การบำบัดดินปนเปื้อนสารไตรคลอโรเอธิลีนโดยใช้การแยกของสารลดแรงตึงผิว และการบำบัดทางชีวภาพ

นางสาวศศิกานต์ เชื้อหอม

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

TRICHLOROETHYLENE CONTAMINATED SOIL CLEAN-UP USING SURFACTANT-BASED SEPARATION TECHNOLOGY AND BIOREMEDIATION

Miss Sasikarn Chuahom

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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ศศิกานต์ เชื้อหอม : การบำบัดดินปนเปื้อนสารไตรคลอโรเอธิลีนโดยใช้การแยกของสารลดแรงตึงผิวและการบำบัดทางชีวภาพ (TRICHLOROETHYLENE CONTAMINATED SOIL CLEAN-UP USING SURFACTANT-BASED SEPARATION TECHNOLOGY AND BIOREMEDIATION) อ. ที่ปรึกษา : ดร.เอกวัล ลือพร้อมชัย, อ.ที่ปรึกษาร่วม : ดร.ปัญจพร เวชยันต์วิวัฒน์, 108 หน้า.

การใช้สารลดแรงตึงผิวและการบำบัดทางชีวภาพถูกนำมาใช้ร่วมกัน เพื่อเพิ่มประสิทธิภาพในการกำจัดสารไตรคลอโรเอธิลีน (ทีซีอี) ออกจากดิน ระบบนี้ได้นำเทคนิคการสกัดแบบขุ่นของสารลดแรงตึงผิวชนิดไม่มีประจุมาใช้เพื่อสกัดสารทีซีอีให้ไปอยู่ในวัฏภาคที่มีความเข้มข้นของสารลดแรงตึงผิวสูง หลังจากนั้นนำวิธีการบำบัดทางชีวภาพมาเสริมในระบบ โดยเติมแบคทีเรียเพื่อย่อยสลายสารทีซีอีที่เหลืออยู่ ได้ทำการศึกษาสารลดแรงตึงผิว 6 ระบบ คือ SURFONIC TDA-5, SURFONIC TDA-6, SURFONIC L24-7, NEODOL 91-5, NEODOL 91-6 ที่ไม่มีเติมสารอิเลคโทรไลต์ และ DTAB/DOWFAX (อัตราส่วนโดยโมลที่ 2:1) ที่มีการเติมโซเดียมคลอไรด์เป็นสารอิเลคโทรไลต์ สารลดแรงตึงผิวเหล่านี้สามารถเกิดการแบ่งวัฏภาค โดยมีวัฏภาคที่มีความเข้มข้นของสารลดแรงตึงผิวสูงปรากฏอยู่ชั้นบนของสารละลาย จึงป้องกันการสะสมของสารลดแรงตึงผิวบนเม็ดดินได้ ผลการทดลองพบว่า SURFONIC TDA-6, SURFONIC L24-7 และ NEODOL 91-6 ไม่ยับยั้งความสามารถของแบคทีเรีย *Rhodococcus* sp. L4 และ *Rhodococcus* sp. P3 ในการย่อยสลายทีซีอี ในขณะที่สารลดแรงตึงผิวชนิดอื่นส่งผลให้แบคทีเรียตาย ทั้งนี้พบว่าแบคทีเรีย *Rhodococcus* sp. L4 สามารถย่อยสลายทีซีอีได้อย่างมีประสิทธิภาพในระบบที่มี SURFONIC TDA-6 ซึ่งทีซีอีความเข้มข้นสิบส่วนในล้านส่วนถูกย่อยสลายได้มากกว่า 58% ภายใน 24 ชั่วโมง โดยเทียบกับชุดควบคุมที่ปราศจากแบคทีเรียซึ่งมีทีซีอีลดลงเพียง 30% จากผลข้างต้นได้เลือก SURFONIC TDA-6 มาศึกษาต่อเพื่อหาสภาวะที่เหมาะสมในการสกัดทีซีอีแบบขุ่น ซึ่งประกอบด้วย ช่วงเวลาสมดุล เวลาในการสัมผัสระหว่างสารละลายของสารลดแรงตึงผิวและดิน และความเข้มข้นเริ่มต้นของสารลดแรงตึงผิว โดยได้ผลของสภาวะที่เหมาะสมดังนี้ ช่วงเวลาสมดุลที่ 72 ชั่วโมง เวลาในการสัมผัสระหว่างสารละลายของสารลดแรงตึงผิวและดินที่ 1 ชั่วโมง และความเข้มข้นเริ่มต้นของสารลดแรงตึงผิว 70 มิลลิโมลาร์ จากนั้นนำสภาวะที่ได้มาศึกษาเพื่อเปรียบเทียบประสิทธิภาพของกระบวนการกำจัดทีซีอีดังต่อไปนี้ (1) การบำบัดทางชีวภาพ (2) การสกัดด้วยสารลดแรงตึงผิว และ (3) เทคนิคร่วมเพื่อทำการประเมินประสิทธิภาพในการกำจัดทีซีอีจากความเข้มข้นของทีซีอีที่เหลืออยู่ในดินหลังการบำบัด พบว่าร้อยละของประสิทธิภาพในการบำบัดดินปนเปื้อนทีซีอีความเข้มข้นหนึ่งร้อยส่วนในล้านส่วน โดยวิธีการบำบัดทางชีวภาพ การสกัดด้วยสารลดแรงตึงผิวและเทคนิคร่วม มีค่าประมาณ 74 74 และ 94 ตามลำดับ นอกจากนี้ยังพบว่าทีซีอีถูกย่อยสลายอย่างสมบูรณ์จากปริมาณคลอไรด์ที่สูงขึ้นหลังจากการบำบัดด้วยวิธีทางชีวภาพและเทคนิคร่วม เมื่อเพิ่มปริมาณทีซีอีเริ่มต้นเป็นสามร้อยส่วนในล้านส่วน พบว่าร้อยละของประสิทธิภาพในการบำบัดด้วยเทคนิคร่วมมีค่าประมาณ 94 ซึ่งสูงกว่าวิธีบำบัดแบบใดแบบหนึ่งประมาณ 30% จากผลการศึกษาพบว่าการบำบัดดินปนเปื้อนด้วยการใช้เทคนิคร่วมมีประสิทธิภาพในการกำจัดทีซีอีได้สูงที่สุด

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต.....ศศิกานต์ เชื้อหอม.....

ปีการศึกษา 2549 ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4889497920 : MAJOR ENVIRONMENTAL MANAGEMENT
KEY WORD: BIOREMEDIATION / SURFACTANT / TRICHLOROETHYLENE / CLOUD POINT EXTRACTION

SASIKARN CHUAHOM : (TRICHLOROETHYLENE CONTAMINATED SOIL CLEAN-UP USING SURFACTANT-BASED SEPARATION TECHNOLOGY AND BIOREMEDIATION. THESIS ADVISOR : EKAWAN LUEPROMCHAI, Ph.D., THESIS COADVISOR : PUNJAPORN WESCHAYANWIWAT, Ph.D., 108 pp.

Surfactant-based separation technology and bioremediation was integrated to enhance the removal of trichloroethylene (TCE) from soil. In this system, cloud point extraction by non-ionic surfactant was conducted to separate high amount of TCE into the surfactant-rich phase and then bioremediation was integrated into the system by adding bacteria to co-metabolize the remaining TCE. Six surfactant systems including SURFONIC TDA-5, SURFONIC TDA-6, SURFONIC L24-7, NEODOL 91-5, NEODOL 91-6 without electrolyte addition and DTAB/DOWFAX (2:1 molar ratio) with 0.8 M NaCl were studied. These surfactants induced a phase separation with the surfactant-rich phase presented on top of the solution thus preventing the accumulation of surfactant on soil particles. The results found that SURFONIC TDA-6, SURFONIC L24-7, and NEODOL 91-6 did not inhibit TCE degradability of either *Rhodococcus* sp. L4 or *Rhodococcus* sp. P3 bacteria while others killed the bacteria. *Rhodococcus* sp. L4 degraded TCE effectively in the presence of SURFONIC TDA-6, in which more than 58% of 10 ppm TCE was reduced within 24 hours compared to only 30% of TCE removal in control treatment (without the bacteria). SURFONIC TDA-6 was then selected for determining the optimal condition for TCE extraction consisting of equilibrium time, contact time between surfactant solution and soil and initial concentration of surfactant. The optimal condition for TCE extraction by cloud point technique were as followed; 72 hours of equilibrium time, 1 hour of contact time between soil and surfactant solution, and 70 mM of initial concentration of surfactant. These acquired conditions were later applied to compare the effectiveness of three TCE treatment processes including: (1) bioremediation, (2) surfactant extraction and (3) integrated technique. TCE removal efficiency was determined from the remaining TCE concentration in soil after treatment. The TCE removal efficiency of 100 ppm TCE contaminated soil by bioremediation, surfactant extraction and integrated technique were about 74%, 74%, and 94%, respectively. Moreover, TCE was mineralized as showed by the increase of chloride ions after remediation by bioremediation and integrated technique. When increased the amount of initial TCE to 300 ppm, the removal efficiency of the integrated technique was about 94%, which was around 30% higher than either technique alone. The result found that soil remediation by the integrated technique had the highest TCE removal efficiency.

Field of Study Environmental Management
Academic year 2006

Student's signature Sasikarn Chuahom
Advisor's signature
Co-advisor's signature

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LIST OF ABBREVATIONS

Abs	Absorbance
ASTP	Aqueous Surfactant Two-Phase System
CMCs	Critical Micelle Concentrations
DCE	Dichloroethene
DNAPL	Dense Non-Aqueous Phase Liquid
DOWFAX	Trade name of Alkyldiphenyloxide disulfonate
DTAB	Dodecyltrimethylammonium Brmide
GC	Gas chromatograph
MSM	Mineral Salt Medium
mL	Millilitre
mM	Millimolar
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PCE	Tetrachloroethylene
ppm	Part per Million
SURFONIC L Series	Linear Alcohol Ethoxylates
SURFONIC TDA Series	Branched Alcohol Ethoxylates (Isotridecyl)
TCE	Trichloroethylene
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
VOCs	Volatile Organic Compounds
VC	Vinyl Chloride

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CHAPTER I

INTRODUCTION

1.1 Statement of Problem

Trichloroethylene (TCE) is commonly used as chlorinated solvent in cleaning and degreasing metal parts and electronic components. It is also used as a solvent or raw material to make other chemicals. The widespread use of TCE in industry and household leads to a high possibility of leakage and contamination to the environment. TCE contamination of large volumes soil becomes an important environmental problem because of its toxic properties. TCE is volatile organic compound (VOC). It has low solubility so it poses a significant risk to accumulate in the body, as it is known animal and suspected human carcinogen (Infante *et al*., 1987).

Many studies have demonstrated that TCE can be biodegraded by aliphatic and aromatic hydrocarbon-degrading bacteria via aerobic co-metabolism (Little *et al*., 1988, Hopkins *et al*., 1993, Fries *et al.*, 1997). Similarly, our laboratory found that *Rhodococcus* sps. which is induced with cumene, a non-toxic, environmentally friendly compound, is able to degrade about 70% of 100 ppm TCE in soil within 4 days (Suttinun *et al*., 2004). Bioremediation is considered as a clean technology because it uses microbes to convert hazardous chemical to environmental friendly products such as water, carbon dioxide, biomass, and salts. It is a friendly alternative choice for TCE removal. However, the success of bioremediation is limited by the low bioavailability of hydrophobic compounds to microorganisms, very high concentrations of pollutants in the contaminated site, and long treatment duration (Guha and Jaffe, 1996; Mihelcic *et al*., 1993; Tiehm *et al.*, 1997). Consequently, soil clean-up by only bioremediation is not the best available treatment method.

TCE is a dense non-aqueous phase liquid (DNAPL), it does not move with the groundwater flow but instead moves downward by gravitational force through an aquifer until reaching an impermeable layer. Thus, remediation strategies are needed to control migration of aqueous-phase TCE through the subsurface and remove TCE from contaminated soil. Remediation of non-aqueous phase liquids (NAPLs) by conventional pump-and-treat methods (i.e., water flushing) is generally considered to

be ineffective due to low water solubilities of NAPLs and to mass-transfer constraints (Haapea and Tuhkanen 2006).

Surfactant-based separation technology is one of the most promising new technologies for cleaning up soil and groundwater contamination as it can greatly increase the apparent solubility of NAPL contaminants (McCray *et al.*, 2001). Many surfactants were used to remove organic carbon pollutants. For example, a nonionic surfactant, POL was used as solvent in soil washing of PCBs (Layton, *et al*., 1998). However, the surfactant only transferred the pollutants from soil into soil wash solution, thus post-treatment of a large volume of this aqueous solution is necessary. Biodegradation of these pollutants and surfactant are usually used as post-treatment (Layton, *et al*., 1998; Haapea and Tuhkanen, 2006). However, the concentration of surfactant necessary for solubilization of hydrophobic compounds may inhibit the bacterial degradation of these compounds (Deschenes *et al*., 1996; Laha and Luthy, 1991). In addition, the cost of surfactant is expensive. The reuse of surfactant would be more appropriated than disposal and biodegradation.

Cloud point technique was applied in this research to overcome those problems. The advantages of cloud point extraction are not only to remove but also pre-concentrate TCE from soil in the surfactant-rich phase, thus the technique reduces the amount of waste for further treatment or disposal. In addition, surfactant in rich phase can be reused. Cloud point extraction is one of the surfactant-based separation technology in which a nonionic surfactant is utilized as a separating agent (Trakultamupatam *et al.,* 2002; Kimchuwanit *et al.,* 2000). When this surfactant is heated above a certain temperature known as the cloud point, the solution separates into two immiscible aqueous phases. The surfactant-rich phase has very high concentrations of surfactant and pollutant, thus the surfactant can be regenerated by vacuum stripping later (Choori *et al.,* 1998). Meanwhile, the surfactant-dilute phase solution contains only small amount of surfactant and pollutant. Using cloud point extraction approach, Kimchuwanit *et al.* (2000) can extract up to 91% of TCE from wastewater. Their results showed that 5.88, 10.49, and 28.15 ppm of TCE still remained in the dilution phase solution from the initial wastewater concentration of 50, 100 and 200 ppm, respectively. There are many studies shown that TCE at this concentration can be degraded by *Pseudomonas cepacia* G4 PR1 (Luu *et al*., 1995), *Rhodococcus* sp. P3 (Suttinun, 2003), and *Rhodococcus* sp. L4 (Luepromchai, 2004).

The combination of surfactant based-separation technology and bioremediation tends to be more effective than either technology alone. Consequently, this proposed research aimed to find an optimal condition for integrating cloud point extraction and bioremediation for clean-up TCE contaminated soil. Type of surfactant was the most important factors in this study since some surfactants may be toxic to TCE-degrading bacteria. Only non-toxic surfactants that induced phase separation with surfactant rich-phase solution on the top of solution were selected to avoid the stickiness on soil after phase separation. Furthermore, an optimal condition upon cloud point extraction of the selected surfactant was determined in order to get the highest TCE extraction efficiency with the least consumption of time, energy and surfactant. The results would open the prospect of applying a selected surfactant and its optimal condition based on cloud point extraction to enhance the efficiency of soil bioremediation treatment to meet the possible lowest level.

1.2 Objective

The main objective of this study was to optimize and integrate surfactant-based separation technology and bioremediation for clean-up TCE contaminated soil. The specific objectives were as follows:

1. To examine the effects of various surfactants on TCE degrading bacteria and select a surfactant that does not inhibit bacterial TCE degradability as well as a strain of bacteria that provides the highest TCE biodegradation rate.
2. To determine an optimal condition upon cloud point extraction of the selected surfactant that gives the highest TCE extraction efficiency.
3. To integrate the extraction technique using surfactant with TCE degrading bacteria for clean-up TCE contaminated soil.

1.3 Hypothesis

An integrated technique of surfactant-based separation technology and bioremediation can enhance the efficiency of TCE contaminated soil clean-up.

1.4 Scope of work

The research was divided into three phases as follows:

Phase 1: The effects of various surfactants on TCE degrading bacteria

- The effects of surfactants on bacterial survival

Six surfactants (5 non-ionic surfactants namely, SURFONIC TDA-5, SURFONIC TDA-6, SURFONIC L24-7, NEODOL 91-5, NEODOL 91-6 without electrolyte addition and a mixture of cationic-anionic surfactant namely, DTAB/DOWFAX (2:1 molar ratio) with 0.8 M NaCl), were screened in this study. After phase separation, only surfactant-dilute phase solution was taken to determine the effects of the surfactants on bacterial growth by plate count technique. Two strains of TCE degrading bacteria including *Rhodococcus* sp. L4 and *Rhodococcus* sp. P3 were used in the study. The promising surfactants were determined by ones which bacteria can survive or tolerate after incubation time.

- The effect of surfactant on TCE biodegradation

Only surfactants which did not make bacteria die from previous study were used in this study. The aims of this experiment were to find surfactant that did not inhibit the activity of bacteria on TCE biodegradation and to select the strain of bacteria that provides the highest TCE biodegradation rate. The initial TCE concentration in the surfactant-dilute phase solution was 10 ppm.

Phase 2: The optimal condition of cloud point extraction on TCE extraction efficiency

The optimal condition on cloud point extraction that provided the highest TCE extraction efficiency from soil was determined. The studied parameters were consisted of equilibrium time, contact time between surfactant solution and soil, and initial surfactant concentration. A selected surfactant from previous study was used in this study. Sandy clay loam soil was used as a model soil sample.

Phase 3: The effectiveness of the integrated process of surfactant-based separation technology and biodegradation in the TCE contaminated soil clean up

The study of TCE biodegradation was performed under the aerobic condition using a selected microorganism, a selected surfactant and its optimal condition to clean up the TCE contaminated soil. Three treatment methods including (a) soil remediation by biodegradation, (b) soil remediation by cloud point extraction, and (c) soil remediation by an integrated technique were conducted to examine their efficiency on TCE removal. TCE removal efficiency was determined from remaining TCE concentration in soil. In addition, chloride ion formation was monitored in order to confirm the mineralization of TCE.

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CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Trichloroethylene (TCE)

2.1.1 General information of TCE

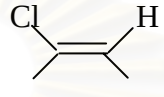
Trichloroethylene (TCE), a chlorinated aliphatic hydrocarbon (CAH) was first synthesized during the preparation of tetrachloroethane in 1864. It is sometimes called by other names, such as trichloroethene, ethylene trichloride, or ethinyl trichloride. It is sold under many different brand names, such as Tri-Clene, Trielene, Trilene, Trichloran, Trichloren, Algylen, Trimar, Triline, Tri, Trethylene, Westrosol, Chlorylen, Gemalgene, and Germalgene.

For most of its history, trichloroethylene (TCE) is commonly used as solvent for cleaning and degreasing of metal parts. It is also found in household products such as in paint, paint stripper and adhesive. Trichloroethylene is an effective solvent for a variety of organic materials. When it was first widely produced in the 1920s, its major use was to extract vegetable oils from plant materials such as soy, coconut, and palm. Other uses in the food industry included coffee decaffeination and the preparation of flavoring extracts from hops and spices. It was also used as a dry cleaning solvent, although tetrachloroethylene (also known as perchloroethylene) surpassed it in this role in the 1950s. Due to concerns about its toxicity, the use of trichloroethylene in the food and pharmaceutical industries has been banned in much of the world since the 1970s (Wikimedia Foundation, Inc., 2006).

Physical and chemical properties of TCE are in Table 2.1. At room temperature, TCE is a colorless liquid with a sweet, chloroform-like odor. It is a volatile organic compound (VOC), producing potentially toxic concentrations at room temperature. It is nearly insoluble in water, but miscible with most organic solvents. It has low water solubility so it poses a significant risk of accumulating in human body. TCE is classified as a dense non-aqueous phase liquid (DNAPL) thus, it does not move with the groundwater flow but tends to migrate down gradient by gravity

through an aquifer until it reaches an impermeable layer (Mackay and Cherry, 1989). Since trichloroethylene decomposes photolytically, it should be stored in cans or dark glass bottles to minimize decomposition. Storage areas should be cool, well ventilated, flame-proof, and shielded from direct sunlight, high-temperature surfaces, or sparks (ATSDR, 2006).

Table 2.1 Physical and chemical properties of TCE

Property	Characteristic
Structure	
Formula	C_2HCL_3 or $Cl_2C=CHCl$
Molecular weight (MW)	134.40
Boiling point (760 mm Hg)	87ºC (189ºF)
Melting point	-73ºC (-99ºF)
Specific gravity at 20ºC (water = 1)	1.465 g/ml
Vapor pressure at 20ºC	58 mm Hg
Gas density (air = 1)	minimal 4.53
Water solubility	0.1% at 77ºF
Description	colorless liquid with a chloroform-like odor
Odor threshold: air	100 ppm
Flammability	Flammable liquid that does not burn easily; at temperatures >600ºF (316ºC), it forms hydrogen chloride and phosgene
Flammable range (concentration in air)	8% to 10.5 %
Partition coefficients: Log Kow Log Koc	2.42 2.03 – 2.66
Henry's law constants: at 20ºC at 25ºC	0.020 atm-m3/mol 0.011 atm-m3/mol

Source: Agency for Toxic Substances and Disease Registry, ASTDR (1997)

2.1.2 Environmental fate of TCE

According to the U.S. Environmental Protection Agency's Toxic Chemical Release Inventory (1995), the biggest source of TCE in the environment is evaporation from metal factories in the process of grease removal. Moreover, TCE can enter the air and water when it is disposed at chemical waste sites. It can also release into the soil through industrial discharges and landfill leachate. Most TCE deposited in surface water or soil surface will volatilize to the atmosphere as a mean of TCE elimination. The process is relatively rapid from surface water more than from soil because TCE is absorbed by the affect of organic content (CEPA, 1993). So, TCE has high mobilization in the soil and this may result in substantial percolation to subsurface region before volatilization occurred.

TCE tend to sink into the soil subsurface by displacing water from soil pores and eventually sinking through the groundwater while leaving behind residual pockets that can contribute to long term contamination (Anderson and Andersen, 1996). At these subsurface environments, TCE is only slowly degraded and is relatively persistent. Under anaerobic condition, TCE may be biotransformed in to dichloroethylene and ultimately to a more potent carcinogen such as vinyl cholrine (Parsons et al., 1994).

In Thailand, TCE is widely used in industry for metal degreasing and electronic parts cleaning as well as in plastic processing. In 1998, Thailand Environment Institute (TEI) surveyed 476 factories and found that 16% of the factories used high amount of TCE and stored the spent chemical in metal or plastic containers before sent to the waste treatment company (TEI, 1998). The study of TCE contamination is limited in our country. Only Milintawisamai et al. (2001) had studied the level of TCE contamination in three Thai factories that utilized TCE in their production process. TCE was found in soil and groundwater from these factories at the concentration ranges from 0.03 - 970 ppm (Milintawisamai et al., 2001). TCE has been released into the environment as a result of improper management in manufacture, storage and disposal i.e., TCE directly discharging to soil around the factory, outdoor storage and accidental leakage from the aged pipe or storage tank. It is also implied that TCE may contaminate other factories in Thailand as well. Therefore, TCE would be a major environmental contaminant and may cause long term health effects in the near future.

2.1.3 Toxicity of TCE

Organochlorine compounds such as trichloroethylene present a potentially serious environmental liability given their great resistance to natural degradation and their high marine toxicity. The active metabolite of trichloroethylene is trichloroethanol, identical to that of chloral hydrate. Therefore, concerns of the carcinogenicity of the latter have been raised, and are subject to on-going debate. (Wikipedia Foundation, Inc., 2006). TCE tends to accumulate in environment by its properties so the organisms in that environment, has risk to expose and accumulate TCE in their bodies. TCE mainly affects the central nervous system (the brain), causing headache, nausea, dizziness, clumsiness, drowsiness, and other effects like those of being drunk. TCE can also damage the facial nerves, and it can cause skin rash. Heavy exposure can damage the liver and kidneys. Data from the Department of health services (1997) shown that TCE causes cancer in animals and may cause cancer in humans. Health affects can be divided into 2 mains effect as following;

- **Acute Exposure**: TCE is thought to depress the central nervous system (CNS) via a solvent effect on lipids and protein components of neural membranes. It sensitizes the heart to epinephrine, making it more susceptible to epinephrine-induced arrhythmias. Direct exposure to liquid trichloroethylene degreases the skin, causing redness, blistering, and scaling. Trichloroethylene can cause respiratory and CNS depression and abnormal heart rhythm. Death may result from respiratory depression. Liver necrosis has been reported for some people exposed to fatal levels of trichloroethylene, but individuals exposed to trichloroethylene as an anesthetic showed only minimal effects on liver function.

- **Chronic Exposure**: Chronic exposure has been reported to be associated with damage to the cranial nerves and neurological effects such as memory loss and impaired cognitive function. However, these studies did not have accurate exposure data and individuals were often exposed to mixtures of chemicals. However, the NTP Board Subcommittee has recommended that it be listed as "reasonably anticipated to be a human carcinogen. And the International Agency for Research on Cancer has determined that trichloroethylene is probably carcinogenic to humans.

The long-term effects of trichloroethylene on human beings are unknown. In animal studies, chronic trichloroethylene exposure has produced liver

cancer in mice, but not in rats. Studies on its effects on reproduction in animals have been similarly inconsistent, and so no conclusive statements about its ability to cause birth defects in humans can be made. Recent studies have shown a correlation between male fertility and exposure to trichloroethylene. Trichloroethylene has been shown to reduce sperm counts in some cases. More recent analyses indicate low-level evidence of a mutagenic or teratogenic effect; thus, it is known that it promotes the formation of tumors, though the exact pathway is not well-understood. Its long-term safe use as a surgical anesthetic did not lead to an increased incidence of cancer as compared to background levels, indicating that any such effect is most probably extremely low-level. It is current categorized as IARC 2A, analogous to trichloromethane—*reasonably anticipated to be a human carcinogen.* More information on the carcinogenic potential of organochlorine compounds may be gleaned from the report on carcinogens.

The Environmental Protection Agency mounted a major effort in the 1990s to assess how dangerous trichloroethylene was to human health. Following four years of study, senior EPA scientists concluded in 2001 that it is 2 to 40 times more likely to cause cancer than the EPA had previously believed. The National Academy of Sciences reported Thursday, July 27, 2006, that significant "evidence on [the] carcinogenic risk and other health hazards from exposure to trichloroethylene has strengthened since 2001." The report goes on to say there is "a large body of epidemiologic data available" on TCE showing the chemical is a possible cause of kidney cancer, reproductive and developmental damage, impaired neurological function and autoimmune disease. TCE is found in about 60 percent of the nation's worst contaminated sites in the Superfund cleanup program, specifically sites maintained by the United States Department of Defense, United States Department of Energy and NASA (Wikimedia Foundation, Inc., 2006).

