เทคนิคร่วมของการสกัดแบบขุ่นและการบำบัดทางชีวภาพสำหรับกำจัดไตรคลอโรเอธิลีน

ในดินปนเปื้อนชนิดต่างๆ

นางสาววิชญา แก้วทิพย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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COMBINED CLOUD POINT EXTRACTION AND BIOREMEDIATION FOR
CLEAN-UP TRICHLOROETHYLENE IN VARIOUS SOIL TYPES

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A Thesis Submitted in Partial Fulfillment of the Requirements
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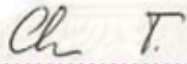
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AND BIOREMEDIATION FOR CLEAN-UP TRICHLOROETHYLENE IN
VARIOUS SOIL TYPES) อ. ที่ปรึกษา: ผศ.ดร. เอกวัล ลือพร้อมชัย, 118 หน้า.

การปนเปื้อนของสารไตรครอโรเอธิลีน(ทีซีอี)ในดินและน้ำใต้ดินได้กลายมาเป็นปัญหาสิ่งแวดล้อมที่
สำคัญเนื่องจากความเป็นพิษและความคงทน ในการศึกษาครั้งนี้ได้นำเทคนิคร่วมของการสกัดแบบขุ่นและการ
บำบัดทางชีวภาพมาประยุกต์ใช้เพื่อเพิ่มประสิทธิภาพในการบำบัดสารทีซีอีออกจากดินต่างชนิดกัน 3 ชนิด คือ
ดินร่วนเหนียวปนทราย ดินเหนียว และดินร่วนปนทราย ในการสกัดแบบขุ่นได้นำสารลดแรงตึงผิวชนิดไม่มี
ประจุ DEHYDOL LS7 TH ซึ่งเป็นสารลดแรงตึงผิวที่ผลิตในประเทศไทย มาทำการกำจัดสารทีซีอีความเข้มข้น
สูงออกจากดินที่ความเข้มข้นเริ่มต้นของสารลดแรงตึงผิว 90 มิลลิโมลาร์ โดยผลการศึกษาค่าสัดส่วนการแบ่งแยก
ความเข้มข้นของสารลดแรงตึงผิวในวัฏภาคที่มีความเข้มข้นสูงและต่ำ ในดินร่วนเหนียวปนทราย และดินร่วน
เหนียวเท่ากับ 14.61 และ 10.71 ตามลำดับ ในขณะที่ดินเหนียวพบว่าไม่สามารถแยกวัฏภาคได้อย่างชัดเจน ซึ่ง
แสดงให้เห็นว่าชนิดของดินมีอิทธิพลต่อการแยกวัฏภาคของสารลดแรงตึงผิว แต่อย่างไรก็ตามพบว่าประสิทธิภาพ
ในการกำจัดสารทีซีอีออกจากดินทั้งสามไม่ต่างกันมากนักและมีปริมาณสารทีซีอีที่เหลือในดินทั้งสามชนิด
หลังจากบำบัดประมาณ 3% ต่อมาได้ทำการเติมสารโซเดียมคลอไรค์ที่ความเข้มข้น 0.2 มิลลิโมลาร์ พบว่า
สัดส่วนการแบ่งวัฏภาคของสารลดแรงตึงผิวในดินทั้งสามชนิดมีค่าเพิ่มมากขึ้น ซึ่งแสดงให้เห็นว่าการเติมเกลือ
ในระบบสามารถเพิ่มค่าสัดส่วนการแบ่งแยกวัฏภาคของทีซีอีได้และเหลือทีซีอีอยู่ในวัฏภาคที่มีความเข้มข้นของ
สารลดแรงตึงผิวต่ำในปริมาณที่น้อย ทั้งนี้พบว่าประสิทธิภาพในการกำจัดสารทีซีอีออกจากดินทั้งสามชนิดมีค่า
ใกล้เคียงกับระบบที่ไม่มีการเติมเกลือ นอกจากนี้ได้ศึกษาปรียบเทียบการพัฒนาหัวเชื้อแบคทีเรีย Rhodococcus
sp. L4 มาใช้ในการบำบัดทางชีวภาพ โดยวิธีตรึงเซลล์ 2 วิธีคือ การตรึงแบคทีเรียบนเมล็ดขี้หร่าและการหุ้ม
แบคทีเรียที่ตรึงบนเมล็ดขี้หร่าด้วยสารเจลแลนกัม เพื่อเพิ่มประสิทธิภาพของแบคทีเรียและป้องกันแบคทีเรียจาก
สภาวะแวดล้อม โดยพบว่า การหุ้มแบคทีเรีย Rhodococcus sp. L4 ด้วยสารเจลแลนกัมมีประสิทธิภาพในการย่อย
สารทีซีอีที่ความเข้มข้นเริ่มต้น 10 ส่วนในล้านส่วนมีค่าเท่ากับ 60 % ซึ่งมีค่ามากกว่าแบคทีเรียที่ถูกตรึงบนเมล็ด
ขี้หร่าเพียงอย่างเดียว นอกจากนี้ยังพบว่าแบคทีเรียที่หุ้มด้วยสารเจลแลนกัมมีความสามารถในการย่อยสลายสารที
ซีอีที่ความเข้มข้นสูงและสามารถทนต่อระบบที่มีสารลดแรงตึงและสภาวะที่มีเกลืออยู่ได้ หลังจากนั้นได้นำ
เทคนิคร่วมของการสกัดแบบขุ่นโดยสารลดแรงตึงผิว DEHYDOL LS7 TH ที่มีการเติมเกลือและการบำบัดทาง
ชีวภาพด้วยแบคทีเรีย Rhodococcus sp. L4 มาใช้ร่วมกันในการบำบัดสารทีซีอีจากดินทั้งสามชนิดที่ความเข้มข้น
เริ่มต้น 1000 ส่วนในล้านส่วน โดยหลังจากการบำบัดด้วยวิธีการสกัดแบบขุ่น สารทีซีอีที่หลงเหลืออยู่ในดินและ
ในวัฏภาคที่มีความเข้มข้นของสารลดแรงตึงผิวต่ำจะถูกกำจัดด้วยการเติมเซลล์อิสระของ Rhodococcus sp. L4
และ Rhodococcus sp. L4 ที่ถูกหุ้มด้วยเจลแลนกัมตามลำดับ โดยการบำบัดด้วยเทคนิคร่วมนี้พบว่าปริมาณของสาร
ทีซีอีลดลงอย่างมีนัยสำคัญและเหลืออยู่ในดินประมาณ 18-19 มิลลิกรัมต่อกิโลกรัมในดินทั้งสามชนิด สำหรับ
ในวัฏภาคที่มีความเข้มข้นของสารลดแรงตึงผิวต่ำพบว่าเหลืออยู่ 6-10 มิลลิกรัมต่อลิตร ซึ่งแสดงให้เห็นว่าเทคนิค
ร่วมทั้งสองนี้สามารถนำไปประยุกต์ใช้ในการกำจัดสารทีซีอีออกดินชนิดต่างๆได้อย่างมีประสิทธิภาพ

