolipid have a greater the adsolubilization capacity than the other type of surfactants.

5.Conclusions

Rhamnolipid biosurfactant-modified surface, having the ability to adsolubilize both styrene and phenanthrene from an aqueous solution, depends on the organic polarity. Findings also demonstrate that both; styrene and phenanthrene were mainly partitioned into the palisade layer, where it was considered as a slightly polar region in the admicelle structure. Additionally, phenanthrene showed a greater adsolubilization capacity than styrene, due to the different partitioning behavior.Styrene has higher dipole-dipole interaction, it then prefered to adsolubilize in the more polar region phase. On the other hand, phenanthrene (the weak dipole-dipole interaction and nonpolar solute) lends itself to partitioning well in the less polar region.

6.Acknowledgements

The authors honored to acknowledge to The Thailand Research Fund, The Office of Higher Education Commission and Burapha University for the grant supported in this work. The appreciation would extend to Faculty of Science Burapha University and Faculty of Environmental Engineering Chulalongkorn University for the valuable guidance and technical supports.

7. References

A.Charoensaeng,D. A. Sabatini, and S. Khaodhiar. 2008. "Styrene Solubilization and Adsolubilization on an Aluminum Oxide Surface Using Linker Molecules and Extended Surfactants." **J. Surfactants Deterg** 11(1): 61–71.

A.Charoensaeng, D. A. Sabatini, and S. Khaodhiar. 2009. "Solubilization and adsolubilization of polar and nonpolar organic solutes by linker molecules and extended surfactants."**J. Surfactants Deterg**. 12(3): 209–217.

B.Z. Fathepure. 2014. "Recent studies in microbial degradation of petroleum hydrocarbons in hypersaline environments." **Front. Microbiol**. 5(173):1-14.

C. Attaphong, E. Asnachinda, A. Charoensaeng, D. A. Sabatini, and S. Khaodhiar. 2010. "Adsorption and adsolubilization of polymerizable surfactants on aluminum oxide." **J. Colloid Interface Sci**. 344(1): 126–131.

C.N. Mulligan. 2005. “Environmental applications for biosurfactants,” **Environ. Pollut**. 133(2):183–198.

C.N.Mulligan. 2007. “Rhamnolipid biosurfactants: solubility and environmental issues,” **Thermodyn. Solubility Environ. Issues**: 279–298.

E. Asnachinda, S. Khaodhiar, and D. A. Sabatini. 2010. “Styrene and phenylethanol adsolubilization of a polymerizable Gemini surfactant.” **J. Surfactants Deterg**. 13(2): 143–148.

J. S. Clifford, M. A. Ioannidis, and R. L. Legge. 2007. “Enhanced aqueous solubilization of tetrachloroethylene by a rhamnolipid biosurfactant.” **J. Colloid Interface Sci**. 305(2):361–365.

K.-H. Shin and K.-W. Kim. 2004 A biosurfactant-enhanced soil flushing for the removal of phenanthrene and diesel in sand. **Environ.Geochem. Health**. 26(1):5–11.

M. Henkel, M. M. Müller, J. H. Kügler, R. B. Lovaglio, J. Contiero, C. Syldatk, and R. Hausmann. 2012 “Rhamnolipids as biosurfactants from renewable resources: concepts for next-generation rhamnolipid production,” **Process Biochem**. 47(8) :1207–1219.

N. Arpornpong, A. Charoensaeng, D. A. Sabatini, and S. Khaodhiar. 2010 “Ethoxy Carboxylate Extended Surfactant: Micellar, Adsorption and Adsolubilization Properties,” **J. Surfactants Deterg**. 13(3): 305–311.

N.Arpornpong,J. Lewlomphaisan, A. Charoensaeng, D. A. Sabatini, and S. Khaodhiar. 2013. “Ethoxy Carboxylate Extended Surfactant: Surface Charge of Surfactant-Modified Alumina, Adsolubilization and Solubilization of Phenylethanol and Styrene.” **J. Surfactants Deterg**. 16(3): 291–298.

N.Arpornpong.2008 **Adsolubilization and solubilization using conventional and extended anionic surfactants on an aluminum oxide surface.** [Online].Available: http://dric.nrct.go.th/bookdetail.php?book_id=227287. [Accessed: 08-Mar-2014].

P. Asvapathanagul, P. Malakul, and J. O’Haver. 2005.“Adsolubilization of toluene and acetophenone as a function of surfactant adsorption,” **J. Colloid Interface Sci**. 292(2) : 305–311.

R. Zhang and P. Somasundaran. 2006. “Advances in adsorption of surfactants and their mixtures at solid/solution interfaces.” **Adv. Colloid Interface Sci**. 123-126: 213–229.

T. F. Guerin, S. Horner, T. McGovern, and B. Davey. 2002 “An application of permeable reactive barrier technology to petroleum hydrocarbon contaminated groundwater,” **Water Res**. 36 (1): 15–24.

Y. Li, S. Tian, H. Mo, and P. Ning 2011. “Reversibly enhanced aqueous solubilization of volatile organic compounds using a redox-reversible surfactant.” **J.Environ. Sci**.23(9) :1486–1490.