2.1.4 Regulations for TCE controlling

Because of the potential toxicity of TCE, the concerns of human health and environment have been raised. Many standards of TCE were set to control and limited the harmfulness of TCE. For example;

- The United States, Environmental Protection Agency (EPA) has set a drinking water standard for TCE to 5 ppb.
- OSHA PEL (permissible exposure limit) for averaged over an 8-hour workshift is 100 ppm
- OSHA ceiling for TCE is 200 ppm
- OSHA STEL (short-term exposure limit) for 5-minute exposure in any 2 hours is 300 ppm
- NIOSH IDLH (immediately dangerous to life or health) for TCE is 1,000 ppm
- AIHA ERPG-2 (emergency response planning guideline) (maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 hour without experiencing or developing irreversible or other serious health effects or symptoms which could impair an individual's ability to take protective action) for TCE is 500 ppm.

In Thailand, Pollution Control Department has set a groundwater standard for TCE to 5 ppb. Moreover, different countries have different TCE standards. The exposure limits of each country were shown in Table 2.2.

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Table 2.2 TCE exposure limits of various countries

Country	OEL (TWA)*	STEL**
Austria	50 ppm	250 ppm
Belgium	50 ppm	200 ppm
Denmark	10 ppm	-
France	75 ppm	200 ppm
Germany	50 ppm	250 ppm
Italy	50 ppm	100 ppm
The Netherlands	35 ppm	100 ppm
Sweden	10 ppm	25 ppm
Switzerland	50 ppm	250 ppm
United Kingdom	100 ppm (MEL)***	150 ppm

Source: Chlorine online information resource or ECSA

*Occupational Exposure Limit (Time Weighted Average): 8 hours per day

**Short Term Exposure Limit (15 minutes)

***Maximum Exposure Limit - obliges users to achieve levels as far below this as possible

2.2 TCE biodegradation

2.2.1 Microorganisms

For the successful of chlorinated aliphatic compound bioremediation, microorganisms that are capable of metabolizing these compounds must be identified. Twenty years ago, most chlorinated aliphatic compounds were considered as nonbiodegradable. Recently, many microorganisms have been discovered in the successes of metabolizing chlorinated aliphatic compounds. For example, *Pseudomonas* sp. CPE1 is capable of metabolizing low-chlorinated biphenyls (Fava, and Grassi1, 1996). *Pseudomonas cepacia* G4 PR1, *Rhodococcus* sp. P3, *Pseudomonas sp*. T1 and *Rhodococcus* sp. L4 are capable of metabolizing 10 ppm of TCE (Luu et al., 1995; Suttinun, 2003; Luepromchai, 2004).

An understanding of mechanisms involved in organohalide metabolism is directly relevant to the design of bioremediation system. For example, one of the

first design decisions is to choose between aerobic and anaerobic biotreatment system. This decision is depended on type of chlorinated pollutants such as a partially chlorinated alkene would likely be treated best aerobically, while perchlorinated alkanes and alkenes would be treated anaerobically (Wackett et al., 1992). Traditionally, chlorinated compounds are degraded by three main pathways.

1. Reductive dehalogenation (electron acceptor reactions): It typically takes place under anaerobic conditions, where the chlorinated solvent acts as an electron acceptor, and one chlorine atom is replaced with a hydrogen atom. This is the most important reaction for highly chlorinated compounds such as tetrachloroethylene (PCE). The chemical reactions are less favorable as the molecule loses chlorine atoms (Alexander, 1994).

2. Electron donor reactions: It typically takes place under aerobic conditions, where the microorganisms utilize less chlorinated compounds (i.e. vinyl chloride) as a primary substrate (Bradley et al., 1997).

3. Co-metabolism: It is a fortuitous modification of one molecule (co-metabolized substrate or co-substrate) by an enzyme which routinely acts on another (primary substrate) molecule. The primary substrate supports growth of the microorganism, while the co-metabolized substrate is usually altered only slightly and does not enter catabolic and anabolic pathways of the microbial cell. Therefore, the responsible organism does not benefit from co-metabolic reactions.

2.2.2 Co-metabolism of TCE

TCE is not a growth supporting substrate for aerobic microorganisms. TCE-degrading microorganisms cannot grow on it but can oxidize it via co-metabolism, which TCE is transformed without concurrent microbial growth. Co-metabolism offers the abilities of microorganisms to transform non-growth-supporting substrates, in the presence of a growth supporting substrate. The metabolism of growing supporting substrates (e.g., methane, propane, butane, toluene, ethylene, and ammonia) is the physiological role of oxygenase enzymes involving in the initiation of TCE degradation. Monooxygenases or dioxygenases are the enzymes responsible for the initiation of this transformation (Arp et al., 2000). Many studies have demonstrated that TCE can be biodegraded by aliphatic and aromatic hydrocarbon-degrading bacteria via aerobic co-metabolism, for example, methane

oxidizing bacteria (Little et al., 1988), ammonia oxidizing bacteria (Hyman et al., 1995), phenol oxidizing bacteria (Hopkins et al., 1993), and toluene oxidizing bacteria (Fries et al., 1997).

Co-metabolism has been reported to take place with TCE, dichloroethylene (DCE) and vinyl chloride (VC), which the less chlorinated compounds (e.g. VC) reacting faster than the higher chlorinated compounds. Example of TCE co-metabolism by toluene-degrading enzymes is shown in Figure 1. Transformation of chlorinated solvents by these enzymes presents the cells with a new set of compounds. Some of these compounds are toxic to cells, others are stable products that are expelled from the cells, and in a few case the cells utilize the products (Alvarez-Cohen and McCarty, 1991).

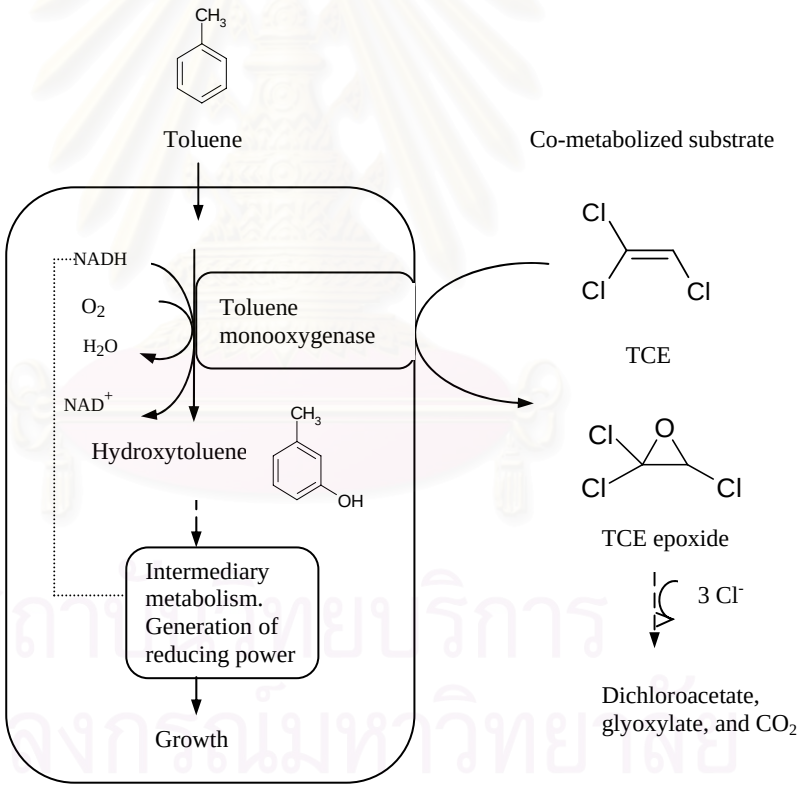


Figure 2.1 Example of TCE co-metabolic pathway by toluene monooxygenase enzyme. The microorganism utilizes toluene as primary substrate while oxidizes TCE to an epoxide and later to CO_2.

(Source: www. wiley-vch.de/books/biotech/pdf/v11b_aero.pdf)

Luu et al. (1995) reported that resting cell suspensions of *Pseudomonas cepacia* G4 PR1 degraded 85 % of 10 ppm TCE in 6 hours. The studies of TCE degradation at the same TCE concentration by cumene, a non-toxic, environmentally friendly compound, as enzyme inducer that performed by Suttinun (2003), showed that *Rhodococcus* sp. P3 and *Psuedomonas* sp. T1 can degrade 76% and 61% of 10 ppm TCE in 24 hours, respectively. The research results corresponds well with the result of Dabrock et al. (1992) and Pflugmacher et al. (1996) which found that the initial TCE oxidation rate of *Pseudomonas* sp. JR1 and *Rhodococcus erythropolis* BD2 increase proportionally with the increasing concentrations of cumene from 3 to 24 mg L^{-1}.

2.3 Surfactant

2.3.1 Characteristics of surfactant

Surfactant is a contraction of a term SURFace ACTive AgeNT. It composes of two dissimilar parts in one molecule called an amphipathic structure. One part is hydrophilic and another one is hydrophobic part. Hydrophobic tail or water-insoluble long-chain hydrocarbon linked to hydrophilic head or water soluble group as shown in Figure 2.2. This combination makes the ambivalent; the hydrophilic head group is attracted to polar environment, for example water, while hydrophobic tail group is attracted to non-polar environment, such as oil. So, it has the ability to solubilize water or oil to create homogenous system (Uppgard, 2002). The outstanding property of this compound, surfactant tend to concentrate at the surface and interfaces of an aqueous solution and to alter the surface properties. It generally reduces the surface tension between two immiscible phases. In distinction it is applied the term of detergent to product of formulation designed for cleaning or laundering (Swisher, 1987).

Surfactant tends to absorb at the surfaces and reduce the surface tension because once surfactant dissolved into an aqueous solution, its hydrophobic will distort the structure of water (by breaking the hydrogen bond between the water molecules) resulting in an increases in the free energy of the system (the minimum work which requires in order to create interface) Then, the system responds in some

ways in order to minimize the free energy and thus, the surfactant is expelled to contact with water and migrates to the surfaces or interfaces of the system.

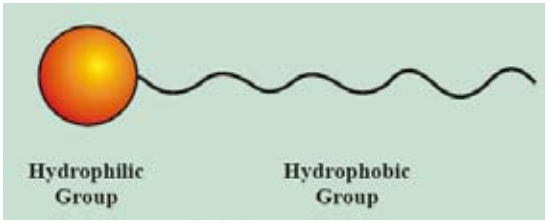


Figure 2.2 Surfactant molecule or monomer (Scamehorn et al., 2004)

2.3.2 Type of surfactants

The classification of surfactant is based on the charge of their hydrophilic head. They can be classified into four types as following; (Milton, 1989; Salager, 2002).

1) Anionic surfactant

Anionic surfactants possess the negative charge at the head portion, usually originating in sulfonate, sulfate, or carboxylate groups. [Eq.(2.1)]. These represent a major fraction of surfactants in commercial use. For example, alkylbenzene sulfonates (detergents), soaps (fatty acid), lauryl sulfate (foaming agent), di-alkyl sulfosuccinate (wetting agent), lignosulfonates (dispersants), etc.

$$RSO_3Na \leftrightarrow RSO_3^- + Na^+ \qquad (2.1)$$

2) Cationic surfactant

Cationic surfactants have positive charge at the head portion. A large proportion of this class corresponds to nitrogen compounds such as fatty amine salts and quaternary ammoniums derivatives, with one or several long chain of the alkyl type, often coming from natural fatty acid [Eq.(2.2)]. In general, these surfactants are more expensive than anionic because of the high pressure hydrogenation reaction used during synthesis. Thus, they represent only a minor fraction of surfactants being used in industry.

$$RMe_3NCl \leftrightarrow RMe_3N^+ + Cl^- \qquad (2.2)$$

3) Nonionic surfactant

Nonionic surfactants have no ionic charge on their hydrophilic groups. They do not ionize appreciably in aqueous solution because their hydrophilic group is a non-dissociable type, such as alcohol, ether, ester, or amide. A large proportion of nonionic surfactant used in industry has their hydrophilic portion as a polyethylene glycol chain, obtained by polycondensation of ethylene oxide (EO). Therefore, they are called polyethoxylate nonionic surfactant. They account for about 45% of the over all industrial production.

4) Zwitterionic surfactant

Zwitterionic surfactants have their charge depending on pH, i.e. they possess positive charge at low pH, negative charge at high pH, and at neutral pH they will have both negative and positive charge. They are commonly expensive and consequently, their use is limited to very special application e.g. cosmetics.

2.3.3 Alcohol Ethoxylates

Alcohol ethoxylate is one of nonionic surfactant which consists of 2 parts of ethylene oxide group (EO) and alcohol compound. The various types of alcohol ethoxylate nonionic surfactant are depended on the number of mole of ethylene oxide group and carbon of alcohol. Therefore, trade names of surfactants are different. Alcohol ethoxylate can be divided into 2 types as follows (Huntsman company, 2007);

(1) Linear Alcohol Ethoxylates

Chemical Structure $CH_3(CH_2CH_2)_xCH_2O(CH_2CH_2O)_nH$

where: x = 2-7 and n = moles of EO

Biodegradability of linear alcohol ethoxylates are classified be readily biodegradable. Linear alcohol ethoxylates, including the SURFONIC L series, undergo rapid and extensive biodegradation under both laboratory and environmental conditions. Their mineralization to CO_2 and water (ultimate biodegradation) is essentially complete during biological wastewater treatments at warm to cold water

temperatures. They are degraded by bacteria in rivers, lakes, groundwater and sediment as well.

The major mechanism of biodegradation is cleavage of the ethoxylate chain from the alkyl group with oxidation of the latter to fatty acid. The fatty acid degrades more rapidly than the ethoxylate chain, which is broken down by sequential oxidation and removal of ethoxylate units.

Alcohol ethoxylates begin to lose their toxicity toward aquatic organisms as soon as biodegradation begins. Water containing degraded surfactant has been shown not to adversely affect fish, invertebrates and algae. Thus, while alcohol ethoxylates are toxic to aquatic organisms, in the event of a spill into a waterway any acute effects would be limited in area and time. SURFONIC L-series surfactants and other linear alcohol ethoxylates pose no serious threat to the environment. They do not accumulate in any environmental compartment and are found, if at all, only at concentrations below chronic effect levels.

(2) Branched Alcohol Ethoxylates

Chemical Structure $CH_3(CH_2)_xCH_2O(CH_2CH_2O)_nH$

where: x = 6, 8, 10 or 11 and n = moles of EO

Biodegradability of branched alcohol ethoxylates including SURFONIC® EH, DA, DDA and TDA series surfactants are classified be inherently biodegradable, approaching requirements for ready biodegradability. Both the isododecyl alcohol (branched alcohol) and its 7-mole ethoxylate have been demonstrated to be rapidly and extensively biodegradable as measured by standard laboratory tests with biodegradation levels meeting the definition for “inherent biodegradability” and approaching that for “ready biodegradability. The 7-mole ethoxylate exhibits aquatic toxicity similar to that of linear alcohol ethoxylates with standard test organisms.

Nomenclature

The SURFONIC alcohol ethoxylates are named using an alphanumeric system. The letter designates the alcohol type.

L Linear (varying carbon ranges)

EH 2-ethylhexanol

DA Isodecyl (branched)

DDA Dodecyl (branched)

TDA Isotridecyl (branched)

For the SURFONIC L series products, the number following the “L” indicates the alcohol blend carbon number range. For example, ethoxylates in the L12 series are based on a blend of C10 and C12 linear alcohols while ethoxylates in the L46 series are based on a blend of C14 and C16 linear alcohols.

For all the alcohol ethoxylates, the number following the hyphen indicates the oxide: alcohol ratio. For example, SURFONIC L12-6 and TDA-6 surfactants are both 6-mole ethoxylates of the specified alcohol or alcohol blend.

SURFONIC L24-7 is seven-mole ethoxylate (average EO groups) of linear, primary 12-14 carbon number alcohol. It is a water-soluble, nonionic surface active agent which is compatible with other nonionic surfactants and with most anionic and cationic surfactants. The product is a clear to slightly turbid liquid.

SURFONIC TDA-6 is six-mole ethoxylate of isotridecyl alcohol. It is a water dispersible, nonionic surface-active agent which is compatible with other nonionic surfactants and with most anionic and cationic surfactants.

NEODOL 91-5 is five-mole ethoxylate of linear, primary 9-11 carbon number alcohol. While, NEODOL 91-6 is the six-mole ethoxylate of linear, primary 9-11 carbon number alcohol.

NEODOL* ethoxylates are colourless and range from liquids to low melting point solids of pasty consistency. They are excellent wetting agents, emulsifiers and detergents, and are moderate foamers. All NEODOL ethoxylates are 100% active, have low colour and a neutral pH (6-7). They also have low water contents (Shell Chemical, 2007).

2.3.4 Mixture of cationic-anionic surfactant

Cationic and anionic surfactant mixtures or catanionic surfactant is a new type of surfactant system which occur phase separation at appropriate initial concentration and composition of mixed surfactant (Zhao and Xiao, 1996; Herrington and Kaler, 1997). Catanionic surfactant mixtures were known as pseudo-nonionic surfactant, they can be induced phase separation by cloud point technique as same as nonionic surfactant.

The phase behavior of catanionic surfactant mixtures of DTAB/DOWFAX were studied by Krutlert (2004). Dodecyltrimethyamonium bromide (DTAB) is a cationic surfactant and Alkyldiphenyloxide disulfonate (ADPODS) or trade name in DOWFAX 8390 is an anionic surfactant. Their chemical structures were shown in Figure 2.3. The results revealed that DTAB and DOWFAX system has a balance in stoichiometry at 2:1 molar ratio behaving as pseudo-nonionic surfactant because DTAB has one cationic head and one alkyl tail while DOWFAX has two anionic heads and one or two alkyl. Moreover, NaCl adding lead to the phase separation since the density of surfactant-rich phase after salt adding is higher than water's.

(a) (b)

Figure 2.3 Chemical structure (a) DTAB (b) DOWFAX

2.3.5 Micellization

The micellization or micelle formation is a phenomenon at which the surfactant forms colloidal-sizes clusters in solution. The concentration of surfactant where is phenomenon takes placed is called the critical micelle concentration (CMC). There are many ways to investigate this critical concentration. Some physical changed at this properties of surfactant solution are obviously change at this

surfactant concentration i.e. surface tension, electrical conductivity, light scattering, osmotic pressure, etc.

At low surfactant concentration in aqueous solution, surfactant present as monomer in the solution as well as adsorbed at the interface because their hydrophobic groups distort the structure of the water leading an increase of free energy of the system. Therefore, the way to reduce this free energy is to migrate to the surface and orient their hydrophobic groups to the air. Thus the free energy can be minimized. If surfactant concentration is increased, the way to minimize the free energy is to form themselves into the clusters (Herrington and Kaler, 1997; Rixt, 2001; Uppard, 2002). When the surfactant concentration exceeds a certain value called the critical micelle concentration (CMC), the simplest new structure formed by the aggregation of monomer is called micelle, where hydrophilic head orient toward the water while their hydrophobic groups direct toward the interior of clusters. The micellar interior is oil-like, formed by hydrocarbon groups surrounded by hydrophilic groups.

2.3.6 Solubility

The solubilization is spontaneous dissolving of a substance (solid, liquid of gas) into the surfactant micelles. After the micellization, the shape of surfactant aggregates bring about interesting properties e.g. their ability to solubilize the solute molecules into the inner core of the micelle, viscosity, cloud point phenomenon (Milton, 1989). The micelles have the ability to dissolve hydrophobic substances into the core of their structure. This is known as micellar solubilization and the solubilized substance being termed the solute or the solubilizate. The solubilization of solute is very slight until the concentration of surfactant reaches a critical concentration at which the solubility increases approximately linearly with the surfactant concentration. That critical concentration is the CMC of the surfactant in the presence of the solubilizate.

Solubilizates are incorporated into the micelles in different location according to their structure. Generally, the polar molecule will solubilize at the outer of the micelle probably at micelle-water interface or at palisade layer, where the nonpolar molecule will solubilize in the inner core of micelle. The palisade layer is

the area between hydrophilic group and first few carbon atom of hydrophobic group. (http://hls.dmu.ac.uk/teaching/gsmith)

(a) Saturated solubilizates such as saturated aliphatic and cyclic hydrocarbon tend to dissolve deep in the interior core of the micelle.

(b) Solubilizates possessed weakly polar or polarizable groups (such as long chain alcohol or any double bonds molecule) tend to locate nearer the micelle surface.

(c) Amphilphillic solubilizates orient along side with surfactant monomers.

(d) Water-soluble solubilizates are throught to form complex with the polyoxyethylene chain, i.e. the solubilizate.

The main factor determining the extent of solubilization of the organic solute in the aqueous micelle solution are the molecule structure of surfactant and organic solute and the condition in the experiment, including temperature and concentration of added electrolyte.

- Structure of surfactant: The longer the hydrophobic tail of surfactant, greater the aggregation number of surfactant micelle resulting in an increase solubilization capacity of nonpolar solubilizate.

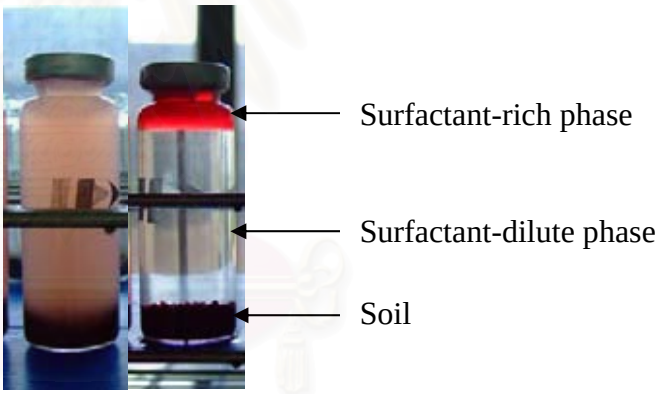
- Structure of solubilizate: The longer the hydrophobic tail of surfactant, greater the aggregation number of surfactant micelle resulting in an increase solubilization capacity of nonpolar solubilizate.

2.3.7 Cloud point extractions

In general, the nonionic surfactant is frequently used to induce the phase separation due to its special characteristic. When the aqueous solutions of nonionic surfactants, which has the concentration more than the critical micelle concentration (CMC), is heated above temperature called the cloud point, the phase separation occurs forming two isotropic phase. The solution can separate into two separate liquid phases as shown in the Figure 2.3. The phase containing most of the surfactant is called the coacervate phase and is really just a very concentrated micellar solution. The surfactant concentration in the diluted phase is slightly above its CMC (Kimchuwanit et al., 2000).

If nonionic surfactant is added to an aqueous solution containing dissolved organic under conditions where the solution temperature is above the Cloud Point, the organic will tend to distribute itself into the surfactant aggregates. The solutes partition between two phases depended on its affinity to the surfactant. Most of surfactant aggregates are concentrated in the surfactant-rich or coacervate phase. This novel separation method is referred to cloud point extraction (Akita et al., 2005; Trakultamupatam et al., 2002; Kimchuwanit et al., 2000) in which the solutes are not only removed from the wastewater but also pre-concentrated in the coacervate phase in a small volume.

A phase separator can be used to separate the coacervate and dilute phase, completing the coacervate/liquid extraction. The solute may be partitioned between the two phases, depending on its affinity to the surfactant. Thus, the two-phase system will provide a separation field, and this novel separation method is referred to cloud point extraction (Akita et al., 2005)



(a) (b)

Figure 2.4 Phase separation by cloud point extraction (a) surfactant solution before heating (b) the phase separation after heating

At surfactant concentrations above the critical micelle concentration, typically below 15 wt%, micellar solutions of nonionic surfactants can exist as homogeneous isotropic liquid phases. Phase separation can be induced in this concentration range by varying the temperature. In many such phase separations, the single isotropic micellar phase separates into two isotropic phases, both of which contain surfactant but which differ in total surfactant concentration. In the surfactant

micellar-rich phase will be concentrated any hydrophobic organic components originally present in the sample subjected to the phase separation step (Hinze, 2005).

2.3.8 Surfactant based separation technology

The two-phase separation of the surfactant solution is known as aqueous surfactant two-phase system (ASTP). Phase behavior results from the competition between internal energy and entropy. The internal energy promotes the separation of micelles while the entropy promotes the miscibility of micelles in water (Liu et al., 1996). Surfactant-based separation process can be effective in the dissolved organics from water. It has advantage of the having modest energy requirement and using non-toxic or biodegradable surfactant as the separating agent (Kimchuwanit et al., 2000).

From the special property of surfactant that can dissolve hydrophobic substances into the core of micelles, it can be applied to remove the organic pollutants of environmental concern from wastewater. It can be the potentially useful method for separation; purification and concentration of the contaminant. Organic pollutants can be extracted and accumulated into surfactant rich phase, and it can be separate out of surfactant aggregate leaving the contaminant-free surfactant for reuse by common procedures, i.e. precipitation for ionic surfactant or vacuum/gas stripping for nonionic surfactant if the contaminants have high enough volatility (Xiao et al., 2000; Trakultamupatum et al., 2002). Kimchuwanit (2000) studied the TCE extraction efficiency by using nonionic surfactant, octylphenoxypoly(ethyleneoxy)ethanol and found that 91% of TCE is extracted in the coacervate phase. Krutlert (2004) studied on BTEX removal using aqueous surfactant two-phase systems technique formed by cationic and anionic surfactant mixtures (A cationic surfactant, dodecyltrimethylammonium bromide (DTAB) and a twin-head anionic surfactant, alkyldiphenyloxide disulfonate (DPDS or trade name of DOWFAX 8390). The results found that the DTAB/DOWFAX at 2:1 molar ratio is a appropriate condition since surfactant partition ratio is highest. Moreover, there is a stable and clear interfacial boundary between two isotropic phases. The results also suggested that addition of electrolyte (NaCl) induce phase separation with coaervate phase on top of solution.

2.4 Integrated technology for soil remediation

In 2006, integrated treatment of contaminated soil has been studied by Haapea and Tuhkanen (2006). Three different methods such as soil washing, ozonation and biological treatment are integrated for remediation of aged oil contaminated with PAHs to below the level of total PAHs in the Finnish guideline. The initial concentration of PAHs was 1,200 mg/kg soil while the target level was 200 mg/kg soil. The result showed that individual method was not able to reach the target level alone (each studied methods has the potential for PAHs reduction of approximately 50%), but several combinations of these methods can achieve 90% reduction of PAHs. Soil washing transfers more than 40% of PAHs into the washing water and then after ozonation, PAHs decreases to 45-65%. After biological post treatment by *Pseudomonas* sp., the amount of PAHs remaining after 12-day incubation is only 6-16% of the initial level. They proposed that ozonation breaks hydrophobic PAHs into more simple form thus, it improves the bioavailability and biologically accessible. Consequently, the ozonation can enhance the biodegradation of PAHs.

In addition, advances in surfactant-enhanced aquifer remediation (SEAR) over the past fifteen years, often resulting from the coupling of laboratory and field studies with economic considerations (The Institute for Applied Surfactant Research, 2006). An early advance looked at surfactant-enhanced solubilization versus mobilization and developed supersolubilization and gradient systems that realized higher removal efficiencies, and thus improved system economics, while avoiding vertical migration concerns for oil phases denser than water. System economics also motivated development / adaptation of separation processes for surfactant recovery and reuse, which is especially important for multiple pore volume (> 3 to 5 pore volumes) surfactant flushes. More recently, low surfactant concentration systems (< 1 wt %) have been developed to reduce surfactant purchase costs and the operating expense of recovering / reusing the surfactant. The most recent development to be discussed is integration of SEAR with a polishing technology such as in situ chemical oxidation (ISCO). This integrated approach was motivated by the field observation that even though significant contaminant mass removed (> 85 to 95%) can reduce ground water concentrations by one to two orders of magnitude, the post-remedial concentrations can still exceed site closure goals. By combining SEAR with an aggressive polishing

step such as ISCO, it is possible to further reduce ground water concentrations toward closure goals in a timely manner.

Two remediation processes, surfactant washing and aerobic biodegradation, have been integrated for use on PCB-contaminated soils from an electric power substation site (Layton, et al., 1998). In this study, a nonionic surfactant, POL was used as solvent in soil washing of PCBs. In a 2-day recycling wash using a 1% (wt/vol) of surfactant solution, greater than 70% of the PCBs were removed from the soil. Then, *Pseudomonas putida* IPL5::TnPCB and *Ralstonia eutropha* B30P4::TnPCB, which utilize surfactants as growth substrates and cometabolize PCBs, were used for PCBs biodegradation. In the biodegradation phase, greater than 90% of the surfactant and 35% of the PCBs were biodegraded in 12 days. The residual PCBs were partitioned onto a solid carrier resulting in greater than 90% removal of PCBs from the bioreactor effluent and a 50-fold reduction in the amount of PCBs-contaminated material. However, this treatment technique still taken a long time for biodegradation and more energy for 2-day recycling wash. Moreover, the reuse of surfactant would be more appropriated than disposal and biodegradation.

From literature reviews, there are many studies about surfactant utilization for organic compound extraction from soil; nevertheless, the use of surfactant to extract TCE from soil by using cloud point extraction technique has never been studied. In addition, there is no report on the use of surfactant to extract TCE from soil incorporated with biodegradation. Therefore, this proposed research aims to investigate the effectiveness of the integration of surfactant-based separation technology (cloud point extraction) and biodegradation in the TCE contaminated soil clean up. The results opened the prospect of applying a selected surfactant and its optimal condition based on cloud point extraction to enhance the efficiency of soil bioremediation treatments to meet the possible lowest level. It also included the use of cumene, a plant derived compound, as an inducer, making this clean-up method environmentally friendly for TCE bioremediation as well as for other chlorinated hydrocarbon pollutants.

CHAPTER III

METHODOLOGY

3.1 Research overview

The research was divided into three phases including the studies on the effects of various surfactants on TCE-degrading bacteria, the optimal condition of cloud point extraction for TCE removal, and the effectiveness of integrated process between surfactant extraction and biodegradation in TCE contaminated soil clean up. The results from the first and second phase were applied in the last phase. Research procedure was illustrated in Figure 3.1.

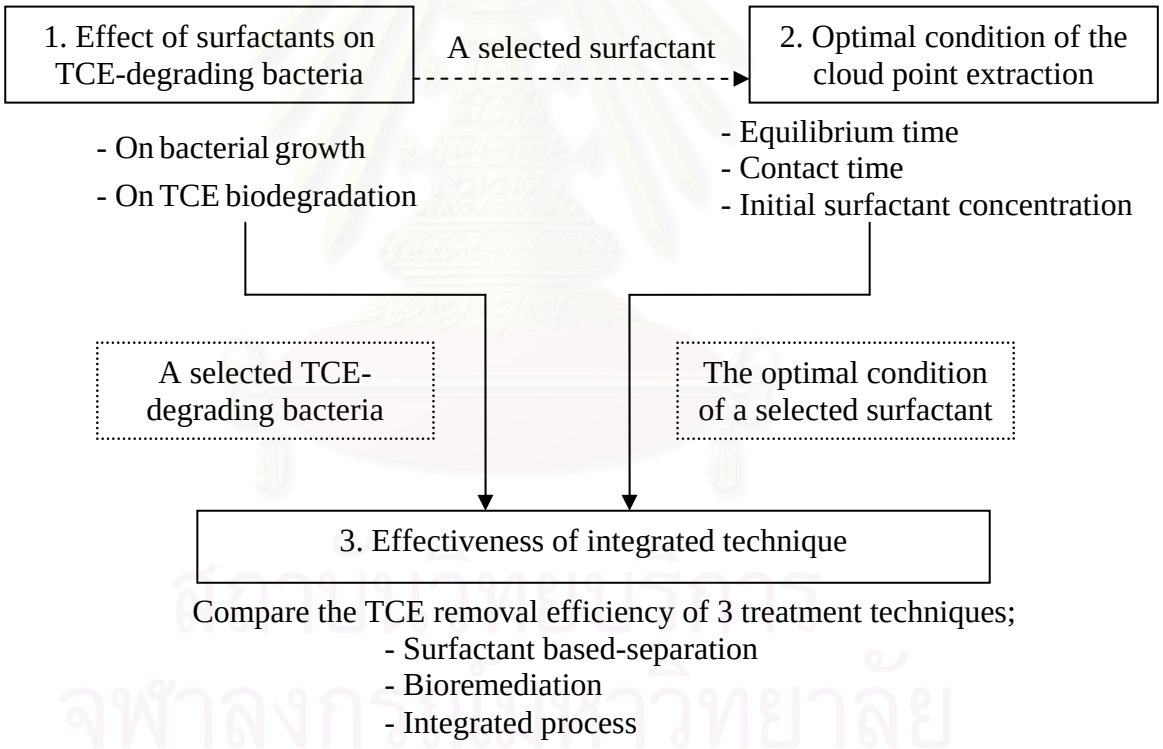


Figure 3.1 Flow chart of the research

3.2 Materials

3.2.1 Microorganisms

Two strain of TCE-degrading bacteria, *Rhodococcus* sp. L4 (TISTR 1542) and *Rhodococcus* sp. P3 (TISTR 1541) were used in this study. These bacteria were deposited at the Microbiological Resources Center, Thailand Institute of Scientific and Technological Research (TISTR). They were isolated earlier by Ekawan Luepromchai from petroleum contaminated soil collected in Bangkok using enrichment culture technique. The partial 16S rRNA gene sequences of *Rhodococcus* sp. L4 and *Rhodococcus* sp. P3 are reported in GenBank as EF527237 and EF450777, respectively.

3.2.2 Surfactants

Six surfactants including 5 nonionic surfactants and a mixture of anionic and cationic surfactant were screened in this study. These surfactants can induce a phase separation, in which the coacervate phase is presented on top of the aqueous solution. Nonionic surfactant namely SURFONIC TDA-5, SURFONIC TDA-6, and SURFONIC L24-7 were contributed by Huntsman Company where NEODOL 91-5 and NEODOL 91-6 were contributed by Shell Company. A cationic surfactant, dodecyltrimethylammonium bromide (DTAB) was purchased from Nanjing Robiot Co., Ltd (China), with a purity of 99% and a twin-head anionic surfactant, alkyldiphenyloxide disulfonate (DPDS or trade name of DOWFAX 8390) was contributed from Dow Chemical Co., Ltd. (USA) with 35% active. Physical and chemical properties of surfactant were shown in Table 3.1.

Table 3.1 Physical and chemical properties of surfactant

Surfactant trade name	SURFONIC TDA-5	SURFONIC TDA-6	SURFONIC L24-7	NEODOL 91-5	NEODOL 91-6	DTAB /DOWFAX with 0.8 M NaCl
Surfactant type	Nonionic	Nonionic	Nonionic	Nonionic	Nonionic	Catanionic
Surfactant class	Branched Alcohol Ethoxylates	Branched Alcohol Ethoxylates	Linear Alcohol Ethoxylates	Linear Alcohol Ethoxylates	Linear Alcohol Ethoxylates	Quaternary Ammonium compound /Sulfonates
MW (g/mol)	550	463	487	387	424	950.35
Density (g/mL) at 25°C	1	0.9715	0.9824	0.964	0.976	> 1
Cloud point (°C)	43	38	49	36	54	30
Solubility in water at 25°C	dispersible	dispersible	soluble	soluble	soluble	Soluble
pH	6.0 − 7.0	6.0 − 7.0	6.0 − 7.0	6.0 − 7.0	6.0 − 7.0	6.0 − 7.0
HLB	10.5	11.4	11.9	11.6	12.5	-
CMC at 25°C (mM)	-	-	0.033	-	-	0.024*
Biodegradable	inherently	inherently	readily	readily	readily	-

Source: Huntsman Company & Shell Company
* Kunanupap (2004)

3.2.3 Chemicals and media

TCE with 99.5% purity, purified cumene solution, and N,N-dimethylfomamide were purchased from Fluka Chemical Industrial. Toluene with 99.5% purity was purchased from Merck Ltd. The mineral salts medium (MSM) was prepared according to the method described by Focht (1994) where all chemicals were in analytical reagent grade and obtained from Merck.

3.2.4 Soil

Sandy clay loam soil was collected from an uncontaminated area (agricultural soil) located in Chiang Mai province, Thailand. All debris was removed and the soil sample was then air dried. Then, the dried soil was sieved by passage through U.S. standard sieve 2.0 mm and 1.0 mm, respectively to get the particle size of soil sample between 1.0-2.0 mm. Properties of soil which determined by the System Development of Soil and Water Analysis Subgroup, Agricultural Chemistry Research Group, Department of Agricultural are shown in Table 3.2. The soil sample was spiked with TCE before being used as a TCE contaminated soil model.

Table 3.2 Properties of soil

Soil properties	Quantity
1. Soil texture	
1.1 Sand (%)	64
1.2 Silt (%)	16
1.3 Clay (%)	20
2. pH	4.40
3. Organic carbon (%)	1.85
4. Cationic exchange capacity-CEC (mol_c/kg)	7.9
5. Electroconductivity-EC (dS/m)	0.115
6. Density for soil compaction (g/L)	1.4

Source: Kraijitmate (2004)

3.3 Experimental procedure

3.3.1 Inoculum preparation

The culture medium used in all experiments was a mineral salt medium (MSM) with detail in Appendix A. *Rhodococcus* sp. L4 and *Rhodococcus* sp. P3 inoculums were prepared as follows.

1) Inoculum preparation for the study of bacterial survival

The bacteria were cultured in 250 mL Erlenmeyer flasks containing 100 mL of MSM. Growth substrate (toluene) was added into an Eppendorf tube which was suspended on top of flask as shown in Figure 3.2. The solution was incubated in an orbital shaker at 200 rpm at room temperature for 2 days. They were then centrifuged at 7,500 rpm for 10 minutes. The harvested cells were washed twice with MSM and resuspended in MSM to give a final concentration of 0.1 OD at 600 nm.

Figure 3.2 *Rhodococcus* sp. P3 culture in MSM-toluene medium

2) Inoculum preparation for the study of TCE biodegradation rate.

The bacteria were cultured in a 250 mL Erlenmeyer flask containing 100 mL MSM. The substrate (toluene) was added into an Eppendorf tube which was suspended on top of flask. The solution was incubated in shaker at 200 rpm at room temperature 2 days (the same procedure with the inoculums preparation for a study of bacterial growth). After that 10 mL of bacteria culture was induced with cumene in the second flasks which containing 90 mL of 4 g/L of a glucose-MSM, in which a stock solution of cumene mixing with N,N dimethylfomamide was later added to give a final concentration of 25 mg/L. Finally, the solution was incubated for 24 hours at room temperature using an orbital shaker at 200 rpm. The bacterial cells were harvested by refrigerated centrifugation at 4800 rpm for 10 minutes. The supernatant was discarded. The bacterial cells were washed once with MSM and resuspended in the same medium. The absorbance of inoculum was adjusted to 1.0 OD at 600 nm.

3.3.2 The effects of surfactants on TCE-degrading bacteria

1) Effects of surfactants on bacterial survival

Six surfactant solutions namely SURFONIC TDA-5, SURFONIC TDA-6, SURFONIC L24-7, NEODOL 91-5, NEODOL 91-6 without electrolyte addition and DTAB/DOWFAX (2:1 molar ratio) with 0.8 M NaCl were screened in this study. All of surfactants were prepared at the same initial concentration, 30 mM. Each surfactant was prepared by precisely weighing in 10 mL beaker and dissolved in deionized distilled water. Then, the solution was added to 100 mL volumetric flask and adjusted the volume accordingly. The sample was mixed homogenously by magnetic stirrer for 30 min. Then, 20 mL of surfactant solution was added to 22 mL screw cap vial and was heated in water bath at the temperature above their cloud point. Surfactants equilibrated in water bath to induce the phase separation shown in Figure 3.3. In this study, 60°C was used because it was the highest cloud point temperature for all surfactants. After the phase separation, the coacervate phase which presents on top was entirely separated out using micropipette. Some portions of the surfactant-dilute phase solution were also removed to make sure that the remaining solution had no coacervate phase contamination. Only surfactant diluted-phase solution was brought to determine the effect on bacterial survival.

The experiment was divided into two sets for *Rhodococcus* sp. L4 and *Rhodococcus* sp. P3. Each experimental set consisted of three samples and a control treatment. Treatments were conducted by adding 1 mL of surfactant-dilute phase solution into a 22 mL vial that contained 4 mL of 0.1 OD bacteria inoculum. Control treatments consisted of 1 mL of distilled water and 4 mL of 0.1 OD bacteria inoculum. The vials were shaked at 200 rpm at room temperature. The sample and control treatment were sampling by sacrificing test vials at 0, 2, and 4 days. To measure the bacterial growth, the number of tested bacteria in each vial was determined by spread plate technique. The samples and control experimental sets were done in triplicate. The surfactants that were not toxic to the bacteria were selected for further study.

Figure 3.3 Surfactants equilibrated in water bath to induce the phase separation

2) Effect of surfactant on TCE biodegradation

Only the dilute phase solutions of selected surfactants were used to determine the effect of surfactant on TCE biodegradation rate. The experiment was divided into two sets for *Rhodococcus* sp. L4 and *Rhodococcus* sp. P3. Each experimental set consisted of three samples of selected surfactant and one control treatment. The control treatment was prepared without the bacteria inoculum. Samples were prepared by adding 1 mL of the surfactant-dilute phase solution to a 22 mL vial with aluminum cap that contained 4 mL of 1.0 OD bacteria inoculum which had been induced with cumene overnight. To maintain the enzyme induction, cumene diluted in N,N dimethylfomamide was further added to make the final concentration of 25 mg/L. The vial was capped immediately with a teflon-lined rubber septum. After that TCE was spiked with a gas-tight syringe to make a final concentration of 10 ppm. The sample and control treatment were sampling by sacrificing test vials at 0, 24, 48 and 96 hours. The samples were analyzed for the remaining TCE by gas chromatography. A surfactant that did not inhibit the activity of bacteria on TCE biodegradation and a strain of bacteria that provides the highest TCE biodegradation rate were selected for further study.

3.3.3 The optimal condition for cloud point extraction

1) Determination of equilibrium time

The contaminated soil was prepared by adding TCE to make the final concentration of 100 ppm in 22 mL vial containing 2.8 g of uncontaminated soil (or 2 mL of uncontaminated soil, this number was calculated from density of soil in order to make the volume ratio of soil volume: surfactant solution of 1:10 v/v). This vial was capped immediately with a teflon-lined rubber septum and leaved overnight to provide the homogenous condition. The initial concentration of a selected surfactant was fixed at 30 mM and then was mixed homogenously by magnetic stirrer for 30 minuets. After that, the surfactant solution was transferred into the 22 mL aluminum cap vial containing contaminated soil until the solution occupied almost all of vial volume to avoid TCE loss at the headspace. The samples were stirred for 30 minutes to provide the contact between soil and surfactant solution. The samples were then equilibrated in a water bath at 60°C. During the 5 equilibrating days, the surfactant-dilute phase concentration was analyzed every 6 hours. The equilibrium time is the time that the surfactant concentration in both phases remains almost constant.

2) Determination of contact time between surfactant solution and soil

The contaminated soil was prepared using the same method as previous experiment. The initial concentration of surfactant was fixed at 30 mM. After that, selected surfactant solution was added into the 22 mL aluminum cap vial containing contaminated soil until the solution occupied almost all of vial volume to avoid the headspace. The samples were stirred to provide a good contact between soil and surfactant solution, stirring time was varied at 15, 30, and 45 minutes, 1, 1.5, 2, 2.5, and 3 hours. The samples were then equilibrated in a water bath at 60°C until the equilibrium time was approached. After the phase separation, 100 µL of rich phase solution was sampled for TCE analysis. Then, the entire rich phase was removed as well as some portions of the dilute phase solution resulting in a total volume of surfactant solution of 6 mL taken out to avoid the contamination of rich phase to

dilute phase solution. Finally, 3 mL of the dilute phase solution was sampled for TCE analysis. TCE concentrations in both solutions were analyzed by gas chromatograph with ECD detector. The optimal contact time indicate the contact time between surfactant solution and contaminated soil that yields the highest TCE concentration in the surfactant-rich phase. Moreover, the surfactant concentration in both phases was also investigated.

3) Determination of optimal initial surfactant concentration

The contaminated soil was prepared as previously mentioned. The initial concentrations of the surfactant were varied at 10, 30, 50, 70, 90, and 110 mM. After that, the surfactant solution was added to the 22 mL aluminum cap vial containing contaminated soil until the solution occupied almost all of vial volume to avoid the headspace. The samples were stirred at the optimal contact time and equilibrated in a water bath controlled at 60°C until the equilibrium condition was approached. After the phase separation, TCE concentrations in both rich- and surfactant-dilute phase solutions were analyzed similar to the above experiment. Moreover, the surfactant concentration in both phases was also investigated by Iodine-Iodide method. The optimal initial surfactant concentration was determined by concentration that can extract and preconcentrate most of TCE into the surfactant-rich phase while leaving the surfactant-dilute phase with the lowest TCE concentration.

3.3.4 Effect of an integrated technique of surfactant extraction and biodegradation for TCE contaminated soil clean-up

Three types of remediation treatment were employed to examine the TCE removal efficiency: (1) soil remediation by bioremediation, (2) soil remediation by surfactant extraction, and (3) soil remediation by an integrated technique (Figure 3.4). The study was performed as soil slurry system; thus after soil remediation, soil was settled and separated into 2 phase, soil phase and aqueous phase. The TCE removal efficiency was determined from the remaining TCE concentration in soil and aqueous phase. Aqueous phase of bioremediation and integrated technique experimental sets was referred to the supernatant solution after the soil particles were

settled at the bottom of the vials, while an aqueous phase of surfactant extraction experimental set was referred to the surfactant surfactant-dilute phase solution. The TCE contaminated soil samples were prepared by spiking TCE (100 or 300 ppm) as previously mentioned. The 300 ppm TCE contaminated soil was conducted to examine the capacity of TCE removal efficiency. Resting cell was used in the experimental sets of bioremediation and integrated technique in order to provide the excess cell. All experiments were done in triplicates.

For bioremediation, 5 mL of 1.0 OD of bacterial inoculums was added directly into the 2.8 g contaminated soil in 22 mL vial. After that cumene in N,N dimethylformamide was added to make the final concentration of 25 mg/L to maintain the enzyme induction. Then, the vials were capped immediately with a teflon-lined rubber septum. The samples were incubated in orbital-shaker at room temperature in the condition that O_2 was sufficiently provided at the beginning of study. Control treatment was prepared without bacterial inoculums. Sampling for TCE and chloride ion analysis was performed by sacrificing the test vials at 0, 24, 48, 72, and 96 hours.

For surfactant extraction, the surfactant solution at the optimal initial concentration was added into the 22 mL vial containing contaminated soil until the solution occupied almost all of vial volume to avoid the headspace. The vials were capped suddenly with teflon-lined rubber septa. The samples were stirred at the optimal contact time and equilibrated for phase separation in a water-bath at 60°C. After the phase separation, the surfactant-rich and surfactant–dilute phases were samples and analyzed for the concentration of remaining TCE similarly to section 3.3.3.

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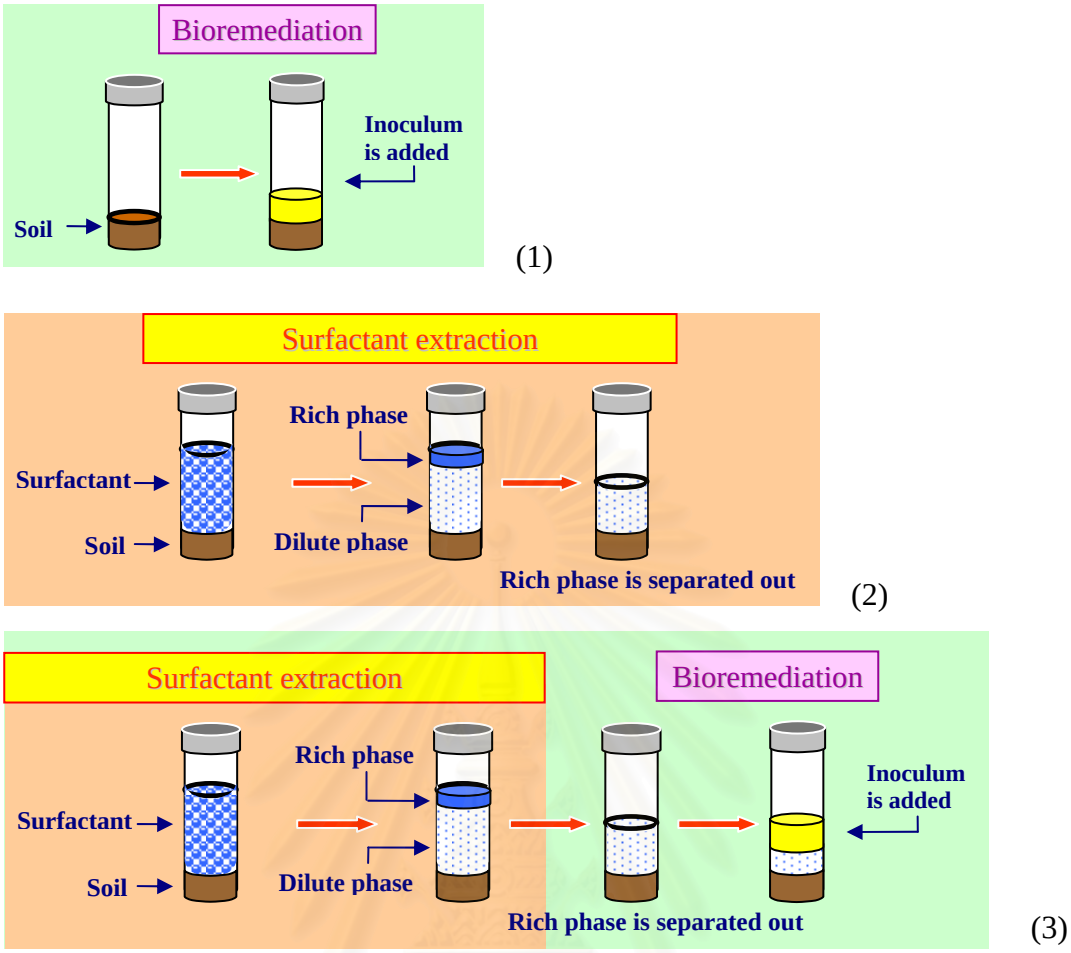

Figure 3.4 Brief procedure for TCE contaminated soil clean-up; (1) bioremediation, (2) surfactant extraction, and (3) integrated technique

For the integrated technique, samples were first prepared by the same process as for surfactant extraction. After the phase separation, 16 mL of the surfactant solution was removed so only small amount of surfactant solution were left in the vial. After that, 2 mL of bacterial inoculums (2.5 OD) was added directly into those vials to make the final OD of 1.0 and total volume of 5 mL. The remaining headspace volume was enough to confer aerobic condition for the bacteria. The vials were process similar to the bioremediation treatment. Control treatment was prepared without bacterial inoculums. Triplicates of samples and control treatments were performed and the test vials were sacrificed at 0, 24, 48, 72, and 96 hours to analyze for the amounts of TCE and chloride ion.

3.4 Analytical methods

3.4.1 Determination of bacterial survival

Bacterial survival was determined from the increasing number of bacterial colonies over time. The procedure consisted of 10-fold dilution with sterile saline solution. Then, 100 µL of diluted sample was spread over MSM agar plate by sterilized glass spreader. Triplicates of each dilution are prepared. Spread plates were incubated at room temperature in glass box supplied with toluene as sole carbon source and energy for one week.

3.4.2 Determination of surfactant concentration

Iodine-Iodide method was used for ethoxylate nonionic surfactant analysis (Baleux, 1972). Briefly, 0.25 mL of KI_3 solution (2% potassium iodide and 1% iodine) was added into 10 mL aqueous sample (1-20 ppm of nonionic surfactant). After 5 minutes, the optical absorption at 500 nm was measured by UV-Visible spectrophotometer (model SPECORD 40 with program winASECT). Standard curve of surfactant concentration was shown in Appendix B.

3.4.3 Determination of TCE concentration

Quantitative analysis of TCE was performed by a headspace gas analysis using a Agilant gas chromatography with ECD detector equipped with a HP-5 (5% Phenyl Methyl Siloxane) fused-silica capillary column (30 m x 0.32 mm ID; thickness, 0.25 µm). The analysis condition was as follows: injector temperature 250 ^{o}C, detector temperature 250 ^{o}C, oven temperature 100 ^{o}C isothermal (4 min). The carrier gas was helium with gas flow rate of 20 mL/min. An injector type was set as splitless. The make up gas was N_2 at 70 mL/min. Samples in 22 mL vial were heated at 93 ^{o}C for 30 minutes before injection. The 100 µL of gaseous sample was directly injected to the GC with a 1000 µL gas-tight microsyringe. Duplicate injections were made for each sample and the readings were averaged.

In the presence of soil, TCE in the aqueous phase was assumed to be in equilibrium with soil. Thus, TCE remaining concentration in soil after treatment was

determined using external standard curve where the known concentrations of TCE were prepared at the same procedure as tested samples. Consequently, two types of TCE standard curves were performed for analysis of TCE during (1) surfactant extraction (in the presence of surfactant) and (2) bioremediation and integrated technique (in the presence of MSM). TCE standard curves for each treatment were shown in Appendix A. The details for those standard curve preparations were as follows;

1) Standard curve of TCE in soil for surfactant extraction

TCE concentration in surfactant extraction system was determined by external standard curve which can be prepared as following; uncontaminated soil was prepared at the same amount (2.8 g) as sample in the vials. The soil was spiked by TCE at 1, 3, 5, 10, 30, and 40 ppm, respectively. Surfactant solution was prepared at the same concentration as the surfactant-dilute phase in the sample and then added into the vials containing the spiked soil. After that, the standard samples were mixed at the optimal contact time and equilibrated in a water-bath at desired temperature as same as sample. The aqueous solution of 3 mL was withdrawn from each vial to determine the concentration of TCE by GC.

2) Standard curve of TCE in soil for bioremediation and integrated technique

TCE concentration in bioremediation and integrated technique system was determined by external standard curve which can be prepared as following; uncontaminated soil was prepared at the same amount as sample (2.8 g) in the vials. Two ranges of TCE concentration were conducted as standard curve for the experimental set of 100 and 300 ppm TCE contaminated soil. TCE was spiked to the soil at the concentrations of 10-40 ppm and 100-300 ppm, respectively and then kept overnight. After that, 5 mL MSM was added into those vials containing the spiked soil. Standard samples were shaked for 1 hour and then they were centrifuged as same as samples. The aqueous solution of 3 mL was withdrawn from each vial to determine the concentration of TCE by GC.

3) Standard curve of TCE in the surfactant-rich phase

The surfactant rich-phase solutions were prepared fresh in 50 mL volumetric flask using an actual surfactant rich-phase concentration of an optimal initial surfactant concentration, 300 mM. Two ranges of TCE concentrations were added into the surfactant rich-phase solutions to make different known concentrations of 100-300 ppm and 500-2000 ppm. The standard samples were mixed for 30 minutes and 100 µL of prepared solution was transferred to 22 mL aluminum cap vials to determine the concentration of TCE by GC.

4) Standard curve of TCE in the surfactant-dilute phase

Due to the concentration of surfactant surfactant-dilute phase is very low; the surfactant at this concentration does not influence TCE volatilization. Consequently, water was used instead of the surfactant surfactant-dilute phase for standard curve preparation. TCE at 5, 10, 20, and 30 were prepared in the volumetric flasks using deionized water as solvent. Only 3 mL of aqueous solution was transferred to 22 mL aluminum cap vials and the concentration of TCE was determined by GC.

3.4.4 Determination of chloride ions

Most of chloride atoms in TCE eventually accumulated in medium as Cl^- ions after biodegradation (Luu et al., 1995). In this study, chloride ion formation was monitored in order to confirm the mineralization of TCE. After incubation, only 3 ml aqueous phase of bioremediation and integrated technique experimental set were taken to determine the generated chloride ions. All of samples were centrifuged at 3,500 rpm for 3 minutes before the sampling. The concentration of generated chloride ions was analyzed by an ion-sensitive chloride combination electrode (Model ionlab terminal 740, Germany). Chloride ion standard (10,000 ppm sodium chloride) was used to make a calibration curve in the range of 20 to 200 ppm chloride. Ionic strength adjustor (2% v/v $NaNO_3$) was added to the 10 mL calibration standards or samples before measuring in a 10 mL beaker which placed inside a 250 mL beaker containing temperature probe (Figure 3.5). The 250 mL beaker contained 100 mL

water was used as water bath to make sure that the temperature of tested sample was constant throughout the analysis.

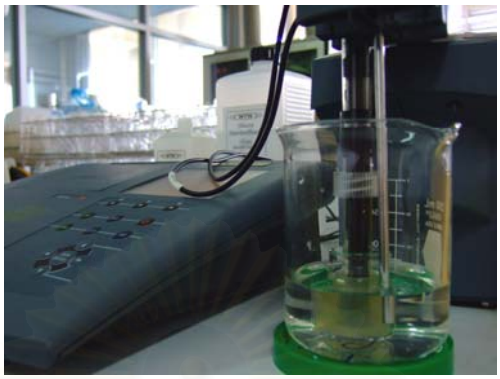

Figure 3.5 Determination of chloride ion concentration

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CHAPTER IV

RESULTS AND DISCUSSION

4.1 Effects of surfactants on TCE-degrading bacteria

4.1.1 Effect of surfactants on bacterial survival

Due to the adverse effects of some surfactants on microorganisms (Franzetti et al., 2005), the surfactant toxicity need to be investigated first in order to select surfactants that do not kill TCE-degrading bacteria. The surfactants namely SURFONIC TDA-5, SURFONIC TDA-6, SURFONIC L24-7, NEODOL 91-5, NEODOL 91-6 and DTAB/DOWFAX (2:1) with 0.8 M NaCl were screened in this study. Only, surfactant-dilute phase solution was taken to determine effect of surfactant in bacterial growth. The result shown that of the six surfactants, only SURFONIC TDA-6, SURFONIC L24-7 and NEODOL 91-6 did not kill *Rhodococcus* sp. L4 and *Rhodococcus* sp. P3. As shown in Table 4.1, both bacteria were found in the presence of these surfactants after 4-day incubation while other surfactants killed these bacteria. Bacterial colonies were found in the presence of surfactant after 4-day incubation shown in Figure 4.1. The results suggested that these surfactants had some adverse effects on bacteria, this correspond with the study of Rothmel et al. (1998) which found %survival of bacteria decreased when surfactant was presented in the system. The low tolerance of ionic surfactant was probably due to the physico-chemical interactions between surfactant and bacterial membrane. For nonionic surfactant, this toxicity was probably related to the membrane-damaging effect, in which surfactant with ethylene oxide chains consisting of fewer than six monomers will bury in the lipid layer of the bacterial liposomes (Cserhati, 1991).

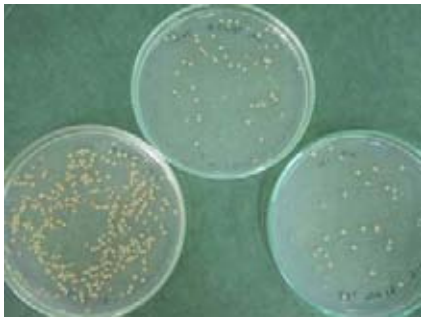

Figure 4.1 *Rhodococcus* sp. L4 were found in the presence of SURFONIC TDA-6 after 4-day incubation

Table 4.1 Number of bacteria after incubating in surfactant solution

	Number of bacteria (x 10^5 CFU /mL)			
	L4		P3	
	2^{nd} day	4^{th} day	2^{nd} day	4^{th} day
Control (no surfactant)	217 ± 193	46 ± 50	3 ± 2	31 ± 24
SURFONIC TDA-5	0	0	0	0
SURFONIC TDA-6	80 ± 4	31 ± 18	4 ± 0	1 ± 0
NEODOL 91-5	0	0	0	0
NEODOL 91-6	91 ± 4	18 ± 19	2 ± 0	43 ± 0
SURFONIC L24-7	81 ± 0	73 ± 13	0.1 ± 0	0.1 ± 0
DTAB/DOWFAX (2:1) with 0.8 NaCl	0	0	0	0

*The initial numbers of L4 and P3 cells were 230 ± 22 and 31 ± 17 (x 10^5 CFU /mL), respectively.

4.1.2 Effect of surfactant on TCE biodegradation

For TCE biodegradation, some surfactants may inhibit TCE biodegradability of bacteria although they do not affect on bacterial cells. The effect of surfactant on TCE biodegradation rate needs to be investigated in order to find a suitable surfactant system that does not inhibit TCE biodegradability of the bacteria. Consequently, only the dilute phase of surfactant solutions from 3 selected surfactants (SURFONIC TDA-6, SURFONIC L24-7 and NEODOL 91-6) were used to determine whether they inhibited TCE degrading activities of either *Rhodococcus* sp. L4 or *Rhodococcus* sp. P3. The TCE degradability of bacteria in the presence of

surfactants was investigated in term of % biodegradation. The absorbance of inoculum was adjusted to 1.0 OD at 600 nm used as resting cell system. The results suggested that *Rhodococcus* sp. L4 had higher TCE degradability than *Rhodococcus* sp. P3 for every tested surfactants (Figure 4.2). Biodegradations of 10 ppm TCE by *Rhodococcus* sp. L4 were 58.43 %, 50.55 % and 48.93 % in the presence of SURFONIC TDA-6, NEODOL 91-6 and SURFONIC L24-7, respectively. The results suggested that these 3 surfactants did not inhibit TCE degradability of bacteria. The results from control treatments (without the bacteria) showed that some TCE was lost by abiotic processes. However, TCE losses in the control treatment were less than the treatments with bacterial cells. *Rhodococcus* sp. L4 degraded TCE effectively in the presence of SURFONIC TDA-6, in which 58.43 % of 10 ppm TCE was reduced within 24 hours compared to 29.35% of TCE removal in control treatment through physical activities. Therefore, *Rhodococcus* sp. L4 and SURFONIC TDA-6 were selected for further study.

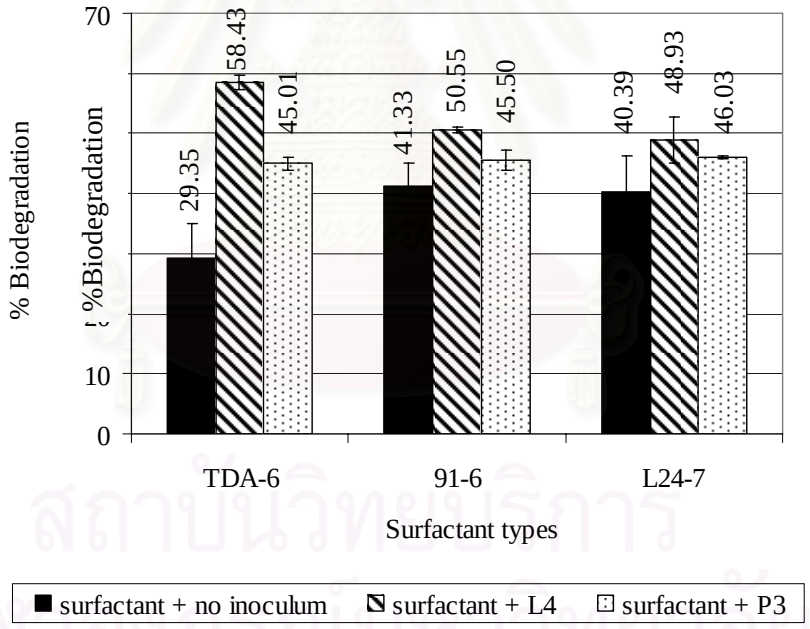

Figure 4.2 Percentage of TCE biodegradation after 24 hr incubation in the presence of various surfactants

TCE biodegradation of 10 ppm TCE in liquid culture without surfactant by *Rhodococcus* sp. L4 and *Rhodococcus* sp. P3 was about 60-70% (Luepromchai, 2001; Suttinun, 2003). Comparing with this study, about 50-60% of TCE was

biodegraded in the presence of surfactant, which was slight lower than the previous study. It suggested that these surfactants had some adverse effects on bacteria. This corresponds with the study of Rothmel et al. (1998) which found %survival of bacteria decreased when surfactant was presented in the system. To prevent the effects of surfactant on TCE biodegradation, the study therefore applied high amounts of bacterial inoculum to TCE contaminated soil as resting cell system (in Section 4.3).

4.2 Optimal condition for TCE removal using surfactant extraction

4.2.1 Equilibrium time

After contaminated soil and SURFONIC TDA-6 were mixed and equilibrated in a water bath, the phase separation occurred. The surfactant concentration in the surfactant-dilute phase was analyzed every 6 hours for 5 days using the Iodine-Iodide method (Baleux, 1972). The optimal equilibrium time was determined by observing the stability of surfactant concentration in surfactant-dilute phase over time. The results showed that surfactant concentration decreased with increasing equilibrated time (Figure 4.3). After 72 hours, surfactant-dilute phase was observed constant. The constant of surfactant-dilute phase was determined by using standard deviation. At the optimal equilibrium time, the standard deviation showed no further change in the surfactant concentration in surfactant-dilute phase with 17.31 %SD. Therefore, the optimal equilibrium time of SURFONIC TDA-6 is 72 hours or 3 days.

Comparing with other studies, the optimal equilibrium time of SURFONIC TDA-6 was hardly different. The equilibrium time of DTAB/DOWFAX was 71.5 hours (Krutlert, 2004) and equilibrium time of $OP(EO)_7$ was about 2 days (Kimchuwanit, 2000).

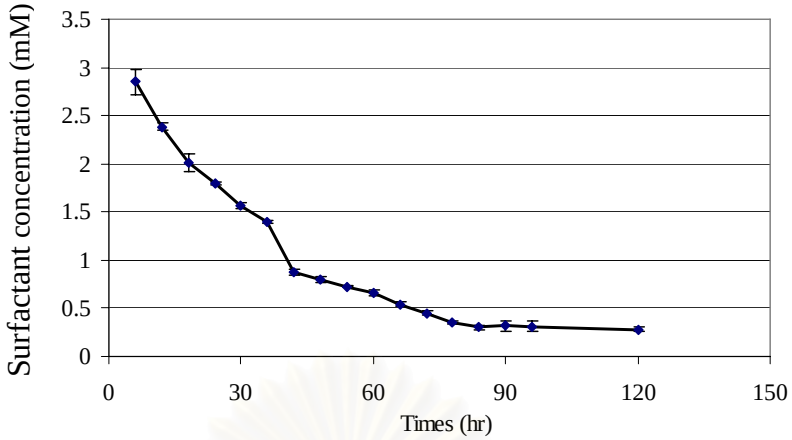

Figure 4.3 Surfactant concentrations in the surfactant-dilute phase over time

4.2.2 Contact time between surfactant solution and soil

Contact time is the time used to mix surfactant solution and soil prior equilibrating at the equilibrium time. In this study, the concentration of TCE was represented by peak area of TCE after GC analysis. The results showed that TCE concentration in both phases increased as the contact time increased (Figure 4.4). The concentration of TCE increased until it reached the plateau region after 1-hour stirring. An increase of TCE concentration in the first period resulted from the release of TCE from soil due to desorption process. The adsorbed TCE in soil particles was gradually desorbed and partitioned into the surfactant aggregates in both phases via an affinity between TCE and surfactant aggregates which was stronger than that of between TCE and soil. When the TCE concentration in both phases reached the plateau region, this condition justified the highest TCE extraction efficiency from soil. Consequently, the contact time of 1 hour was chosen to be applied into the next studies.

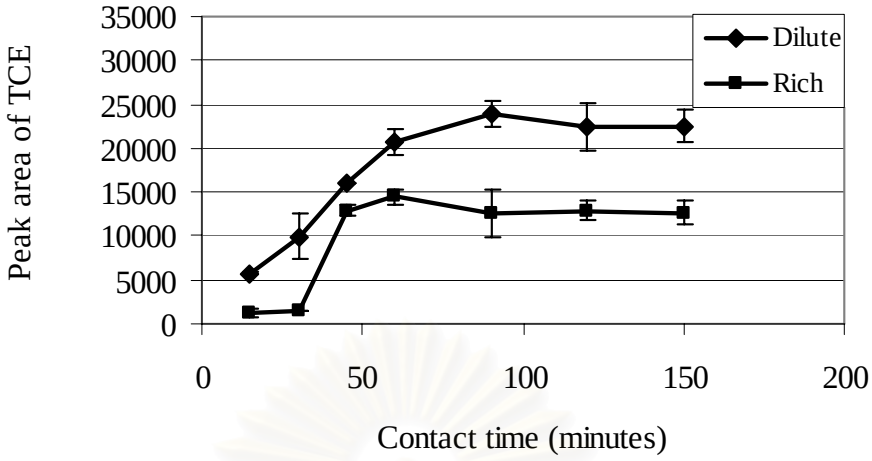

Figure 4.4 Peak area of TCE in the surfactant-rich and surfactant-dilute phases at various contact time

From Figure 4.4, the peak area of TCE in surfactant-rich phase was lower than in surfactant-dilute phase, this is resulted from the lower sample volume used for TCE analysis and the effects of higher surfactant concentration on TCE volatility. With cloud point extraction, high amount of TCE and surfactant aggregates are pre-concentrated in a small volume of surfactant-rich phase while leave only small amount of them in surfactant-dilute phase (Kimchuwanit et al., 2000). Therefore, in this study, 100 µL of surfactant-rich phase and 3 mL of surfactant-dilute phase were taken to determine the amount of TCE. The very high concentration of surfactant in surfactant-rich phase trapped TCE tightly in the surfactant aggregates. Consequently, it affected the volatility of TCE in the surfactant-rich phase and reduced the peak area of TCE when the samples were heated for a headspace gas analysis using gas chromatography.

4.2.3 Effect of the initial surfactant concentration

Since, surfactant cost is quite expensive, initial concentration of surfactant is one of the most important factors affecting the remediation cost. In addition, surfactant concentration influences TCE removal efficiency. When surfactant is used at too low concentration, high amount of TCE still remains in soil while using too high surfactant concentration will lead to a high surfactant remaining

in the effluent as Kimchuwanit et al. (2000) found that when surfactant concentration was increased, remaining surfactant concentration in surfactant-dilute phase solution was increased while surfactant concentration in rich-phase still remain constant. Excessive surfactant concentration is not only cost ineffectiveness but also has a toxic effect on microorganisms (Franzetti et al., 2005). Therefore, the initial concentration of surfactant was determined in this study in order to use the least amount of surfactant that satisfies the high TCE removal efficiency.

The experimental treatments at various initial concentration of surfactant were performed by using optimal condition achieved previous study. After experimental treatments were mixed for 1 hr and equilibrated in water bath for 72 hours, the optimal initial surfactant concentration was determined from the concentration that can extract and preconcentrate highest amount of TCE into the surfactant-rich phase while leaving the lowest TCE concentration in surfactant-dilute phase or effluent water. Furthermore, surfactant partition ratio, which is the ratio of surfactant in the surfactant-rich phase to that of in the dilute phase, is another parameter used to determine the optimal initial surfactant concentration. The higher the surfactant partition ratio, the greater amount of surfactant presents in the rich phase. The results showed that both TCE remaining in soil and in surfactant-dilute phase decreased with increasing initial surfactant concentration due to the fact that the amount of the extracting agent increased. However, insignificant reduction in the remaining TCE in soil and surfactant-dilute phase was observed when the initial surfactant concentration higher than 70 mM (Figure 4.5). In addition, the surfactant partition ratio was found to be the highest of 124.12 at the initial surfactant concentration at 70 mM (Table 4.2). Consequently, the initial surfactant concentration of 70 mM was chosen to use in the next studies.

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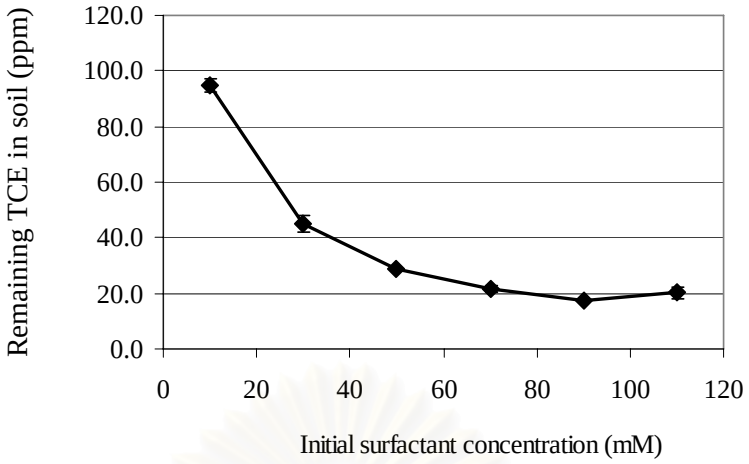
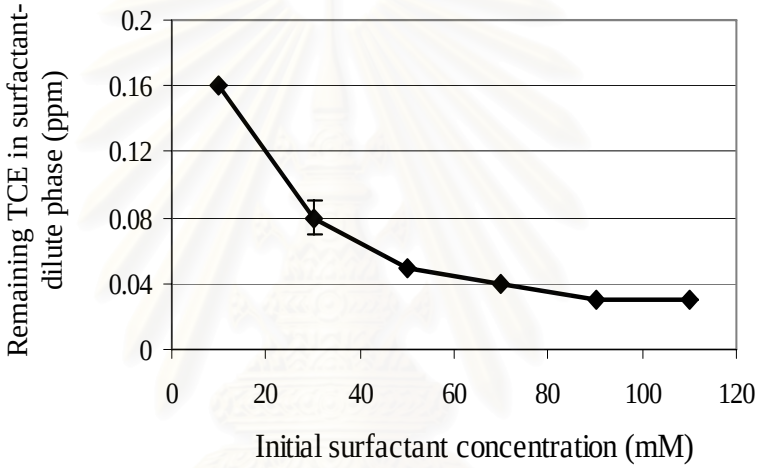


(a)



(b)

Figure 4.5 Remaining TCE concentration at various initial surfactant concentrations after phase separation; (a) the remaining TCE concentration in soil and (b) the remaining TCE concentration in surfactant-dilute phase

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Table 4.2 The remaining surfactant concentrations in surfactant-rich and surfactant-dilute phase; and the surfactant partition ratio at various initial surfactant concentrations

Initial surfactant Concentration (mM)	Remaining surfactant concentration (mM)		Surfactant partition ratio
	dilute phase	rich phase	
0	0	0	0
10	0.15 ± 0.02	ND	-
30	1.84 ± 0.10	154.98 ± 11.20	84.13
50	2.50 ± 0.07	276.88 ± 5.36	110.83
70	2.78 ± 0.08	345.37 ± 32.94	124.12
90	5.08 ± 0.46	355.64 ± 26.21	70.06
110	6.07 ± 0.37	327.19 ± 9.22	53.94

ND = not determined. The rich-phase was not clearly separated.

At the initial surfactant concentration of 10 mM, the phase separation did not occur. In addition, it was observed that at this surfactant concentration, most surfactant was adsorbed at soil surface and at the wall of vial more than at other concentrations. Moreover, the adsorption of surfactant at the wall also occurred at the surfactant concentration of 110 mM. The results summarized in Table 4.2 revealed that the surfactant concentration in the dilute phase drastically increased with the increasing initial surfactant concentration meanwhile that of in the rich phase was slightly increased. Thus, the surfactant partition ratio increased with increasing the surfactant concentration until it reached the maximum value at the initial surfactant concentration of 70 mM and declined upon further increasing the surfactant concentration. Therefore, % surfactant recovery was investigated to check the adsorption as shown in Appendix B (Table B-13).

Furthermore, an experimental set without soil was conducted to assure the effect of adsorption. Pictures demonstrating the phase separation of surfactant system at various initial surfactant concentrations of both experimental sets (with soil and without soil) were shown in Figure 4.6. Actually, SURFONIC TDA-6 has no color. The red color of surfactant rich-phase comes from dye, which added for clear vision.

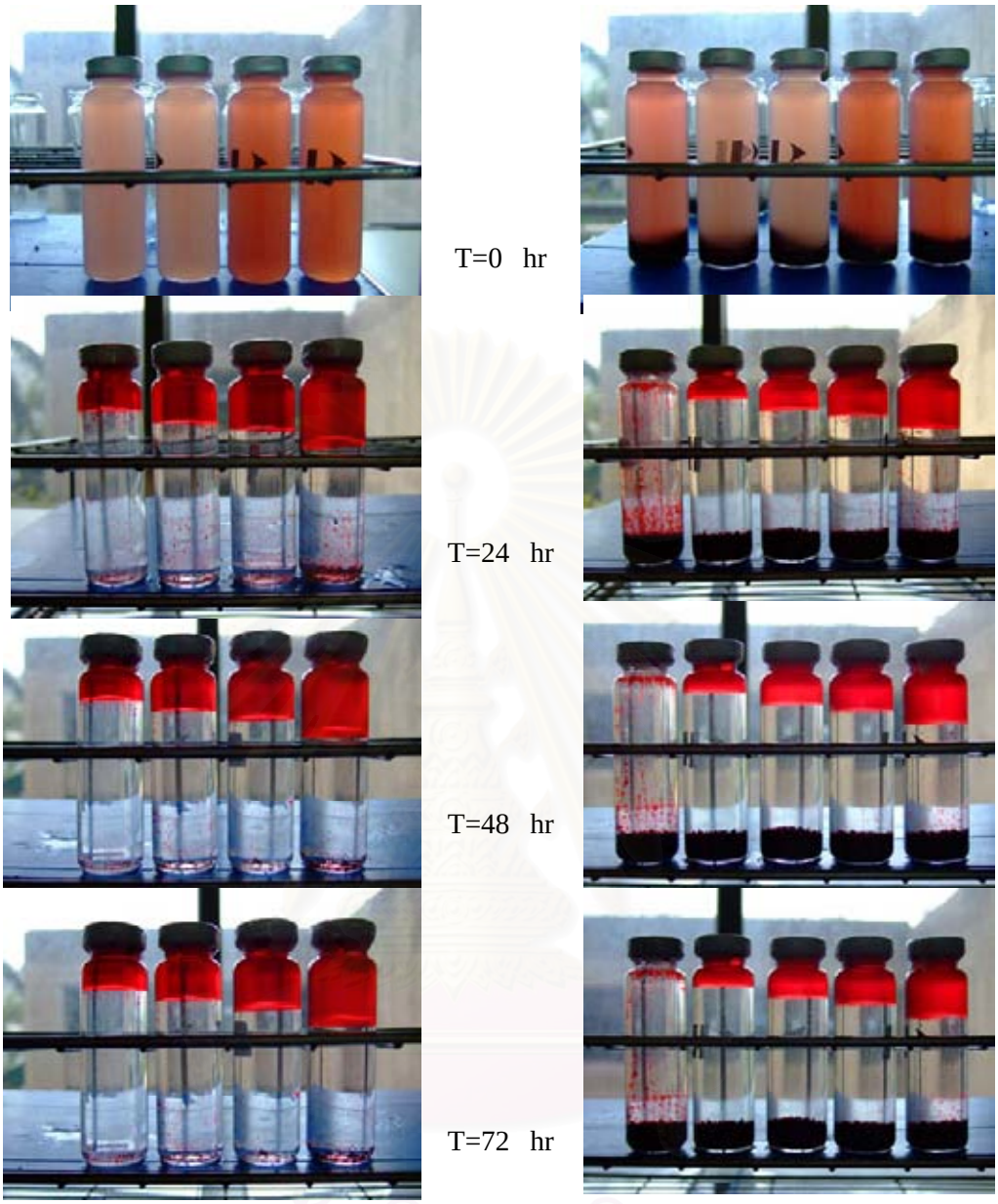

Figure 4.6 Phase separation of surfactant system at various initial surfactant concentrations (a) without soil and (b) with soil

4.3 The effectiveness of an integrated technique of surfactant-based separation technology and biodegradation for TCE contaminated soil clean-up

4.3.1 Soil clean-up by bioremediation

For bioremediation, contaminated soil was treated by adding *Rhodococcus* sp. L4 inoculum directly to the soil sample. Control and treatment samples were incubated on the orbital shaker and sampled when the desire incubated time approach. TCE biodegradation was taken place under aerobic conditions in soil slurry system. For 100 ppm TCE contaminated soil, the results shown the increasing of TCE concentration in the first period probably resulted from desorption. TCE which adsorb in soil particles was gradually extracted into surfactant-dilute phase via interaction between surfactant solution and soil. After 24 hours incubated time, TCE concentration was decreased continuously. The loss of TCE in treated samples was mainly due to the activity of added *Rhodococcus* sp. L4 since the remaining TCE in this condition was lower than control without the inoculum. The decreasing of TCE in control treatment may probably due to abiotic activities such as volatilization and adsorption of TCE. However, further investigation is needed to assure this explanation.

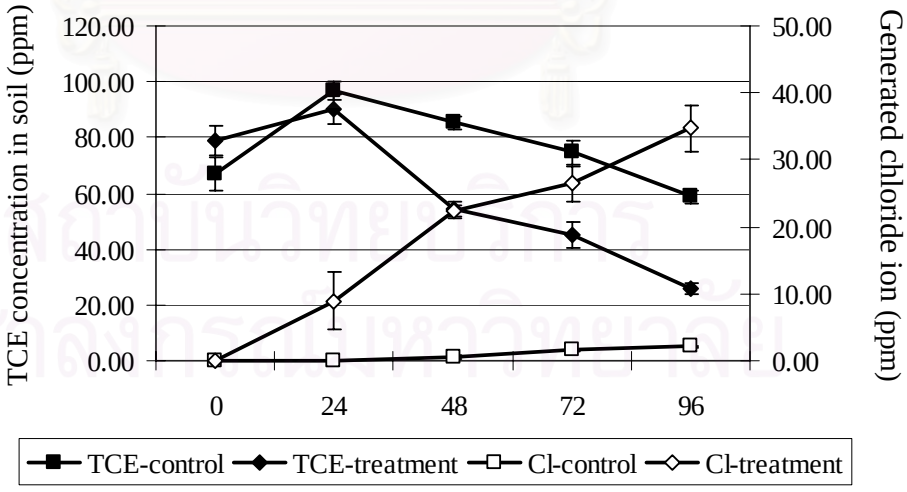

Figure 4.7 Interactions between chlorine ion generation and TCE degradation during the bioremediation treatment of 100 ppm contaminated soil

To confirm the extent of complete TCE mineralization, generated chlorine ion during TCE aerobic biodegradation process was monitored. The results from the treated samples showed the increased chlorine ion concentration with the increase incubating time (Figure 4.8). The amount of chlorine ion of bioremediation treatment and control treatment after 96 hours incubating time were 34.67 ppm and 2.10 ppm, respectively. Control treatment shown slightly increasing of the generated chloride ion after 48 hours incubating time however this increasing was not significant.

In addition, bioremediation of 300 ppm TCE contaminated soil was conducted to examine the capacity of TCE removal efficiency. The results shown a tendency of TCE degradation similar with bioremediation of 100 ppm TCE contaminated soil (Figure 4.9). However, %biodegradation rate of 300 ppm TCE contaminated soil was lower than in 100 ppm contaminated soil. This may be resulted from the higher TCE concentration. High amount of TCE was dissolved from soil into aqueous phase and probably toxic to the TCE-degrading bacteria.

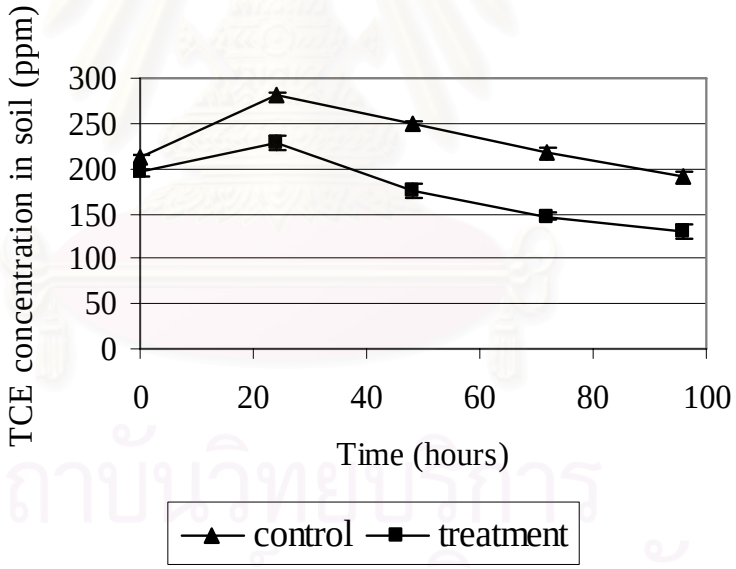

Figure 4.8 The concentrations of remaining TCE (ppm) after bioremediation of 300 ppm contaminated soil

4.3.2 Soil clean-up by surfactant extraction

For surfactant extraction, the optimal condition which achieve from the optimal condition study was applied in this remediation. SURFONIC TDA-6 at the optimal initial concentration was added into contaminated soil. Then, the samples were stirred at the optimal contact time. Finally, these samples were equilibrated in water bath at equilibrium time to induce phase separation by cloud point technique. After phase separation, remaining TCE in soil, surfactant-rich phase, and surfactant-dilute phase were analyzed to determine TCE removal efficiency. From the initial TCE concentration of 100 ppm in soil, the results shown that the amounts of remaining TCE in soil, surfactant rich-phase and surfactant-dilute phase were 26.46, 285.71 and 0.09 ppm, respectively (Table 4.3). Moreover, from the initial TCE concentration of 300 ppm in soil, the results shown that the amount of remaining TCE in soil, surfactant rich-phase and surfactant-dilute phase were 122.16, 1101.13 and 0.44 ppm, respectively. The results show that most of TCE was removed from soil through solubilization into the surfactant aggregates and pre-concentration in the surfactant rich-phase, and left only small amount of TCE in surfactant-dilute phase.

Table 4.3 The concentrations of remaining TCE (ppm) in soil and surfactant solutions after surfactant extraction

Initial TCE concentration in soil (ppm)	Remaining TCE in each phase (ppm)		
	soil	rich phase	dilute phase
100	26.46 ± 3.21	285.71 ± 42.21	0.09 ± 0.01
300	122.16 ± 12.47	1101.13 ± 132.98	0.44 ± 0.04

4.2.3 Soil clean-up by integrated technique

For integrated technique, bioremediation was combined as a post treatment to solvent extraction method. *Rhodococcus* sp. L4 inoculum was added to the contaminated soil after the entire coacervate phase and some portions of surfactant-dilute phase solution were separated out to ensure no contamination of surfactant coacervate phase. Control and treatment were incubated on the orbital shaker and sampled when the desire incubated time approach. TCE biodegradation

was taken place under aerobic conditions in soil slurry system. Remediation of 100 ppm TCE contaminated soil shown tendency of TCE decreasing similar with that occurred in bioremediation, TCE concentration was increased in the first period and after 24 hours incubated time, TCE concentration was decreased continuously. The loss of TCE in treatment was still mainly due to the activity of added bacteria since the remaining TCE in treatments was lower than control without the inoculum.

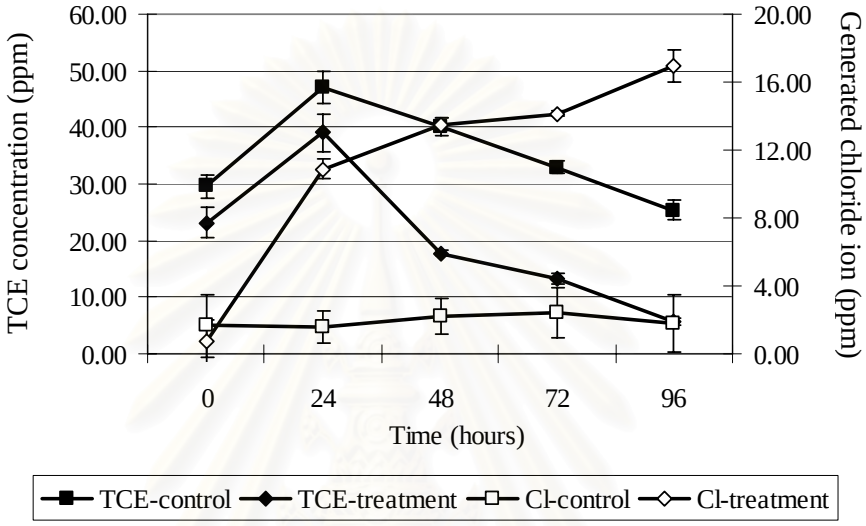

Figure 4.9 Chlorine ion generation and TCE concentration by integrated technique treatment of 100 ppm contaminated soil via TCE aerobic biodegradation

To confirm the extent of complete TCE mineralization, generated chlorine ion during TCE aerobic biodegradation process was monitored. The results shown generated chlorine ion concentration increased with the increase incubating time (Figure 4.10). Meanwhile remaining TCE concentration increased with increasing incubating time. Generated chlorine ion of bioremediation treatment and control treatment after 96 hours incubating time, there were 16.96 ppm and 1.78 ppm, respectively. Control treatment did not show increasing of the generated chloride ion but the presence of chloride ion still remained at the same concentration at the begining although time was increased. It may be result of some interferences or the error from calibration curve preparation. If the chloride ion generation of control treatment is not detected, it can confirm that TCE decreasing in control treatment may come from abiotic process because of no chloride ion generation.

In addition, bioremediation of 300 ppm TCE contaminated soil conducted to examine the capacity of TCE removal efficiency shown tendency of TCE decreasing similar with bioremediation of 100 ppm TCE contaminated soil (Figure 4.11). Biodegradation of 300 ppm TCE contaminated soil was as same as the 100 ppm TCE contaminated soil. This may be resulted from the high TCE concentration was firstly removed by surfactant extraction. Large amount of TCE was removed from soil in to surfactant rich-phase and then left only small amount of TCE in surfactant surfactant-dilute phase and soil. Consequently, bacteria could effectively degrade the remaining TCE.

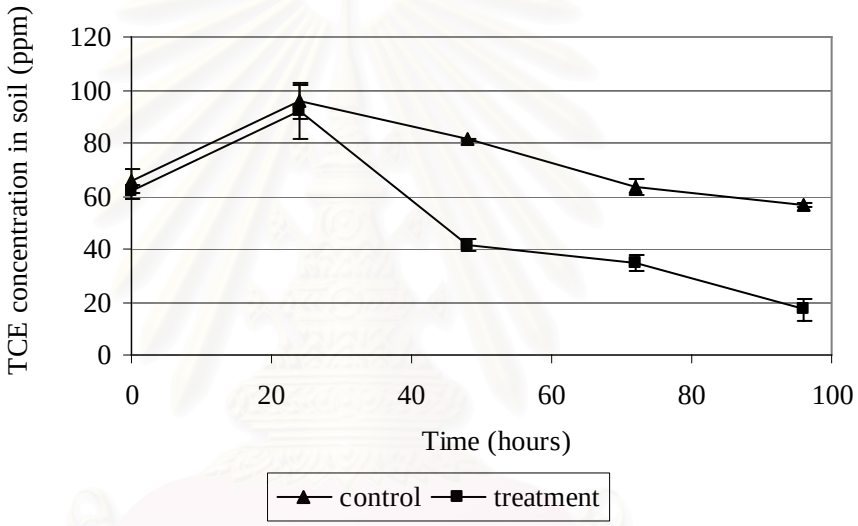

Figure 4.10 The remaining TCE concentrations with incubating time after remediate 300 ppm contaminated soil by integrated process

4.2.4 Comparison of TCE removal efficiency

TCE removal efficiency was determined by comparing the amount of remaining TCE in soil after treatment. TCE concentrations in soil after remediation were shown in Table 4.4. After remediate the 100 ppm TCE contaminated soil by bioremediation, surfactant extraction, and integrated technique treatment for 96 hr, there were 25.97 ppm, 26.45 ppm, and 5.64 ppm of TCE, respectively. After remediate the 300 ppm TCE contaminated soil by these 3 different treatments for 96 hr, there were 129.80 ppm, 122.16 ppm, and 17 ppm of TCE, respectively. The loss of

TCE of both bioremediation and integrated technique treatment was mainly due to the activity of added bacteria since the amounts of remaining TCE were lower than the control without the inoculum.

Table 4.4 The amounts of TCE in soil and aqueous phases after remediating with different treatment methods

Initial TCE concentration (ppm)		TCE remaining in each phase (ppm)					
		Soil phase			Aqueous phase		
		Bioremediation	Surfactant extraction	Integrated technique	Bioremediation	Surfactant extraction	Integrated technique
100	control	58.88 ± 2.23	ND	25.41 ± 1.86	0.90 ± 0.03	ND	0.39 ± 0.03
	treatment	25.97 ± 2.17	26.45± 3.21	5.64 ± 0.68	0.40 ± 0.03	0.09±0.01	0.09 ± 0.01
300	control	191.10±4.29	ND	56.30 ± 0.78	10.86 ±0.54	ND	0.86 ± 0.01
	treatment	129.80±7.08	122.16±12.47	17.00 ± 4.00	3.19 ± 0.88	0.44±0.04	0.26 ± 0.06

*Control was used to determine the removal of TCE by non-biological activities.
ND = not determined

Furthermore, amount of TCE remaining in aqueous phase was also detected in order to select the best available treatment method. The concentrations of remaining TCE in aqueous phase after remediation were also shown in Table 4.4. After remediate the 100 ppm TCE contaminated soil by bioremediation, surfactant extraction, and integrated technique treatment for 96 hours, there were 0.40 ppm, 0.09 ppm, and 0.09 ppm of TCE, respectively. After remediate the 300 ppm TCE contaminated soil with these 3-different treatments for 96 hours, there were 3.19 ppm, 0.44 ppm, and 0.26 ppm of TCE, respectively. Similar to soil, the amounts of remaining TCE in bioremediation and integrated technique was lower than control without the inoculum. This study reported the concentration of TCE as ppm since the unit is commonly used in environmental standards. Although, the amount of TCE in aqueous phase after treatment was still higher than groundwater standard (5 ppb), the remaining TCE could be later removed by natural processes such as volatilization, sorption, and dilution.

Alternatively, TCE removal efficiency can be calculated by comparing the amount of remaining TCE with the initial TCE concentration in soil. The TCE removal efficiency of 100 ppm TCE contaminated soil by bioremediation, surfactant extraction, and integrated technique treatment were about 74%, 74%, and 94%,

respectively whereas TCE removal efficiency of 300 ppm TCE contaminated soil these 3-different treatments were 57%, 59%, and 94%, respectively (Table 4.5).

Table 4.5 TCE removal efficiency (%) of different treatment methods

Initial TCE concentration (ppm)	TCE removal efficiency in soil phase (%)		
	Bioremediation	Surfactant extraction	Integrated technique
100	74 ± 2	74 ± 3	94 ± 1
300	57 ± 2	59 ± 4	94 ± 1

TCE removal efficiency of bioremediation in 300 ppm TCE contaminated soil clean-up was lower than that in 100 ppm TCE contaminated soil. This may be because the large amount of TCE was dissolved from soil into aqueous phase and probably toxic to the bacteria. This result suggested that bioremediation was not effective for clean-up high level of TCE contaminated soil. Bioremediation was suitable for low level of TCE contaminated site. The decreasing of TCE removal efficiency when increased TCE concentration in soil was also found in the remediation by surfactant extraction. This is resulted from the limitation of surfactant; firstly, type of surfactant and secondly, initial concentration of surfactant. The 70 mM initial surfactant concentration was optimal for 100 ppm TCE contaminated soil. Thus, the surfactant at this concentration may not enough for removal of higher TCE concentration from soil, so some TCE still remain in soil and aqueous phase. For higher TCE contaminated soil clean-up, further works should be studied, for example to find the optimal condition or new surfactants that have more potency. For integrated technique, TCE removal efficiencies of soil with different initial TCE concentrations were similar. This is due to the combination of surfactant extraction and bioremediation. After surfactant extraction, only small amount of TCE was dissolved from soil into aqueous phase, thus it did not affect the activities of TCE-degrading bacteria. Moreover, surfactant increased the bioavailability of TCE so the bacteria could degrade it easier. These results suggest that the integrated technique can solve the problem of the remediation of high pollutant contamination.

The results were comparable with other studies. For example an integrated treatment of PAH contaminated soil by soil washing, ozonation and biological treatment showed 90% PAHs reduction (Haapea et al., 2006). The study also indicated that each individual method achieved only 50% PAHs reduction. TCE removal efficiency of bioremediation was less than the integrated process; which was probably due to the low bioavailability of TCE in this treatment. The improvement of biodegradation is assumed to be the result of an increased mobilization of TCE from soil matrix by surfactant (McCray et al., 2001; Haapea et al., 2006). TCE removal efficiency of bioremediation treatment in this study was higher than Suttinun (2003), in which 30% of TCE remained after 96 hr incubation for soil microcosms. It may because of the differences in soil treatment system. This study worked with soil slurry system, in which the samples were shaking during the incubating time, thus accelerating the reactions between bacterial enzymes and TCE. TCE removal efficiency of surfactant-based separation technology was lower than Kimchuwanit et al. (2000), which was able to extract 90% of TCE from wastewater containing 100 ppm TCE. This may be resulted from the limitation of selected surfactants and adsorption of TCE on soil.

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CHAPTER V

CONCLUSIONS

The comparative study of three TCE treatment processes, including: (1) bioremediation, (2) surfactant extraction and (3) integrated technique of bioremediation and surfactant extraction indicated that soil remediation by the integrated technique had the highest TCE removal efficiency. The effectiveness of each treatment was determined from the remaining TCE concentration in soil. Treatments of both 100 ppm and 300 ppm TCE contaminated soil by the integrated technique shown 95% TCE removal efficiency, which was about 30% higher than either technique alone. Moreover, TCE was mineralized as showed by the increase of chloride ions after remediation by bioremediation and integrated technique. In the integrated system, cloud point extraction by SURFONIC TDA-6 was conducted to separate high amount of TCE into the surfactant-rich phase and then bioremediation was integrated into the system by adding *Rhodococcus* sp. L4 to co-metabolize the remaining TCE.

Remediation by each individual method has its own limitations. Soil remediation by surfactant based-separation technology was limited by TCE extraction efficiency of the selected surfactant and the optimal condition that will be varied in different contaminated sites. On the other hand, bioremediation was limited by the low bioavailability of TCE, and the adverse effects of pollutant and surfactant on bacterial cells. The disadvantages of bioremediation are long time treatment and the failure on remediation of sites where there are very high concentrations of pollutants. Here, an integrated technique of surfactant extraction and bioremediation was developed and showed to enhance the efficiency of TCE contaminated soil clean-up. When using the surfactant based-separation technology with cloud point extraction technique, large amount of TCE was removed from soil through solubilization into surfactant aggregates and pre-concentration in the coacervate-phase. Remaining TCE in the surfactant diluted-phase was later degraded by *Rhodococcus* sp. bacteria. These results indicate that surfactant can enhance biodegradability of TCE by desorbing them from the soil matrix into more bioavailable form. Consequently, bioremediation was used as biological post treatment to reduce TCE to the possible lowest level.

Surfactant based-separation technology by cloud point technique can remediate sites with very high concentration of TCE. Advantage of cloud point extraction is not only the removal but also the pre-concentration of TCE from soil into the surfactant rich-phase. Consequently, the remaining concentrations of TCE and surfactant after removal of the surfactant rich-phase will not toxic to bacterial cells or inhibit TCE biodegradation. In addition, cloud point technique reduces the amount of waste for further treatment or disposal. The surfactant in rich-phase can be reused afterward.

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CHAPTER VI

RECOMMENTDATIONS FOR FUTURE WORK

There were many factors affecting the amount of TCE removal in this study. For example, the percentage of actual TCE reduction may be overestimated because not only microbial activity caused TCE degradation, but abiotic process such as volatilization could also affected its diminishing. In addition, the sorption of TCE to soil and bacterial cells may influence the amount of TCE removal. So, further study is needed to determine the extent of TCE sorption on bacterial cells. Moreover, this study did not use sterlilized soil because soil property can be changed if it is sterilized. Consequently, TCE reduction may also due to the activity of soil indigenous bacteria.

Based on the results of this study, some recommendations for further study are proposed as follows; first, the concentration of TCE in contaminated soil should be increased in order to examine the capacity of TCE removal efficiency. The increasing of TCE concentration in contaminated soil may clearly differentiated the TCE removal efficiency of each treatment method. Secondly, the detailed effects of surfactant on bacteria should be determined. Thirdly, new surfactant which has the lower temperature of Cloud point (°C) may be screened in order to save energy. Surfactant which can induce phase separation at room temperature should be recommended. Fourthly, gas chromatography with ECD detector equipped with headspace auto-sampler should be used rather than the headspace manual-sampler for TCE analysis in order to avoid human error. Finally, use of bacterial carrier materials may enhance the potential for successful bioremediation by increasing the survival of bacteria that have been inoculated into contaminated soil.

In application aspect, the integrated technique of surfactant-based separation technology and bioremediation can be applied for clean-up contaminated site. The combination of these techniques may be conducted as both in-situ and ex-situ remediation. The part of surfactant-based separation technology is an ex-situ remediation, in which the surfactant solution will be injected to the contaminated site to solubilize and mobilize the trapped organic pollutant into surfactant aggregates as surfactant flushing. Then, this surfactant solution containing pollutant will be pumped up and induced phase separation by using cloud point technique in a reactor.

Surfactant in the concentrated form may be reused while the surfactant-dilute phase may be pumped back to the site together with bacteria inoculum as the biological post treatment. So, bioremediation will act as an in-situ remediation. In addition, the efficiency of added bacteria may be enhanced by adding some nutrients and air along with the surfactant-dilute phase solution and bacteria inoculum to the site.

This integrated technique can be applied to remediate the contaminated site containing various types of soil such as sand, silt or clay soil. However the removal efficiencies may not be the same due to the differences in soil properties such as organic matter content, hydraulic conductivity, size of soil particle, etc. For example, the sorption capacity of organic pollutant on the soil is strongly increased in soil containing high amount of natural organic matter and thereby causing the loss of surfactant during treatment (Ferraz et al., 2005). Clay soil has the small particle size and low hydraulic conductivity; thus decreased the ability of surfactant flushing and pollutant-degrading bacteria that may result to the low removal efficiency. Consequently, the optimal condition of remediation technique for individual soil type may be investigated for the best removal efficiency.

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APPENDICES

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APPENDIX A

1. Mineral salts medium (MSM) preparation

MSM used in all experiments of this research was consisted of following components per liter.

Table A-1 Composition of MSM

Stock dolution	Additional volume (mL)	Final concentration (mM)
$(NH_4)_2SO_4$	10	10
$Fe(NO_3)_3$	0.01	0.01
$Ca(NO_3)_2$	0.1	0.1
NaH_2PO_4	3	3
$MgSO_4$	1	1
K_2HPO4	10	10
Trace minerals	1	
$MnSO_4$	1 mM	0.001
$ZnSO_4$	1 mM	0.001
$CuSO_4$	1 mM	0.001
$NiSO_4$	0.1 mM	0.0001
$CoSO_4$	0.1 mM	0.0001
Na_2MoO_4	0.1 mM	0.0001

Source: Focht (1994)

MSM was prepared in a 1 L beaker by adding about 0.5 L of distilled water before adding any of the stock solutions above, or precipitates will form, and then make a final volume to 1 L. MSM was autoclaved at 121 °C for 15 min. as sterilization. For solid media, 15 g/l of agar was added. Glucose MSM was prepared by adding 4 g/l of glucose into MSM and it was autoclaved at 110 °C for 15 min.

2. Standard curve of TCE in soil for contaminated soil remediation by surfactant extraction

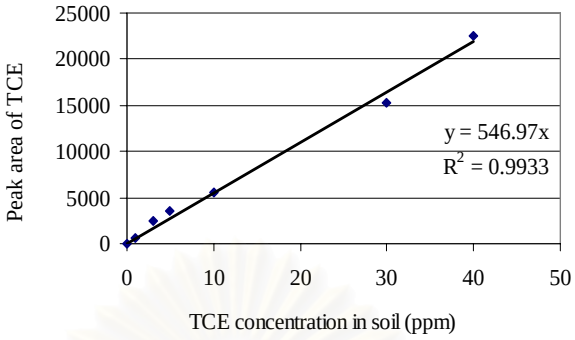
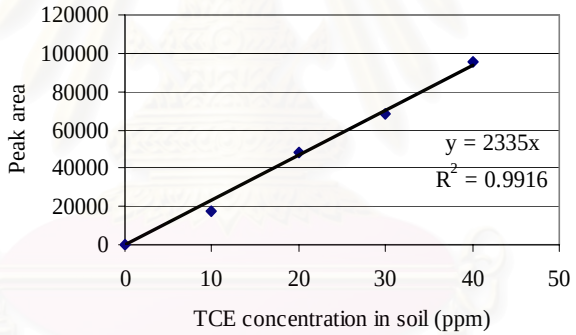
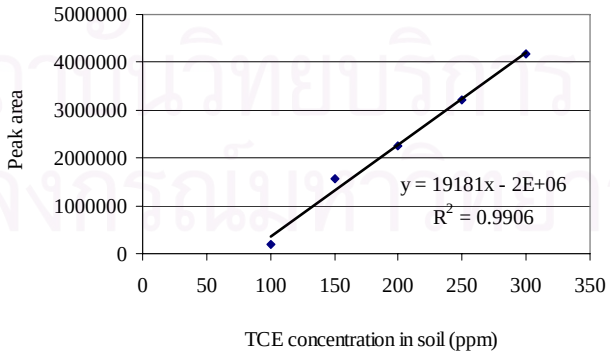


Figure A-1 Standard curve of TCE in soil for contaminated soil remediation by surfactant extraction

3. Standard curve of TCE in soil for contaminated soil remediation by bioremediation and integrated technique



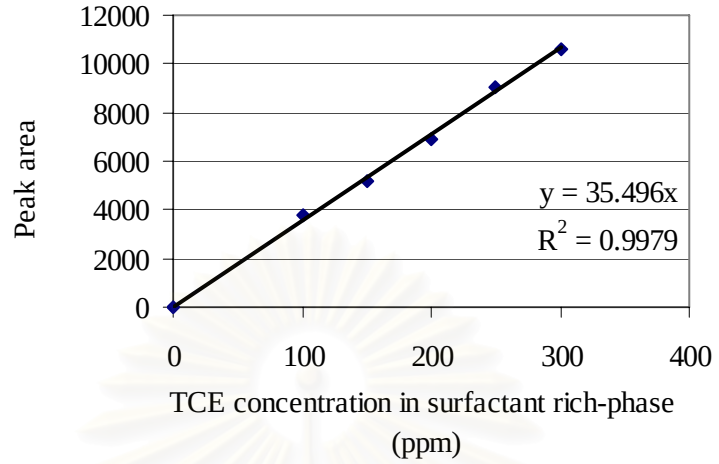
(a)



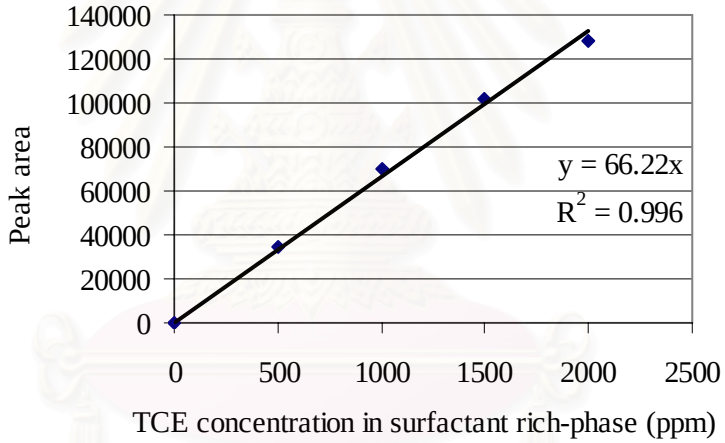
(b)

Figure A-2 Standard curve of TCE in soil for contaminated soil remediation by bioremediation and integrated process (a) 10 – 40 ppm of TCE concentration in soil (b) 100 – 300 ppm of TCE concentration in soil.

4. Standard curve of TCE in rich-phase –phase



(a)



(b)

Figure A-3 Standard curve of TCE in rich-phase –phase (a) 100 – 400 ppm of TCE concentration in soil (b) 500 –2000 ppm of TCE concentration in soil; Surfactant concentration in rich phase was 300 mM

5. Standard curve of TCE in the surfactant-dilute phase

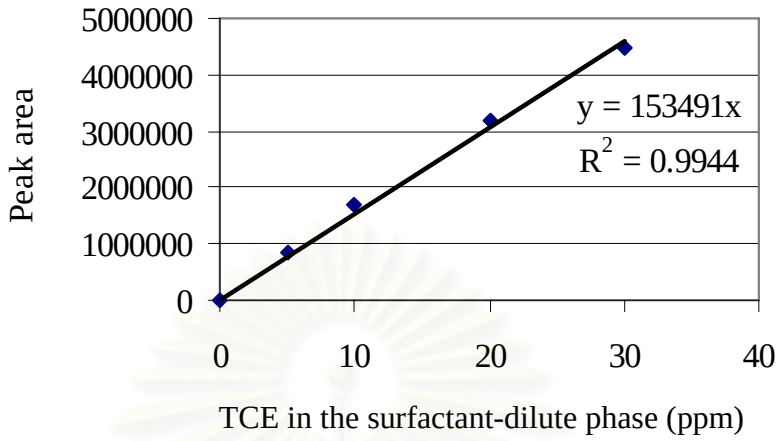


Figure A-4 Standard curve of TCE in the surfactant-dilute phase

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APPENDIX B

1. Effect of surfactant on bacterial survial study

Amount of bacteria *Rhodococcus* sp. L4 and *Rhodococcus* sp. P3 found in the presence of various surfactants after 4-day incubation were shown in table B-1.

Table B-1 Bacterial number in the presence of various surfactants

	t=0			**t=2**			**t=4**		
	x 10^5	x 10^6	x 10^7	x 10^5	x 10^6	x 10^7	x 10^5	x 10^6	x 10^7
1) P3	>300	43	14	120	59	7	>300	48	26
	188	27	8	118	30	2	138	18	4
2) P3 + TDA-5				0	0	0	0	0	0
				0	0	0	0	0	0
3) P3 + TDA-6				39	3	3	14	3	1
				5	0	0	2	1	0
4) P3 + 91-5				4	0	0	0	0	0
				11	0	0	0	0	0
5) P3 + 91-6				19	4	2	51	4	1
				10	0	0	8	0	0
6) P3 + L24-7				>300	0	0	1	0	1
				1	0	1	0	0	0
6) P3 + D/D				0	0	0	0	0	0
				0	0	0	0	0	0
1) L4	>300	>300	21	>300	110	44	>300	81	17
	>300	252	24	>300	102	11	108	11	0
2) L4 + TDA-5				0	0	0	0	0	0
				0	0	0	0	0	0
3) L4 + TDA-6				>300	77	10	179	43	16
				>300	83	7	36	18	12
4) L4 + 91-5				0	0	0	0	0	0
				0	0	0	0	0	0
5) L4 + 91-6				>300	85	23	>300	93	7
				>300	98	14	>300	66	7
6) L4 + L24-7				>300	81	14	93	30	4
				>300	23	10	72	10	1
6)L4 + D/D				0	0	0	0	0	0
				0	0	0	0	0	0

2. Effect of surfactant on TCE biodegradation study

% TCE biodegradation after 24-hour incubation of treatment and control where 3- selected surfactants presence were show in table B-2. In addition, % TCE biodegradation rate for 96-hour incubation of each surfactant was shown respectively. For control treatment, bacterial inoculums was substituted by MSM

Table B-2 % TCE biodegradation at 24 hours in the presence of various surfactants

	Area		**%Biodegradation**
Control	***t=0***	***t=24 hr***	
1) Ms + L24-7 (TCE + cumene)	22,454.7	14,308.3	36.28
	21,844.1	12,122.6	44.50
	22,149.4	**13,215.5**	**40.39**
2) Ms + 91-6 (TCE + cumene)	20,603.0	11,526.2	44.06
	20,538.2	12,610.6	38.60
	20,570.6	**12,068.4**	**41.33**
3) Ms + TDA-6 (TCE + cumene)	20,506.9	15,294.4	25.42
	19,741.3	13,169.3	33.29
	20,124.1	**14,231.9**	**29.35**
1) L4 + L24-7 (TCE + cumene)	18,096.1	9,731.8	46.22
	17,703.2	8,559.9	51.65
	17,899.7	**9,145.9**	**48.93**
2) L4 + 91-6 (TCE + cumene)	24,209.9	13,904.0	42.57
	24,209.9	10,040.3	58.53
	24,209.9	**11,972.2**	**50.55**
3) L4 + TDA-6 (TCE + cumene)	20,110.2	8,186.4	59.29
	19,001.4	8,064.5	57.56
	19,555.8	**8,125.5**	**58.43**
1) P3 + L24-7 (TCE + cumene)	22,725.8	12,219.0	46.23
	22,174.0	12,010.3	45.84
	22,449.9	**12,114.7**	**46.03**
2) P3 + 91-6 (TCE + cumene)	22,404.6	12,474.5	44.32
	22,393.2	11,940.5	46.68
	22,398.9	**12,207.5**	**45.50**
3) P3 + TDA-6 (TCE + cumene)	22,091.2	12,310.7	44.27
	22,008.2	11,940.5	45.75
	22,049.7	**12,125.6**	**45.01**

Table B-3a Peak area of TCE for 96-hour incubation in the presence of SURFONIC L24-7

	Area			
Sample	***t=0***	***t=24 hr***	***t=48 hr***	***t=4 d***
1) Ms + L24-7 (TCE + cumene)	22,454.7	14,308.3	7,653.1	3,908.4
	21,844.1	12,122.6	7,268.4	3,873.6
	22,149.4	**13,215.5**	**7,460.8**	**3,891.0**
2) L4 + L24-7 (TCE + cumene)	18,096.1	9,731.8	4,207.1	1,229.3
	17,703.2	8,559.9	4,167.4	1,193.4
	17,899.7	**9,145.9**	**4,187.3**	**1,211.4**
3) P3 + L24-7 (TCE + cumene)	22,725.8	12,219.0	5,122.6	2,877.1
	22,174.0	12,010.3	4,406.2	2,692.4
	22,449.9	**12,114.7**	**4,764.4**	**2,784.8**

Table B-3b % TCE biodegradation rate for 96-hour incubation in the presence of SURFONIC L24-7

	% Biodegradation			
Sample	***t=0***	***t=24 hr***	***t=48 hr***	***t=4 d***
1) Ms + L24-7 (TCE + cumene)	(C0-Ct)/C0	36.28	65.92	82.59
		44.50	66.73	82.27
Average	**0**	**40.39**	**66.32**	**82.43**
SD	**0**	**5.8**	**0.6**	**0.2**
2) L4 + L24-7 (TCE + cumene)		46.22	76.75	93.21
		51.65	76.46	93.26
Average	**0**	**48.93**	**76.61**	**93.23**
SD	**0**	**3.8**	**0.2**	**0.0**
3) P3 + L24-7 (TCE + cumene)		46.23	77.46	87.34
		45.84	80.13	87.86
Average	**0**	**46.03**	**78.79**	**87.60**
SD	**0**	**0.3**	**1.9**	**0.4**

Table B-4a Peak area of TCE for 96-hour incubation in the presence of NEODOL 91-6

	Area			
Sample	***t=0***	***t=24 hr***	***t=48 hr***	***t=4 d***
1) Ms + 91-6 (TCE + cumene)	20,603.0	11,526.2	5,988.7	3,031.6
	20,538.2	12,610.6	5,648.8	2,320.7
	20,570.6	**12,068.4**	**5,818.8**	**2,676.2**
2) L4 + 91-6 (TCE + cumene)	24,209.9	12,904.0	6,119.3	1,648.9
	24,209.9	11,040.3	5,568.6	1,478.5
	24,209.9	**11,972.2**	**5,844.0**	**1,563.7**
3) P3 + 91-6 (TCE + cumene)	22,404.6	12,474.5	8,309.5	2,824.6
	22,393.2	11,940.5	8,061.1	2,621.9
	22,398.9	**12,207.5**	**8,185.3**	**2,723.3**

Table B-4b Peak area of TCE for 96-hour incubation in the presence of NEODOL 91-6

	% Biodegradation			
Sample	***t=0***	***t=24 hr***	***t=48 hr***	***t=4 d***
1) Ms + 91-6 (TCE + cumene)	(C0-Ct)/C0	44.06	70.93	85.29
		38.60	72.50	88.70
Average	**0**	**41.33**	**71.71**	**86.99**
SD	**0**	**3.9**	**1.1**	**2.4**
2) L4 + 91-6 (TCE + cumene)		46.70	74.72	93.19
		54.40	77.00	93.89
Average	**0**	**50.55**	**75.86**	**93.54**
SD	**0**	**5.4**	**1.6**	**0.5**
3) P3 + 91-6 (TCE + cumene)		44.32	62.91	87.39
		46.68	64.00	88.29
Average	**0**	**45.50**	**63.46**	**87.84**
SD	**0**	**1.7**	**0.8**	**0.6**

Table B-5a Peak area of TCE for 96-hour incubation in the presence of SURFONIC TDA-6

	Area			
Sample	***t=0***	***t=24 hr***	***t=48 hr***	***t=4 d***
1) Ms + TDA-6 (TCE + cumene)	20,506.9	15,294.4	6,825.6	3,234.3
	19,741.3	13,169.3	6,471.2	3,039.9
	20,124.1	**14,231.9**	**6,648.4**	**3,137.1**
2) L4 + TDA-6 (TCE + cumene)	20,110.2	8,186.4	4,895.8	1,883.9
	19,001.4	8,064.5	4,863.3	1,220.9
	19,555.8	**8,125.5**	**4,879.6**	**1,552.4**
3) P3 + TDA-6 (TCE + cumene)	22,091.2	12,310.7	7,016.1	2,874.0
	22,008.2	11,940.5	6,351.4	1,916.1
	22,049.7	**12,125.6**	**6,683.8**	**2,395.1**

Table B-5b % TCE biodegradation rate for 96-hour incubation in the presence of SURFONIC TDA-6

	% Biodegradation			
Sample	***t=0***	***t=24 hr***	***t=48 hr***	***t=4 d***
1) Ms + TDA-6 (TCE + cumene)	(C0-Ct)/C0	25.42	66.72	84.23
		33.29	67.22	84.60
Average	**0**	**29.35**	**66.97**	**84.41**
SD	**0**	**5.6**	**0.4**	**0.3**
2) L4 + TDA-6 (TCE + cumene)		59.29	75.66	90.63
		57.56	74.41	93.57
Average	**0**	**58.43**	**75.03**	**92.10**
SD	**0**	**1.2**	**0.9**	**2.1**
3) P3 + TDA-6 (TCE + cumene)		44.27	68.24	86.99
		45.75	71.14	91.29
Average	**0**	**45.01**	**69.69**	**89.14**
SD	**0**	**1.0**	**2.1**	**3.0**

3. Optimal condition of surfactant study

3.1 The equilibrium time

Table B-6 The data of SURFONIC TDA-6 concentration in surfactant-dilute phase sampled every 6 hours for 5 days

		Absorbance			Abs x dilute factor			Concentration (mM)		
		Surfactant-dilute phase			Surfactant-dilute phase			Surfactant-dilute phase		
	vial (triplicates)	1	2	3	1	2	3	1	2	3
t=0	1	0.0321	0.0331		321.00	331.00		27.79	28.65	
	2	0.0322	0.0334		322.00	334.00		27.87	28.91	
	3	0.0325	0.0333		325.00	333.00		28.13	28.83	
(1000x)	Avg	**0.0323**	**0.0333**		**322.67**	**332.67**		**27.93**	**28.80**	
t=6	1	0.3292	0.3730	0.3292	32.92	37.30	32.92	2.85	3.23	2.85
	2	0.3269	0.3165	0.3269	32.69	31.65	32.69	2.83	2.74	2.83
	3	0.3281	0.3096	0.3281	32.81	30.96	32.81	2.84	2.68	2.84
(100x)	Avg	**0.3281**	**0.3330**	**0.3281**	**32.81**	**33.30**	**32.81**	**2.84**	**2.88**	**2.84**
t=12	1	0.2669	0.2761	0.2796	26.69	27.61	27.96	2.31	2.39	2.42
	2	0.2692	0.2761	0.2796	26.92	27.61	27.96	2.33	2.39	2.42
	3	0.2726	0.2796	0.2819	27.26	27.96	28.19	2.36	2.42	2.44
(100x)	Avg	**0.2696**	**0.2773**	**0.2804**	**26.96**	**27.73**	**28.04**	**2.33**	**2.40**	**2.43**
t=18	1	0.2206	0.2310	0.2414	22.06	23.10	24.14	1.91	2.00	2.09
	2	0.2253	0.2345	0.2414	22.53	23.45	24.14	1.95	2.03	2.09
	3	0.2241	0.2195	0.2530	22.41	21.95	25.30	1.94	1.90	2.19
(100x)	Avg	**0.2233**	**0.2283**	**0.2453**	**22.33**	**22.83**	**24.53**	**1.93**	**1.98**	**2.12**
t=24	1	0.2056	0.2045	0.2079	20.56	20.45	20.79	1.78	1.77	1.80
	2	0.2056	0.2056	0.2102	20.56	20.56	21.02	1.78	1.78	1.82
	3	0.2079	0.2068	0.2114	20.79	20.68	21.14	1.80	1.79	1.83
(100x)	Avg	**0.2064**	**0.2056**	**0.2098**	**20.64**	**20.56**	**20.98**	**1.79**	**1.78**	**1.82**

Table B-6 (cont.) The data of SURFONIC TDA-6 concentration in surfactant-dilute phase every 6 hours for 5 days

		Absorbance			Abs x dilute factor			Concentration (mM)		
		Surfactant-dilute phase			Surfactant-dilute phase			Surfactant-dilute phase		
	vial (triplicate)	1	2	3	1	2	3	1	2	3
t=30	1	0.3627	0.3604	0.3604	18.14	18.02	18.02	1.57	1.56	1.56
	2	0.3674	0.3581	0.3558	18.37	17.91	17.79	1.59	1.55	1.54
	3	0.3720	0.3466	0.3674	18.60	17.33	18.37	1.61	1.50	1.59
(50x)	Avg	**0.3674**	**0.3550**	**0.3612**	**18.37**	**17.75**	**18.06**	**1.59**	**1.54**	**1.56**
t=36	1	0.3281	0.3235	0.3211	16.41	16.18	16.06	1.42	1.40	1.39
	2	0.3281	0.3211	0.3188	16.41	16.06	15.94	1.42	1.39	1.38
	3	0.3235	0.3211	0.3165	16.18	16.06	15.83	1.40	1.39	1.37
(50x)	Avg	**0.3266**	**0.3219**	**0.3188**	**16.33**	**16.10**	**15.94**	**1.41**	**1.39**	**1.38**
t=42	1	0.1895	0.2056	0.2033	9.48	10.28	10.17	0.82	0.89	0.88
	2	0.1918	0.2056	0.2033	9.59	10.28	10.17	0.83	0.89	0.88
	3	0.1987	0.2079	0.2056	9.94	10.40	10.28	0.86	0.90	0.89
(50x)	Avg	**0.1933**	**0.2064**	**0.2041**	**9.67**	**10.32**	**10.20**	**0.84**	**0.89**	**0.88**
t=48	1	0.1895	0.1825	0.1779	9.48	9.13	8.90	0.82	0.79	0.77
	2	0.1918	0.1825	0.1802	9.59	9.13	9.01	0.83	0.79	0.78
	3	0.1987	0.1848	0.1802	9.94	9.24	9.01	0.86	0.80	0.78
(50x)	Avg	**0.1933**	**0.1833**	**0.1794**	**9.67**	**9.16**	**8.97**	**0.84**	**0.79**	**0.78**
t=54	1	0.1687	0.1687	0.1710	8.44	8.44	8.55	0.73	0.73	0.74
	2	0.1687	0.1687	0.1710	8.44	8.44	8.55	0.73	0.73	0.74
	3	0.1663	0.1640	0.1687	8.32	8.20	8.44	0.72	0.71	0.73
(50x)	Avg	**0.1679**	**0.1671**	**0.1702**	**8.40**	**8.36**	**8.51**	**0.73**	**0.72**	**0.74**
t=60	1	0.1571	0.1571	0.1409	7.86	7.86	7.05	0.68	0.68	0.61
	2	0.1571	0.1571	0.1432	7.86	7.86	7.16	0.68	0.68	0.62
	3	0.1571	0.1594	0.1456	7.86	7.97	7.28	0.68	0.69	0.63
(50x)	Avg	**0.1571**	**0.1579**	**0.1432**	**7.86**	**7.89**	**7.16**	**0.68**	**0.68**	**0.62**
t=66	1	0.1271	0.1155	0.1294	6.36	5.78	6.47	0.55	0.50	0.56
	2	0.1271	0.1201	0.1294	6.36	6.01	6.47	0.55	0.52	0.56
	3	0.1248	0.1225	0.1317	6.24	6.13	6.59	0.54	0.53	0.57
(50x)	Avg	**0.1263**	**0.1194**	**0.1302**	**6.32**	**5.97**	**6.51**	**0.55**	**0.52**	**0.56**

Table B-6 (cont.) The data of SURFONIC TDA-6 concentration in surfactant-dilute phase every 6 hours for 5 days

		Absorbance			Abs x dilute factor			Concentration (mM)		
		Surfactant-dilute phase			Surfactant-dilute phase			Surfactant-dilute phase		
	vial (triplicate)	1	2	3	1	2	3	1	2	3
t=72	1	0.0993	0.1040	0.1109	4.97	5.20	5.55	0.43	0.45	0.48
	2	0.0993	0.1017	0.1086	4.97	5.09	5.43	0.43	0.44	0.47
	3	0.1017	0.1017	0.1086	5.09	5.09	5.43	0.44	0.44	0.47
(50x)	Avg	**0.1001**	**0.1025**	**0.1094**	**5.01**	**5.12**	**5.47**	**0.43**	**0.44**	**0.47**
t=78	1	0.0816	0.0832	0.0762	4.08	4.16	3.81	0.35	0.36	0.33
	2	0.0837	0.0878	0.0786	4.19	4.39	3.93	0.36	0.38	0.34
	3	0.0818	0.0878	0.0809	4.09	4.39	4.05	0.35	0.38	0.35
(50x)	Avg	**0.0824**	**0.0863**	**0.0786**	**4.12**	**4.31**	**3.93**	**0.36**	**0.37**	**0.34**
t=84	1	0.0670	0.0762	0.0647	3.35	3.81	3.24	0.29	0.33	0.28
	2	0.0670	0.0762	0.0647	3.35	3.81	3.24	0.29	0.33	0.28
	3	0.0670	0.0786	0.0670	3.35	3.93	3.35	0.29	0.34	0.29
(50x)	Avg	**0.0670**	**0.0770**	**0.0655**	**3.35**	**3.85**	**3.27**	**0.29**	**0.33**	**0.28**
t=90	1	0.0647	0.0893	0.0670	3.24	4.47	3.35	0.28	0.39	0.29
	2	0.0647	0.0893	0.0670	3.24	4.47	3.35	0.28	0.39	0.29
	3	0.0624	0.0917	0.0670	3.12	4.59	3.35	0.27	0.40	0.29
(50x)	Avg	**0.0639**	**0.0901**	**0.0670**	**3.20**	**4.51**	**3.35**	**0.28**	**0.39**	**0.29**
t=96	1	0.0762	0.0832	0.0531	3.81	4.16	2.66	0.33	0.36	0.23
	2	0.0762	0.0878	0.0554	3.81	4.39	2.77	0.33	0.38	0.24
	3	0.0786	0.0878	0.0554	3.93	4.39	2.77	0.34	0.38	0.24
(50x)	Avg	**0.0770**	**0.0863**	**0.0546**	**3.85**	**4.31**	**2.73**	**0.33**	**0.37**	**0.24**
t=5d	1	0.0647	0.0601	0.0716	3.24	3.01	3.58	0.28	0.26	0.31
	2	0.0647	0.0601	0.0716	3.24	3.01	3.58	0.28	0.26	0.31
	3	0.0624	0.0601	0.0693	3.12	3.01	3.47	0.27	0.26	0.30
(50x)	Avg	**0.0639**	**0.0601**	**0.0708**	**3.20**	**3.01**	**3.54**	**0.28**	**0.26**	**0.31**

Standard curve of surfactant concentration

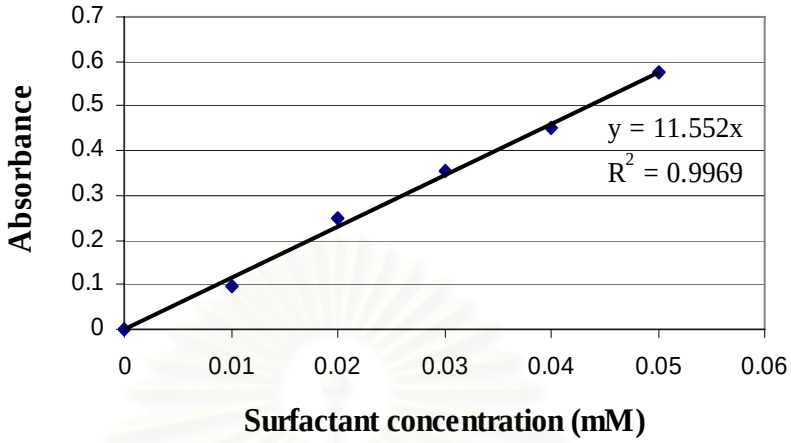

Figure B-1 Standard curve of remaining surfactant concentration in dilute phase

4.2.2 The contact time between surfactant solution and soil

Table B-7a The average and standard deviation of TCE peak area in surfactant both-phase solution

	Peak area			
Contact time (hr)	dilute	rich	SD (di)	SD (ri)
15	5731.2	1333.3	184.8	512.4
30	9914.0	1489.9	2587.3	108.1
45	15991.8	12878.5	81.4	606.7
60	20706.4	14425.3	1382.9	835.7
90	23814.0	12524.4	1451.7	2656.4
120	22520.8	12857.8	2720.3	1112.7
150	22520.8	12663.6	1898.4	1409.0
180	13641.3	14149.4	725.0	1900.4

Table B-7b Data of TCE peak area in surfactant both-phase solution

	Surfactant-dilute phase		Surfactant-rich phase	
Contact time	time (min)	Area	time (min)	Area
15	2.723	5873.9	2.733	1380.6
	2.721	5864.3	2.733	1327.1
	2.725	5707.8	2.736	1939.1
	2.723	5478.6	2.732	686.4
30	2.725	12890.0	2.720	1460.5
	2.730	8653.3	2.724	1399.6
	2.732	8198.6	2.723	1609.7
45	2.725	15984.6	2.721	12034.4
	2.724	15914.2	2.720	13102.8
	2.726	16076.6	2.720	13461.6
			2.722	12915.2
1 hr	2.721	19264.7	2.725	13953.3
	2.722	22021.8	2.721	13932.4
	2.721	20832.8	2.720	15390.2
1(1/2)	2.775	22594.5	2.72	10698.6
	2.772	22983.3	2.720	10588.1
	2.774	22837.4	2.719	10529.1
	2.721	25448.5	2.720	15945.7
	2.720	23138.0	2.721	14860.5
	2.777	25882.1		
2	2.721	24055.3	2.721	14372.3
	2.72	20031.9	2.721	12779.1
	2.723	25566.6	2.722	12578.0
	2.723	20429.4	2.728	11701.8
2(1/2)	2.722	21636.3	2.723	11746.1
	2.72	21690.1	2.723	12101.2
	2.729	20255.5	2.723	11824.2
	2.721	18400.4	2.721	15125.0
	2.722	17487.2	2.721	12521.7
3	2.778	13413.5	2.723	15756.6
	2.774	13154.1	2.722	15538.6
	2.776	14845.0	2.775	11311.7
	2.774	13746.7	2.776	15036.4
	2.773	13047.1	2.777	13103.5

The optimal contact time was determined by TCE extraction efficiency which was referred from TCE in surfactant-dilute phase and surfactant-rich phase. TCE can be comparable when surfactant-rich phase concentration of each treatment is equal. Therefore, surfactant concentration in surfactant-dilute phase and surfactant-rich phase were monitoring as data-base for TCE comparison.

Table B-8a The average and standard deviation of surfactant concentration in surfactant both-phase solution

	Surfactant concentration (mM)		Standard deviation (SD)	
Contact time (min)	surfactant-dilute phase	rich-phase	surfactant-dilute phase	rich-phase
15	1.0835	73.5535	0.0330	4.0416
30	1.2996	57.8199	0.0295	2.0181
45	3.0769	126.1810	0.2579	3.2721
60	2.0748	150.4386	0.0391	5.2053
90	2.0984	136.6006	0.0583	2.7060
120	2.0655	104.0967	0.0279	1.0460
150	2.1609	108.5431	0.2645	20.2344
180	2.4789	110.5472	0.1141	10.3102
average	2.0423	108.4726		

Table B-8b The data of surfactant concentration in surfactant-dilute phase solution

		Surfactant-dilute phase (50x)								
		Absorbance			Abs x dilute factor			Concentration (mM)		
	vial (triplicates)	1	2	3	1	2	3	1	2	3
CT15	1	0.2644	0.5065	0.2830	13.22	25.33	14.15	1.03	1.98	1.10
	2	0.2694	0.5083	0.2854	13.47	25.42	14.27	1.05	1.98	1.11
	3	0.2773	0.5097	0.2857	13.87	25.49	14.29	1.08	1.99	1.12
	Avg	**0.2704**	**0.5082**	**0.2847**	**13.52**	**25.41**	**14.24**	**1.06**	**1.98**	**1.11**
CT30	1	0.3305	0.3211		16.53	16.06		1.29	1.25	
	2	0.3371	0.3279		16.86	16.40		1.32	1.28	
	3	0.3456	0.3351		17.28	16.76		1.35	1.31	
	Avg	**0.3377**	**0.3280**		**16.89**	**16.40**		**1.32**	**1.28**	
CT45	1	0.8421	3.1069	0.7223	42.11	155.35	36.12	3.29	12.13	2.82
	2	0.8531	3.1069	0.7254	42.66	155.35	36.27	3.33	12.13	2.83
	3	0.8541	3.1069	0.7317	42.71	155.35	36.59	3.33	12.13	2.86
	Avg	**0.8498**	**3.1069**	**0.7265**	**42.49**	**155.35**	**36.32**	**3.32**	**12.13**	**2.84**
CT1hr	1	0.5357	0.7027	0.5163	26.79	35.14	25.82	2.09	2.74	2.02
	2	0.5395	0.7057	0.5244	26.98	35.29	26.22	2.11	2.76	2.05
	3	0.5449	0.7092	0.5279	27.25	35.46	26.40	2.13	2.77	2.06
	Avg	**0.5400**	**0.7059**	**0.5229**	**27.00**	**35.29**	**26.14**	**2.11**	**2.76**	**2.04**
$CT1_{(1/2)}$	1	0.5185	0.5501	0.5473	25.93	27.51	27.37	2.02	2.15	2.14
	2	0.5176	0.5497	0.5458	25.88	27.49	27.29	2.02	2.15	2.13
	3	0.5161	0.5478	0.5445	25.81	27.39	27.23	2.01	2.14	2.13
	Avg	**0.5174**	**0.5492**	**0.5459**	**25.87**	**27.46**	**27.29**	**2.02**	**2.14**	**2.13**

Table B-8b (cont.) The data of surfactant concentration in surfactant-dilute phase solution

		Dilute phase (50x)								
		Absorbance			Abs x dilute factor			Concentration (mM)		
	vial (triplicates)	1	2	3	1	2	3	1	2	3
CT2	1	0.5240	1.1216	0.5212	26.20	56.08	26.06	2.05	4.38	2.03
	2	0.5275	1.1244	0.5258	26.38	56.22	26.29	2.06	4.39	2.05
	3	0.5311	1.1273	0.5448	26.56	56.37	27.24	2.07	4.40	2.13
	Avg	**0.5275**	**1.1244**	**0.5306**	**26.38**	**56.22**	**26.53**	**2.06**	**4.39**	**2.07**
$CT2_{(1/2)}$	1	0.6336	0.4907	0.5130	31.68	24.54	25.65	2.47	1.92	2.00
	2	0.6488	0.4972	0.5192	32.44	24.86	25.96	2.53	1.94	2.03
	3	0.6495	0.5022	0.5273	32.48	25.11	26.37	2.54	1.96	2.06
	Avg	**0.6440**	**0.4967**	**0.5198**	**32.20**	**24.84**	**25.99**	**2.51**	**1.94**	**2.03**
CT3	1	0.5950	0.6574	0.6556	29.75	32.87	32.78	2.32	2.57	2.56
	2	0.5965	0.6558	0.6517	29.83	32.79	32.59	2.33	2.56	2.54
	3	0.5949	0.6565	0.6510	29.75	32.83	32.55	2.32	2.56	2.54
	Avg	**0.5955**	**0.6566**	**0.6528**	**29.77**	**32.83**	**32.64**	**2.32**	**2.56**	**2.55**

Table B-8c The data of surfactant concentration in surfactant-rich phase solution

		Rich phase (10,000)								
		Absorbance			Abs x dilute factor			Concentration (mM)		
	vial (triplicates)	1	2	3	1	2	3	1	2	3
CT15	1	0.0846	0.0055	0.0936	846.0	55.0	936.0	66.06	4.29	73.09
	2	0.0910	0.0083	0.0971	910.0	83.0	971.0	71.05	6.48	75.82
	3	0.0981	0.0097	0.1008	981.0	97.0	1008.0	76.60	7.57	78.71
	Avg	**0.0912**	**0.0078**	**0.0972**	**912.3**	**78.3**	**971.7**	**71.24**	**6.12**	**75.87**
CT30	1	0.0707	0.0711		707.0	711.0		55.20	55.52	
	2	0.0762	0.0738		762.0	738.0		59.50	57.62	
	3	0.0785	0.0740		785.0	740.0		61.29	57.78	
	Avg	**0.0751**	**0.0730**		**751.3**	**729.7**		**58.67**	**56.97**	
CT45	1	0.1595	0.0080	0.1526	1595.0	80.0	1526.0	124.54	6.25	119.15
	2	0.1640	0.0082	0.1634	1640.0	82.0	1634.0	128.05	6.40	127.59
	3	0.1656	0.0094	0.1645	1656.0	94.0	1645.0	129.30	7.34	128.45
	Avg	**0.1630**	**0.0085**	**0.1602**	**1630.3**	**85.3**	**1601.7**	**127.30**	**6.66**	**125.06**
CT1hr	1	0.1964	0.1806	0.2194	1964.0	1806.0	2194.0	153.35	141.02	171.31
	2	0.1984	0.1888	0.2245	1984.0	1888.0	2245.0	154.92	147.42	175.29
	3	0.1994	0.1924	0.2285	1994.0	1924.0	2285.0	155.70	150.23	178.42
	Avg	**0.1981**	**0.1873**	**0.2241**	**1980.7**	**1872.7**	**2241.3**	**154.66**	**146.22**	**175.01**
$CT1_{(1/2)}$	1	0.1668	0.1726	0.1785	1668.0	1726.0	1785.0	130.24	134.77	139.38
	2	0.1725	0.1749	0.1786	1725.0	1749.0	1786.0	134.69	136.57	139.45
	3	0.1774	0.1778	0.1754	1774.0	1778.0	1754.0	138.52	138.83	136.96
	Avg	**0.1722**	**0.1751**	**0.1775**	**1722.3**	**1751.0**	**1775.0**	**134.48**	**136.72**	**138.60**

Table B-8c (cont.) The data of surfactant concentration in surfactant-rich phase solution

		Rich phase (10,000)								
		Absorbance			Abs x dilute factor			Concentration (mM)		
	vial (triplicates)	1	2	3	1	2	3	1	2	3
CT2	1	0.1315	0.0343	0.1312	1315.0	343.0	1312.0	102.68	26.78	102.44
	2	0.1337	0.0398	0.1341	1337.0	398.0	1341.0	104.40	31.08	104.71
	3	0.1350	0.0399	0.1344	1350.0	399.0	1344.0	105.41	31.15	104.94
	Avg	**0.1334**	**0.0380**	**0.1332**	**1334.0**	**380.0**	**1332.3**	**104.16**	**29.67**	**104.03**
$CT2_{(1/2)}$	1	0.1047	0.1603	0.1364	1047.0	1603.0	1364.0	81.75	125.17	106.50
	2	0.1068	0.1666	0.1460	1068.0	1666.0	1460.0	83.39	130.09	114.00
	3	0.1088	0.1717	0.1498	1088.0	1717.0	1498.0	84.95	134.07	116.97
	Avg	**0.1068**	**0.1662**	**0.1441**	**1067.7**	**1662.0**	**1440.7**	**83.37**	**129.77**	**112.49**
CT3	1	0.1412	0.1573	0.1219	1412.0	1573.0	1219.0	110.25	122.82	95.18
	2	0.1407	0.1562	0.1275	1407.0	1562.0	1275.0	109.86	121.96	99.55
	3	0.1468	0.1550	0.1276	1468.0	1550.0	1276.0	114.62	121.03	99.63
	Avg	**0.1429**	**0.1562**	**0.1257**	**1429.0**	**1561.6**	**1256.6**	**111.58**	**121.94**	**98.12**

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4.2.3 The initial concentrations of surfactants

Table B-9 The data of peak area, standard deviation, and remaining TCE concentration in soil and effluent of various initial surfactant concentrations

	Dilute phase		Rich phase		remaining TCE conc. (ppm)	
Initial Conc.	time (min)	Area	time (min)	Area	Soil	Effluent
10	2.77	24405.3	2.778	1623.5	92.31	0.16
	2.776	25774.2	2.775	1467.3	97.49	0.17
	2.776	25448.4	2.774	1503.6	96.25	0.17
	2.776	24823.4	2.774	1577.8	93.89	0.16
30	2.775	11098.9	2.776	6095.8	41.98	0.07
	2.778	12557.3	2.781	7143.9	47.50	0.08
	2.787	12599.4	2.78	7703.4	47.65	0.08
	2.779	12685.0	2.777	7976.7	47.98	0.08
	2.775	11311.7	2.778	8099.0	42.78	0.07
	2.776	10869.0			41.11	0.07
50	2.775	7546.2	2.780	9378.3	28.54	0.05
	2.775	7637.9	2.781	9990.0	28.89	0.05
	2.776	7470.4	2.78	9790.6	28.26	0.05
	2.775	7524.6	2.779	9534.4	28.46	0.05
70	2.778	6112.9	2.774	8609.6	23.12	0.04
	2.778	6573.4	2.772	8744.3	24.86	0.04
	2.784	5610.8	2.776	6794.7	21.22	0.04
	2.788	5524.6	2.775	7129.0	20.90	0.04
	2.778	5459.5	2.773	6796.0	20.65	0.04
	2.776	5618.8	2.776	6823.8	21.25	0.04
90	2.773	4716.2	2.773	5874.6	17.84	0.03
	2.774	4746.6	2.774	5767.1	17.95	0.03
	2.779	4513.7	2.775	5852.5	14.81	0.03
	2.778	4640.3	2.775	5962.6	14.51	0.03
			2.779	5619.8		
110	2.772	5854.4	2.776	5700.5	22.14	0.04
	2.774	5870.1	2.774	5640.3	22.20	0.04
	2.774	4714.1	2.774	5458.3	17.83	0.03
	2.772	4637.3	2.773	5416.8	17.54	0.03
	2.772	5426.3	2.779	5618.8	20.52	0.04
	2.774	5461.8	2.776	5369.9	20.66	0.04

Table B-10 Peak area, standard deviation, and remaining TCE concentration in soil and effluent of various initial surfactant concentrations

Initial concentration of surfactant (mM)	Peak area		remaining TCE conc. (ppm)	
	dilute	rich	Soil	Effluent
10	25112.8 ± 614.92	1543.1 ± 70.65	95.0 ± 2.32	0.16 ± 0
30	11853.6 ± 845.61	7403.8 ± 818.49	45.0 ± 3.02	0.08 ± 0.01
50	7544.8 ± 69.79	9673.3 ± 271.03	28.5 ± 0.26	0.05 ± 0
70	5816.7 ± 436.99	7482.9 ± 934.42	21.6 ± 1.01	0.04 ± 0
90	4654.2 ± 103.79	5815.3 ± 129.56	17.6 ± 0.39	0.03 ± 0
110	5327.3 ± 538.98	5534.1 ± 136.09	20.1 ± 2.04	0.03 ± 0

Table B-11 Remaining surfactant concentration in surfactant-dilute phase of various initial surfactant concentrations

		Dilute phase (50x)								
		Absorbance			Abs x dilute factor			Concentration (mM)		
	vial (triplicates)	1	2	3	1	2	3	1	2	3
IC10	1	0.0391	0.0300	0.0449	1.96	1.50	2.25	0.15	0.12	0.18
	2	0.0340	0.0349	0.0478	1.70	1.75	2.39	0.13	0.14	0.19
	3	0.0388	0.0349	0.0486	1.94	1.75	2.43	0.15	0.14	0.19
	Avg	**0.0373**	**0.0333**	**0.0471**	**1.87**	**1.66**	**2.36**	**0.15**	**0.13**	**0.18**
IC30	1	0.4922	0.4893	0.4419	24.61	24.47	22.10	1.92	1.91	1.73
	2	0.4937	0.4879	0.4355	24.69	24.40	21.78	1.93	1.90	1.70
	3	0.4865	0.4860	0.4337	24.33	24.30	21.69	1.90	1.90	1.69
	Avg	**0.4908**	**0.4877**	**0.4370**	**24.54**	**24.39**	**21.85**	**1.92**	**1.90**	**1.71**
IC50	1	0.6210	0.6566	0.6321	31.05	32.83	31.6	2.42	2.56	2.47
	2	0.6187	0.6598	0.6359	30.94	32.99	31.8	2.42	2.58	2.48
	3	0.6259	0.6651	0.6442	31.30	33.26	32.2	2.44	2.60	2.52
	Avg	**0.6219**	**0.6605**	**0.6374**	**31.09**	**33.03**	**31.9**	**2.43**	**2.58**	**2.49**
IC70	1	0.6990	0.6840	0.7242	34.95	34.20	36.21	2.73	2.67	2.83
	2	0.7360	0.6930	0.7265	36.80	34.65	36.33	2.87	2.71	2.84
	3	0.7370	0.6874	0.7272	36.85	34.37	36.36	2.88	2.68	2.84
	Avg	**0.7240**	**0.6881**	**0.7260**	**36.20**	**34.41**	**36.30**	**2.83**	**2.69**	**2.83**
IC90	1	0.7344	0.5920	0.6530	73.44	59.20	65.30	5.73	4.62	5.10
	2	0.7240	0.6360	0.5980	72.40	63.60	59.80	5.65	4.97	4.67
	3	0.7205	0.5950	0.5980	72.05	59.50	59.80	5.63	4.65	4.67
	Avg	**0.7263**	**0.6077**	**0.6163**	**72.63**	**60.77**	**61.63**	**5.67**	**4.74**	**4.81**
IC110	1	0.7279	0.8753	0.7720	72.79	87.53	77.20	5.68	6.83	6.03
	2	0.7288	0.8074	0.7760	72.88	80.74	77.60	5.69	6.30	6.06
	3	0.7310	0.8157	0.7580	73.10	81.57	75.80	5.71	6.37	5.92
	Avg	**0.7292**	**0.8328**	**0.7687**	**72.92**	**83.28**	**76.87**	**5.69**	**6.50**	**6.00**

Table B-12 remaining surfactant concentration in surfactant-rich phase of various initial surfactant concentrations

		Rich phase (10,000)								
		Absorbance			Abs x dilute factor			Concentration (mM)		
	vial (triplicates)	1	2	3	1	2	3	1	2	3
IC10	1 2 3 Avg	uncompleted separation phase								
IC30	1	0.1802	0.2132	0.1977	1802.0	2132.0	1977.0	140.70	166.47	154.37
	2	0.1885	0.2186	0.1957	1885.0	2186.0	1957.0	147.19	170.69	152.81
	3	0.1798	0.2158	0.1969	1798.0	2158.0	1969.0	140.39	168.50	153.74
	Avg	**0.1828**	**0.2159**	**0.1968**	**1828.3**	**2158.7**	**1967.7**	**142.76**	**168.55**	**153.64**
IC50	1	0.3666	0.3506	0.3468	3666.0	3506.0	3468.0	286.25	273.76	270.79
	2	0.3640	0.3532	0.3478	3640.0	3532.0	3478.0	284.22	275.79	271.57
	3	0.3588	0.3539	0.3497	3588.0	3539.0	3497.0	280.16	276.33	273.05
	Avg	**0.3631**	**0.3526**	**0.3481**	**3631.3**	**3525.7**	**3481.0**	**283.54**	**275.29**	**271.80**
IC70	1	0.3995	0.4845	0.4325	3995.0	4845.0	4325.0	311.94	378.31	337.71
	2	0.3932	0.4985	0.4336	3932.0	4985.0	4336.0	307.02	389.24	338.56
	3	0.4021	0.5031	0.4338	4021.0	5031.0	4338.0	313.97	392.83	338.72
	Avg	**0.3983**	**0.4954**	**0.4333**	**3982.7**	**4953.7**	**4333.0**	**310.98**	**386.79**	**338.33**
IC90	1	0.4569	0.4106	0.4853	4569.0	4106.0	4853.0	356.76	320.61	378.93
	2	0.4526	0.4276	0.4988	4526.0	4276.0	4988.0	353.40	333.88	389.47
	3	0.4538	0.4132	0.5004	4538.0	4132.0	5004.0	354.34	322.64	390.72
	Avg	**0.4544**	**0.4171**	**0.4948**	**4544.3**	**4171.3**	**4948.3**	**354.83**	**325.71**	**386.38**
IC110	1	0.4307	0.4143	0.4062	4307.0	4143.0	4062.0	336.30	323.49	317.17
	2	0.4377	0.4174	0.4082	4377.0	4174.0	4082.0	341.77	325.92	318.73
	3	0.4310	0.4218	0.4040	4310.0	4218.0	4040.0	336.53	329.35	315.45
	Avg	**0.4331**	**0.4178**	**0.4061**	**4331.3**	**4178.3**	**4061.3**	**338.20**	**326.25**	**317.12**

Mass balance calculation of surfactant concentration

Mass balance = ((Vd/Vt) x [surfactant]di) + ((1-(Vd/Vt) x [surfactant]ri)

% recovery = (Mass balance/ [initial surfactant conc.])*100

Table B-13 % Recovery of surfactant concentration in the optimal initial concentration of surfactant study

initial surfactant concentration (mM)	Mass balance	% Recovery
10	0.14	1.37
30	8.82	29.41
50	16.33	32.67
70	58.06	82.94
90	90.98	101.09
110	107.73	97.94

Standard curve of %recovery calculation in the optimal initial concentration of surfactant study

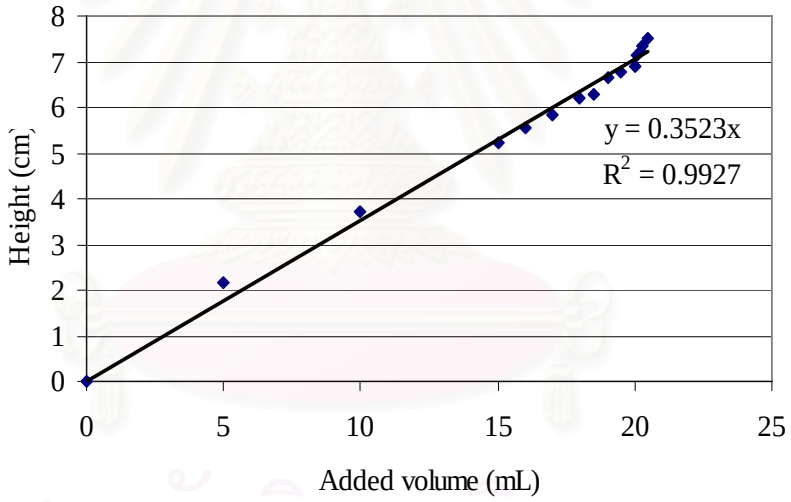

Figure B-2 relation between added volume of water into soil containing vial (mL) and height (cm)

Table B-14 Volume of surfactant in both phase solution which were calculated from height of surfactant

Initial surfactant concentration (mM)	Height of surfactant (cm)		Calculated volume (mL)	
	Dilute phase	Rich phase	Dilute phase	Rich phase
0				
10	7.5	0	20.47	0.03
30	6.8	0.7	19.56	0.94
50	6.2	1.3	19.30	1.20
70	5.8	1.7	16.89	3.61
90	5.3	2.2	15.22	5.28
110	4.9	2.6	13.90	6.60

Table B-15 Calibration of height of surfactant in the soil containing vial

Adding surfactant volume (mL)	Averaged surfactant height (cm)	Triplicate of surfactant height		
0	0	0	0	0
5	2.17	2.15	2.15	2.2
10	3.72	3.7	3.7	3.75
15	5.23	5.2	5.2	5.3
16	5.53	5.5	5.5	5.6
17	5.83	5.8	5.8	5.9
18	6.20	6.2	6.2	6.2
18.5	6.30	6.3	6.3	6.3
19	6.63	6.6	6.6	6.7
19.5	6.78	6.75	6.8	6.8
20	6.92	6.85	6.85	7.05
20.1	7.15	7.1	7.1	7.25
20.2	7.23	7.2	7.2	7.3
20.3	7.33	7.25	7.3	7.45
20.5	7.50	7.5	7.5	7.5

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4.3 The effectiveness of an integrated process study

4.3.1 Soil remediation by bioremediation

Table B-16 data of remaining TCE concentration (ppm) in soil and effluent after remediate 100 ppm contaminated soil by bioremediation

	Area		TCE conc. in soil (ppm)		Conc. in effluent (ppm)	
Time (hr)	Control	Sample	Control	Sample	Control	Sample
0	170972.2	196854.1	73.22	84.31	1.11	1.28
	156449.1	182899.0	67.00	78.33	1.02	1.19
	141694.0	172864.5	60.68	74.03	0.92	1.13
24	228342.2	208802.5	97.79	89.42	1.49	1.36
	217805.8	222836.2	93.28	95.43	1.42	1.45
	232337.4	199331.9	99.50	85.37	1.51	1.30
48	203073.4	133050.0	86.97	56.98	1.32	0.87
	193266.2	126318.2	82.77	54.10	1.26	0.82
	200991.7	121016.7	86.08	51.83	1.31	0.79
72	183892.3	116124.7	78.75	49.73	1.20	0.76
	174588.8	104679.5	74.77	44.83	1.14	0.68
	164347.8	94319.3	70.38	40.39	1.07	0.61
96	135294.2	64004.6	57.94	27.41	0.88	0.42
	143420.8	54810.2	61.42	23.47	0.93	0.36
	133734.8	63093.6	57.27	27.02	0.87	0.41

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Table B-17data of remaining TCE concentration (ppm) in soil and effluent after remediate 300 ppm contaminated soil by bioremediation

	Area		TCE conc. in soil (ppm)		Conc. in aqueous solution (ppm)	
Time (hr)	Control	Sample	Control	Sample	Control	Sample
0	1998402.7	1753089.6	208.46	195.67	13.02	11.42
	2125796.4	1666574.9	215.10	191.16	13.85	10.86
	2058470.0	1832349.0	211.59	199.80	13.41	11.94
24	3441703.5	2478703.2	283.70	233.50	22.42	16.15
	3304499.0	2201741.3	276.55	219.06	21.53	14.34
		2465365.3		232.80	0.00	16.06
48	2862232.8	1499787.2	253.49	182.46	18.65	9.77
	2762576.4	1316012.3	248.30	172.88	18.00	8.57
	2736437.7	1198315.8	246.93	166.74	17.83	7.81
72	2061283.9	904858.0	211.73	151.44	13.43	5.90
	2140705.1	763630.1	215.88	144.08	13.95	4.98
	2289776.4	765552.2	223.65	144.18	14.92	4.99
96	1741592.5	642427.2	195.07	137.76	11.35	4.19
	1678917.0	383104.6	191.80	124.24	10.94	2.50
	1578332.9	443127.2	186.56	127.37	10.28	2.89

4.3.2 Soil remediation by surfactant based-separation technology

Table B-18 data of peak area and concentration of remaining TCE in soil and effluent after remediate 100 ppm contaminated soil by surfactant based-separation technology

	Peak area of TCE		Remaining TCE (ppm)		
vial	dilute	rich	soil	rich	dilute
1.1	13560.4	12479.1	24.79	351.56	0.09
1.2	15098.4	11377.7	27.60	320.53	0.10
2.1	12945.5	9583.2	23.67	269.98	0.08
2.2	14197.2	9904.3	25.96	279.03	0.09
3.1	13316.4	8723.8	24.35	245.77	0.09
3.2	17702.4	8780.7	32.36	247.37	0.12
		average	26.45	285.71	0.09
		SD	3.21	42.21	0.01

Table B-19 Data of peak area and concentration of remaining TCE in soil and effluent after remediate 300 ppm contaminated soil by surfactant based-separation technology

	Peak area of TCE		Remaining TCE (ppm)		
vial	dilute	rich	soil	rich	dilute
1.1	70798.8	71086.3	129.44	1073.49	0.46
1.2	72107.5	74345.5	131.83	1122.70	0.47
2.1	53863.8	87128.4	98.48	1315.74	0.35
2.2	65733.8	77053.7	120.18	1163.60	0.43
3.1	71240.9	64997.2	130.25	981.53	0.46
3.2	67158.1	62890.1	122.78	949.71	0.44
		average	122.16	1101.13	0.44
		SD	12.47	132.98	0.04

4.3.3 Soil remediation by integrated technique

Table B-20 Data of peak area and concentration of remaining TCE in soil and effluent after remediate 100 ppm contaminated soil by integrated process

	Area		TCE concentration in soil (ppm)		TCE concentration in aqueous solution (ppm)	
Time (hr)	Control	Sample	Control	Sample	Control	Sample
0	64976.1	56354.1	27.83	24.13	0.42	0.37
	68136.4	58733.0	29.18	25.15	0.44	0.38
	73836.6	46864.0	31.62	20.07	0.48	0.31
24	104491.8	99366.3	44.75	42.56	0.68	0.65
	107141.5	84251.8	45.89	36.08	0.70	0.55
	117188.3	89704.8	50.19	38.42	0.76	0.58
48	98024.9	41605.6	41.98	17.82	0.64	0.27
	92267.8	42554.2	39.52	18.22	0.60	0.28
	91039.2	40111.7	38.99	17.18	0.59	0.26
72	79725.2	32159.9	34.14	13.77	0.52	0.21
	74895.5	31658.7	32.08	13.56	0.49	0.21
	75929.8	28109.7	32.52	12.04	0.49	0.18
96	64063.3	12106.9	27.44	5.18	0.42	0.08
	58343.3	12396.8	24.99	5.31	0.38	0.08
	55555.5	14985.6	23.79	6.42	0.36	0.10

Table B-21 Data of peak area and concentration of remaining TCE in soil and effluent after remediate 300 ppm contaminated soil by integrated process

	Area		Conc.		Conc. in aqueous solution (ppm)	
Time (hr)	Control	Sample	Control	Sample	Control	Sample
0	164292.5	145393.0	70.36	62.27	1.07	0.95
	142871.7	136535.5	61.19	58.47	0.93	0.89
	154440.6	149563.1	66.14	64.05	1.01	0.97
24	239824.0	222222.4	102.71	95.17	1.56	1.45
	220969.7	233378.7	94.63	99.95	1.44	1.52
	208424.3	187768.6	89.26	80.41	1.36	1.22
48	191383.9	103509.6	81.96	44.33	1.25	0.67
	188669.5	95436.3	80.80	40.87	1.23	0.62
	188835.1	93473.6	80.87	40.03	1.23	0.61
72	154960.2	83104.6	66.36	35.59	1.01	0.54
	149220.0	86300.0	63.91	36.96	0.97	0.56
	140452.0	72207.3	60.15	30.92	0.92	0.47
96	129645.3	29600.8	55.52	12.68	0.84	0.19
	133262.9	47978.6	57.07	20.55	0.87	0.31
	131592.9	41779.8	56.36	17.89	0.86	0.27

Chlorine ion generation

Table B-22 Data of Generated chlorine ion (ppm) after remediate 100 ppm contaminated soil by bioremediation (dilute 2x)

	Generated chlorine ion (ppm)				
treatment	0	24	48	72	96
C1	0	0	0.268	0.722	0.958
	0	0	0.469	0.859	1.158
	0	0	0.228	0.717	1.028
S1	0	2.056	10.672	14.578	19.190
	0	5.458	11.196	11.982	17.094
	0	5.910	11.514	13.038	15.722

C1 = control treatment of bioremediation experimental set

S1 = sample treatment of bioremediation experimental set

Table B-23 Data of Generated chlorine ion concentration (ppm) after remediate 100 ppm contaminated soil by bioremediation

	Generated chlorine ion concentration (ppm)	
Time (hr)	Control (C1)	Sample (S1)
0	0.00	0.00
24	0.00	8.95 ± 4.21
48	0.64 ± 0.26	22.25 ± 0.85
72	1.53 ± 0.16	26.40 ± 2.61
96	2.10 ± 0.20	34.67 ± 3.49

Table B-24 Data of Generated chlorine ion (ppm) after remediate 100 ppm contaminated soil by integrated process (dilute 5x)

	Generated chlorine ion (ppm)				
treatment	0	24	48	72	96
C3	0.723	0.256	0.654	0.310	0.170
	0.269	0.537	0.415	0.310	0.149
	0.000	0.167	0.233	0.818	0.750
S3	0.020	2.046	2.616	2.859	3.478
	0.000	2.202	2.732	2.815	3.167
	0.446	2.272	2.747	2.813	3.528

C3 = control treatment of integrated process experimental set
S3 = sample treatment of integrated process experimental set

Table B-25 Data of Generated chlorine ion concentration (ppm) after remediate 100 ppm contaminated soil by integrated process

	Generated chlorine ion concentration (ppm)	
Time (hr)	Control (C3)	Sample (S3)
0	1.65 ± 1.83	0.78 ± 1.26
24	1.60 ± 0.97	10.87 ± 0.58
48	2.17 ± 1.06	13.49 ± 0.36
72	2.40 ± 1.47	14.15 ± 0.13
96	1.78 ± 1.71	16.96 ± 0.98

APPENDIX C

Full paper in the 6th National Environmental Conference (March, 7-9th 2007) on the topic of Integrated Technique for Trichloroethylene (TCE) Contaminated Soil Clean-up Using Surfactant-Based Separation and Bioremediation. This full paper was presented as oral presentation by Miss Sasikarn Chuahom.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

07R4-11

เทคนิคร่วมสำหรับการบำบัดดินปนเปื้อนสารไตรคลอโรเอธิลีน โดยใช้สารลดแรงตึงผิวและการบำบัดทางชีวภาพ

Integrated Technique for Trichloroethylene (TCE) Contaminated Soil Clean-up using Surfactant-based Separation Technology and Bioremediation

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บทคัดย่อ

เทคโนโลยีร่วมระหว่างการใช้สารลดแรงตึงผิวและการบำบัดทางชีวภาพ ถูกพัฒนาขึ้นเพื่อเพิ่มประสิทธิภาพการแยกสารมลพิษที่ปนเปื้อนออกจากดิน (Soil washing) ในการศึกษาได้นำเทคนิคการสกัดแบบขุ่นของสารลดแรงตึงผิวชนิดไม่มีประจุมาใช้แยกสารไตรคลอโรเอธิลีน (ทีซีอี) ปริมาณมากให้ไปอยู่ในวัฏภาคที่มีความเข้มข้นของสารลดแรงตึงผิวสูง อย่างไรก็ดียังคงมีทีซีอีในระดับความเข้มข้นที่เป็นอันตรายเหลืออยู่ในวัฏภาคที่มีความเข้มข้นของสารลดแรงตึงผิวต่ำ ดังนั้นจึงนำวิธีการบำบัดทางชีวภาพมาเสริมในระบบ โดยเติมแบคทีเรียเพื่อย่อยสลายสารทีซีอีที่เหลืออยู่ หลังจากแยกวัฏภาคที่มีความเข้มข้นของของสารลดแรงตึงผิวสูงออกแล้ว การศึกษาในเบื้องต้นพบว่า สารลดแรงตึงผิว 6 ระบบ ได้แก่ SURFONIC TDA-5, TDA-6, L24-7, NEODOL 91-5, 91-6 ที่ไม่มีเติมสารอิเลคโทรไลต์และ DTAB/DOWFAX (อัตราส่วนโดยโมลที่ 2:1) ที่มีการเติมโซเดียมคลอไรด์เป็นสารอิเลคโทรไลต์ สามารถเกิดการแบ่งวัฏภาค โดยมีวัฏภาคที่มีความเข้มข้นของสารลดแรงตึงผิวสูงปรากฏอยู่ชั้นบนของสารละลาย จึงได้นำสารลดแรงตึงผิวดังกล่าวมาศึกษาถึงผลยับยั้งความสามารถของแบคทีเรีย *Rhodococcus* sp. L4 และ *Rhodococcus* sp. P3 ในการย่อยสลายทีซีอี ผลการทดลองพบว่า SURFONIC TDA-6, L24-7 และ NEODOL 91-6 ไม่ยับยั้งความสามารถของแบคทีเรียในการย่อยสลายทีซีอี ในขณะที่สารลดแรงตึงผิวชนิดอื่นส่งผลให้แบคทีเรียตาย ทั้งนี้พบว่าแบคทีเรีย *Rhodococcus* sp. L4 สามารถย่อยสลายทีซีอีได้อย่างมีประสิทธิภาพในระบบที่มี SURFONIC TDA-6 ซึ่งทีซีอีความเข้มข้นสิบส่วนในล้านส่วนถูกย่อยสลายได้มากกว่า 58% ภายใน 24 ชั่วโมง โดยเทียบกับชุดควบคุมที่ปราศจากแบคทีเรียซึ่งทีซีอีมีลดลง 30% เนื่องจากกิจกรรมทางกายภาพ จากผลข้างต้นได้เลือก SURFONIC TDA-6 มาศึกษาต่อเพื่อหาสภาวะที่เหมาะสมในการสกัดแบบขุ่นเพื่อสกัดทีซีอีซึ่งประกอบด้วย ช่วงเวลาสมดุลความเข้มข้นเริ่มต้นของสารลดแรงตึงผิว และเวลาในการสัมผัสระหว่างสารละลายของสารลดแรงตึงผิวและดิน จากนั้นนำสภาวะที่ได้มา

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ศึกษาเพื่อเปรียบเทียบประสิทธิภาพของกระบวนการกำจัดทีซีอีทั้งสามวิธีดังต่อไปนี้ (I) เทคโนโลยีการแบ่งวัฏภาคของสารลดแรงตึงผิว (II) การบำบัดทางชีวภาพ และ (III) เทคนิคร่วมระหว่างเทคโนโลยีการแบ่งวัฏภาคของสารลดแรงตึงผิวและการบำบัดทางชีวภาพ ทำการประเมินประสิทธิภาพในการกำจัดทีซีอีจากความเข้มข้นของทีซีอีที่เหลืออยู่ และการเกิดคลอไรด์อิออน จากผลการศึกษาพบว่าการบำบัดดินปนเปื้อนด้วยการใช้เทคนิคร่วมมีประสิทธิภาพในการกำจัดทีซีอีได้สูงที่สุด

คำสำคัญ : การบำบัดทางชีวภาพ; สารลดแรงตึงผิว; ไตรคลอโรเอธิลีน; การสกัดแบบขุ่น

Abstract

Integrated surfactant-based separation technology and bioremediation was developed to enhance the efficiency of soil washing approach. In this system, cloud point extraction by non-ionic surfactant was conducted to separate high amount of Trichloroethylene (TEC) into the surfactant-rich phase. Meanwhile, TCE at harmful level still presented in the surfactant-dilute phase, thus bioremediation was integrated into the system by adding bacteria to co-metabolize the remaining TCE after the surfactant-rich phase was separated out. Preliminary results showed that six surfactant systems including SURFONIC TDA-5, TDA-6, L24-7, NEODOL 91-5, 91-6 without electrolyte addition and DTAB/DOWFAX (2:1 molar ratio) with 0.8 M NaCl could induce a phase separation with the surfactant-rich phase presented on top of the solution. Therefore, the surfactants were used to determine whether they inhibited TCE degrading activities of either *Rhodococcus* sp. L4 or *Rhodococcus* sp. P3 bacteria. The result found that SURFONIC TDA-6, L24-7, and NEODOL 91-6 do not inhibit bacterial TCE degradability while others killed the bacteria. *Rhodococcus* sp. L4 degraded TCE effectively in the presence of SURFONIC TDA-6, in which more than 58% of 10 ppm TCE was reduced within 24 hours compared to 30% of TCE removal in control treatment (without the bacteria) through physical activities. SURFONIC TDA-6 was then selected for determining the optimal condition for TCE extraction consisting of equilibrium time and contact time between surfactant solution and soil. The acquired condition was later applied to compare the effectiveness of three TCE treatment processes including: (I) surfactant-based separation technology, (II) bioremediation, and (III) an integrated process of surfactant-based separation technology and bioremediation. TCE removal efficiency was determined from the remaining TCE concentration and the formation of chloride ions. The result found that soil remediation by the integrated technique has the highest TCE removal efficiency.

Keywords : Bioremediation; surfactant; Trichloroethylene; cloud point extraction

Introduction

Trichloroethylene (TCE) is commonly used as solvent for cleaning and degreasing of metal parts. It is also found in household products such as in paint, paint stripper and adhesive. The widespread use of TCE in industries and households results in a high possibility of leaking and contaminating to the environment. TCE is a volatile organic compound (VOC) and known as carcinogen [1]. It has low water solubility so it poses a significant risk of accumulating in human body. TCE is classified as a dense non-aqueous phase liquid (DNAPL) thus, it does not move with the groundwater flow but tends to

migrate down gradient by gravity through an aquifer until it reaches an impermeable layer. Surfactant-based separation technology is one of the most promising technologies for clean up of soil and groundwater contaminations. It can increase the solubility of an organic pollutant and bioavailability [2] since the organic pollutant will solubilize into the surfactant aggregates. The cloud point extraction is one of surfactant-based separation technology in which a nonionic surfactant is utilized as a separating agent [3]. When a solution of nonionic surfactant is heated above a certain temperature known as the cloud point, the solution separates into two immiscible aqueous phases, which are the surfactant-rich or coacervate phase and the surfactant-dilute phase. The surfactant-rich phase has very high concentrations of surfactant and pollutant. The surfactants in the concentrated form can possibly be regenerated by vacuum stripping if the pollutants have high enough volatility such as VOCs [4]. The surfactant-dilute phase solution is generally leaved as effluent water because it contains only small amount of surfactant and pollutant and is not cost effectiveness for reuse or further treatment.

From the study using cloud point extraction, Kimchuwanit (2000) found that TCE at harmful level still presented in the surfactant-dilute phase [5]. Meanwhile, TCE at that concentration can be removed by bacteria via co-metabolism [6, 7]. Biodegradation of TCE would result in the complete destruction of this pollutant [8]. So, it is possible to combine surfactant-based separation technology and biodegradation for clean up TCE contaminated soil to the possible lowest level. Bioremediation is usually used after physical or chemical treatments to completely remove the pollutants. Integrated treatment such as soil washing, ozonation, and bioremediation has been successfully carried out for remediation of aged soil contaminated with PAHs [9]. Nevertheless, the incorporation of surfactant based on cloud point extraction and biodegradation to remove TCE from soil has never been studied. Therefore, this research was conducted to optimize and integrate surfactant-based separation technology and bioremediation for clean up TCE contaminated soil. An integrated technique of surfactant-based separation technology and biodegradation is expected to enhance the efficiency of TCE contaminated soil clean-up.

Materials and Methods

Inoculum preparation

To study bacterial growth, *Rhodococcus* sp. L4 and *Rhodococcus* sp. P3, the known TCE degrading bacteria, were grown in MS medium [10] which has toluene in an Eppendorf tube suspended on top of flask. The cultures were incubated on shaker at 200 rpm, room temperature and overnight. They were centrifuged at 7,500 rpm for 10 minutes. The harvested cells was washed twice and resuspended in MS medium to have a final optical density of 0.1 at 600 nm.

To study TCE biodegradation rate, the bacteria were induced with 25 mg/L cumene in the flasks containing 4 g/L of a glucose-MS medium. The absorbance of inoculums was adjusted to 1.0 at 600 nm (approximately 10^8 CFU per mL).

Effect of surfactants on bacterial inoculum

1) The study on the effects of surfactants on bacterial growth

Six surfactants (SURFONIC TDA-5, TDA-6, L24-7, NEODOL 91-5, 91-6 without electrolyte addition and DTAB/DOWFAX (2:1 molar ratio) with 0.8 M NaCl) were prepared at the concentration of 30 mM. They were heated in a water-bath at a temperature above their cloud points. After phase separation, the coacervate phase was separated out. One mL of the surfactant-dilute phase solution was added into the 22 mL vial that contained 4 mL of 0.1 OD bacteria

inoculums. Control treatments consist of 1 mL of dilute phase surfactant solution and 4 mL of MS. The study were done in triplicate. After incubation on orbital shaker at 200 rpm, room temperature, the amounts of bacteria in each vial were determined by dilution plate count method on toluene-MS agar.

2) The study on the effect of surfactant on TCE biodegradation

The surfactants which did not harm the bacteria were used. One mL of the surfactant-dilute phase solution was added into the 22 mL vial that contained 4 mL of 1.0 OD bacteria inoculums. Then, cumene was added to make the final concentration of 25 mg/L to maintain enzyme induction. The vial was capped suddenly with a Teflon-lined rubber septum. After that TCE was spiked with a gas-tight syringe to make a final concentration of 10 ppm. Control treatment was prepared without bacteria inoculums. The samples were taken by sacrificing test vials at 0, 24, 48 and 96 hours. The remaining TCE was extracted using hexane and then analyzed by GC.

Optimal conditions for surfactant-based separation technology

1) The study of the equilibrium time

The 100 ppm TCE contaminated soil was prepared by adding TCE to 2.8 g uncontaminated soil. This vial was capped immediately and leaved it overnight to provide the homogenous condition. Then, 30 mM surfactant was added to those vials until full. The samples were stirred for 30 minutes and then equilibrated in a water bath at 60°C. After the phase separation, surfactant concentrations in surfactant-dilution phases were analyzed by Iodine-Iodide method [11] every 6 hours for 5 days.

2) The study of the contact time between surfactant solution and soil

The 100 ppm TCE contaminated soil was prepared as the previous experiment. The initial concentration of surfactant was fixed at 30 mM. Stirring time was varied at 15, 20, 30, and 45 minutes, 1, 1 ½, 2, 2 ½, and 3 hours. After equilibrating, the surfactant-dilute phase solution was transferred by decantation to the second vial and sealed with rubber septa. TCE concentration in the surfactant-dilute phase solution was analyzed.

Effectiveness of an integrated process of in TCE contaminated soil clean up

Three types of treatments for TCE contaminated soil remediation were employed: (I) surfactant-based separation technology, (II) bioremediation, and (III) integrated process of surfactant-based separation technology and biodegradation (Figure 1). The TCE removal efficiency was determined by the amounts of remaining TCE in aqueous solution.

For treatment (I), 30 mM surfactant concentration was added into the contaminated soil in 22-mL vial until full and then the vials were capped suddenly. The samples were stirred at the optimal contract time and equilibrated in a water-bath at desired temperature. After phase separation, the entire coacervate phase and some portions of surfactant dilute-phase solution were separated out. The concentration of remaining TCE in the surfactant-dilute phase was determined. For treatment (II), 5 ml of 1.0 OD bacteria inoculums was added into the contaminated soil. After that, cumene was added to make the final concentration of 25 mg/L. Then, the vials were capped and incubated on shaker at room temperature. Control treatment was prepared without bacteria inoculums. The vials were centrifuged at 3,000 rpm 3 minutes to separate soil before the concentration of remaining TCE in the aqueous solution was measured. For treatment (III), samples were prepared by the same process of treatment (I). After the phase separation, coacervate phase and some

portions of surfactant dilute-phase solution were separated out to provide a head-space volume for aerobic condition. After that, 2 ml of 2.5 OD of bacteria inoculums was added directly into those vials. Cumene was added before the vials were capped. Control treatment was prepared without bacteria inoculums. The remaining TCE concentration after degradation was representing the effectiveness of soil remediation by integrated process (III).

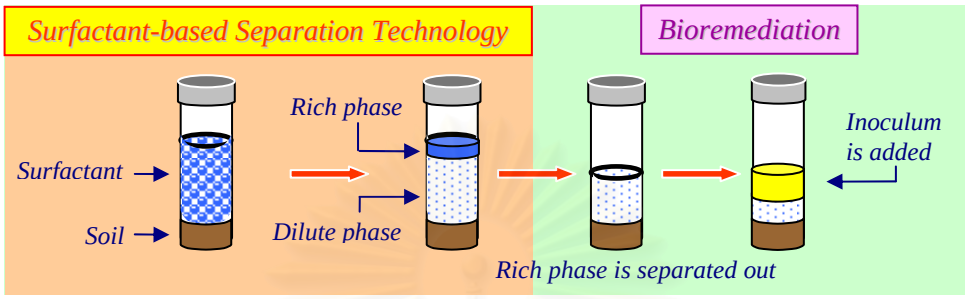

Figure 1 Overall procedure for clean-up TCE contaminated soil by integrated process

Analytical methods

TCE concentration was analyzed through gas chromatograph with ECD detector equipped with headspace manual-sampler and a HP-5 (5% Phenyl Methyl Siloxane) column (30 m x 0.32 mm ID x 0.25 µm). The analysis condition: oven, 100°C isothermal (4 min); injector, 100 µL splitless 250°C; detector, 250°C. Samples were heated at 93°C for 30 min. The carrier gas was helium with gas flow of 20 ml/min, and the make up gas was N_2 at 70 ml/min.

Results and Discussion

Effect of surfactants on bacterial inoculum

1) The effects of surfactants on bacterial growth

Of the six surfactants, only SURFONIC TDA-6, L24-7 and NEODOL 91-6 were not toxic to *Rhodococcus* sp. L4 and *Rhodococcus* sp. P3. Both bacteria were found in the presence of these surfactants after 4-day incubation while other surfactants killed these bacteria. The low tolerance of ionic surfactant was probably due to physico-chemical interactions between surfactant and bacterial membrane. For nonionic surfactant, this toxicity related to membrane-damaging effect: surfactant with ethylene oxide chains consisting of fewer than six monomers bury in the lipid layer of the liposomes [12].

2) The effect of surfactant on TCE biodegradation

The TCE degradability of bacteria in the presence of surfactants was investigated through %biodegradation. *Rhodococcus* sp. L4 had TCE degradability more than *Rhodococcus* sp. P3 in every surfactant presences (Figure 2). %Biodegradation of 10 ppm TCE by *Rhodococcus* sp. L4 was 58.43%, 50.55% and 48.93% in the presence of SURFONIC TDA-6, NEODOL 91-6 and SURFONIC L24-7, respectively. The results from control treatments (without the bacteria) shown some TCE losses caused by abiotic process. However, TCE losses in control treatment were less than %biodegradation of both bacteria. The results suggested that three of surfactants did not inhibit TCE degradability of

bacteria. *Rhodococcus* sp. L4 degraded TCE effectively in the presence of SURFONIC TDA-6, in which 58.43 % of 10 ppm TCE was reduced within 24 hours compared to 29.35% of TCE removal in control treatment through physical activities. Therefore, *Rhodococcus* sp. L4 and SURFONIC TDA-6 were selected for further study.

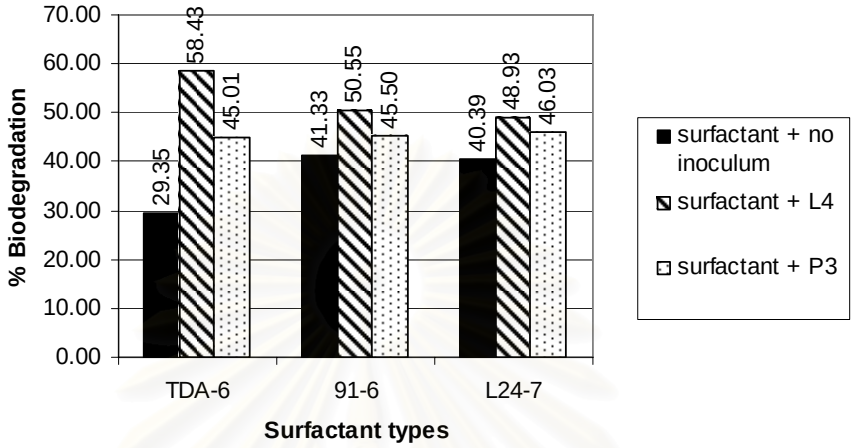

Figure 2 Percentages TCE degradation after 24 hr incubation in the presence of various surfactants

Optimal conditions for surfactant-based separation technology

1) The equilibrium time

After contaminated soil and SURFONIC TDA-6 were mixed and equilibrated in a water bath, the phase separation occurs. The surfactant concentration in surfactant-dilute phase was analyzed. Surfactant concentration decreased with the increase equilibrated time (Figure 3a). After 72 hours, there was no further change in the surfactant concentration in both phases. Therefore, the optimal equilibrium time of TDA-6 is 72 hours or 3 days.

2) The contact time between surfactant solution and soil

Contact time is the time used to stirred surfactant solution and soil before equilibrated at the equilibrium time. The results shown that TCE concentration in surfactant-dilute phase increased as the stirring time increased (Figure 3b). At stirring time more than 1 hr, TCE concentration was decreased until it remained relatively constant after 2-hr stirring time. The increasing of TCE concentration in the first period probably resulted from desorption. TCE which adsorb in soil particles was gradually extracted into surfactant-dilute phase via interaction between surfactant solution and soil. When stirring time was longer, TCE in surfactant-dilute phase decreased. The possible explanation was TCE re-adsorbed into soil particle. When the stirring time increases, the soil grains were beat into very fine particles, thus the interfacial area of soil drastically increases potentially leading to a higher adsorption of TCE back onto the soil. However, the further investigation needed to be studied to assure this explanation. When TCE concentration was observed constant, it is defined as the condition where no further increase in the TCE extraction efficiency. Consequently, the contact time of 2 hours was chosen to be applied into next studies.

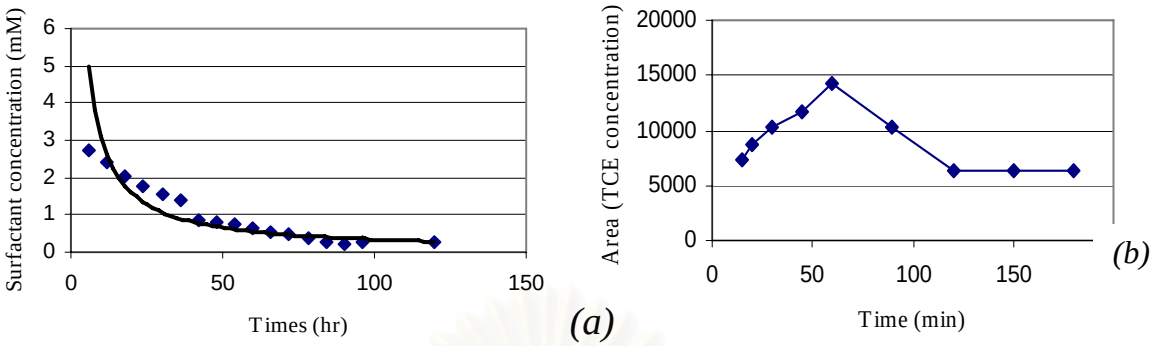

Figure 3 The optimal equilibrium time (a) and optimal contact time (b) between surfactant solution and soil

Effectiveness of an integrated process in TCE contaminated soil clean up

TCE removal efficiency was determined by the remaining TCE concentration in aqueous solution. TCE concentrations in aqueous solution after remediation were shown in table 1. After remediate by treatment (I), (II), and (III) for 96 hr, there were 6.37 ppm, 4.18 ppm, and 1.05 ppm of TCE, respectively. The loss of TCE in treatment (II) and (III) was mainly due to the activity of added bacteria since the remaining TCE in both treatments was lower than control without the inoculum. Alternatively, the results can be calculated as TCE removal efficiency by comparing TCE in aqueous solution before (at T=0) and after remediation (at T=96 hr). The TCE removal efficiency of treatment (I), (II), and (III) were 59.27%, 73.26%, and 93.27%, respectively in which initial TCE concentration in aqueous solution was 15.64 ppm. These indicated that the integrated process (III) was the most effectiveness. The results were comparable with other studies. For example an integrated treatment of PAH contaminated soil by soil washing, ozonation and biological treatment showed 90% PAHs reduction [9]. The study also indicated that each individual method achieved only 50% PAHs reduction. TCE removal efficiency of bioremediation (II) was less than the integrated process (III); which was probably due to the low bioavailability of TCE in this treatment. The improvement of biodegradation is assumed to be the result of an increased mobilization of TCE from soil matrix by surfactant [2, 9]. TCE removal efficiency of treatment (II) was similar with the study of Suttinun [7] that found 30% of TCE remained after 96 hr incubation for soil microcosms. TCE removal efficiency of surfactant-based separation (I) was lower than Kimchuwanit *et al*. [5], which was able to extract 90% of TCE from wastewater containing 100 ppm TCE. This may be resulted from the limitation of selected surfactants and soil adsorption of TCE.

Table 1 Amount of TCE in aqueous solution of the soil containing vials

Remediation approach	Amount of remaining TCE (ppm)		TCE removal efficiency (%)
	Control*	Treatment	Treatment
I. Surfactant-based separation	ND	6.37 ± 0.17	59.27
II. Bioremediation	13.20 ± 0.75	4.18 ± 1.29	73.26
III. Integrated process	3.19 ± 0.11	1.05 ± 0.21	93.27

*Control was used to determine the removal of TCE by non-biological activities. ND = not determined

Conclusions

Remediation by individual method has own limitations. Soil remediation by surfactant based-separation technology was limited by TCE extraction efficiency of surfactant; while bioremediation was limited by bioavailability of bacteria. Here, an integrated technique of surfactant-based separation technology and bioremediation was developed and showed to enhance the efficiency of TCE contaminated soil clean-up. When using surfactant based-separation technology with cloud point extraction technique, large amount of TCE was removed from soil through solubilization into surfactant aggregates and pre-concentration in coacervate-phase. Remaining TCE in surfactant diluted-phase was later degraded by *Rhodococcus* sps bacteria. These results indicate that surfactant can enhance biodegradability of TCE by desorbing them from the soil matrix into more bioavailable form. Consequently, bioremediation was used as biological post treatment to reduce TCE to the possible lowest level.

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ทำเนียบวิทยากร

ชื่อบทความ เทคนิคร่วมสำหรับการบำบัดดินปนเปื้อนสารไตรคลอโรเอธิลีนโดยใช้สารลดแรงตึงผิวและการบำบัดทางชีวภาพ

Integrated Technique for Trichloroethylene (TCE) Contaminated Soil Clean-up using Surfactant-based Separation Technology and Bioremediation

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ตำแหน่งหน้าที่ปัจจุบัน -

ประวัติการทำงาน -

ประสบการณ์ -

ผลงาน -

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Miss Sasikarn Chuahom was born on December 17, 1982 in Songkla, Thailand. She graduated with a Bachelor of Science in Environmental Science and Technology from the Faculty of Environmental and Resource Studies of Mahidol University, Thailand. Later, she pursued her Master's degree study in the International Program in Environmental Management, National Research Center for Environmental and Hazardous Waste Management (NRC-EHWM), Chulalongkorn University, Thailand in May 2005. She received Master's degree of Science in Environmental Management in May 2007.

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