สาขาวิชา การจัดการสิ่งแวดล้อม    ลายมือชื่อนิสิต.........................

ปีการศึกษา 2551    ลายมือชื่ออาจารย์วิทยานิพนธ์หลัก...................

# # 5087542620 : MAJOR ENVIRONMENTAL MANAGEMENT
KEYWORDS:/ TRICHLOROETHYLENE/ CLOUD POINT EXTRACTION/ BIOREMEDIATION/ IMMOBILIZATION

WITCHAYA KAEWTIP : (COMBINED CLOUD POINT EXTRACTION AND BIOREMEDIATION FOR CLEAN-UP TRICHLOROETHYLENE IN VARIOUS SOIL TYPES). THESIS ADVISOR: ASST.PROF EKAWAN LUEPROMCHAI, Ph.D., 118 pp.

The contamination of trichloroethylene (TCE) in soil and groundwater has become an important environmental problem because of their toxicity and persistence. In this study, a combination of cloud point extraction and bioremediation was applied to enhance TCE removal efficiency from three soil types including sandy clay loam, sandy loam and clay soil. Dehydol LS7 TH, a nonionic surfactant synthesized in Thailand, was used in cloud point extraction technique for removing high TCE concentration out of the soil. Using an initial concentration of 90 mM Dehydol LS7 TH, the surfactant partition ratio on sandy clay loam and sandy loam were 14.61 and 10.71, respectively. Meanwhile, this surfactant could not separate well in clay soil. It was indicated that soil types can affect the phase separation. However the different in TCE removal efficiency from each soil types was minor and about 3% of the initial TCE was remained in those soil samples. The addition of 0.2 M sodium chloride (NaCl) was found to enhance the phase separation and increase the surfactant partition ratio of Dehydol LS7 TH in all three types of soil. The addition of NaCl also increased TCE partition ratio, in which only small amount of TCE was remained in the surfactant dilute phase. TCE removal efficiencies of this condition in all soil types were similar to the cloud point extraction without adding NaCl. The inoculum of Rhodococcus sp. L4 was later developed for bioremediation by immobilization technique i.e. attachment of cells on cumin seeds and encapsulation of cumin seed-attached cells in gellan gum beads. The immobilization was to enhance TCE removal and protect the cells from environmental stress. The result showed that cumin seed-Rhodococcus sp. L4 encapsulated in gellan gum was capable of degrading 60% of 10 ppm TCE. This encapsulated cell had higher TCE removal efficiency than that of cumin seed-attached cells alone and was more tolerant to high TCE concentration in the presence of surfactant and NaCl. The combination of cloud point extraction process using Dehydol LS7 TH in presence of NaCl and bioaugmentation with Rhodococcus sp. L4 inoculums were later performed to clean up 1,000 ppm TCE in those soil samples. After cloud point extraction, the residual TCE in soil was degraded by free cells of Rhodococcus sp. L4 and the residual TCE in surfactant dilute-phase was degraded by cumin seed-Rhodococcus sp. L4 encapsulated in gellan gum. The amounts of TCE after the combined treatment were decreased significantly to only 18-19 mg/kg in all three soil types and to 6-10 mg/L in the dilute phase surfactant. The results suggested that the combination of these techniques could be effectively applied for TCE removal from various soil types.

Field of Study   Environmental Management   Student's Signature...Witchaya Kaewtip
Academic Year 2008   Advisor's Signature...Ekawan...

# ACKNOWLEDGEMENTS

# CONTENTS

page

# LIST OF TABLES

Table

# LIST OF FIGURES

Figure <span style="float:right">page</span>

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

## LIST OF ABBREVATIONS

| | |
|---|---|
| ATSDR | Agency for Toxic Substances and Disease Registry |
| CMCs | Critical Micelle Concentrations |
| DEHYDOL LS7 TH | Fatty Alcohol C12-14, (7) Ethoxylates |
| DNAPL | Dense Non-Aqueous Phase Liquid |
| GC | Gas chromatograph |
| Kg | Kilogram |
| MSM | Mineral Salt Medium |
| mL | Millilitre |
| mM | Millimolar |
| O.D. | Optical Density |
| ppm | Part per Million |
| SURFONIC TDA | Branched Alcohol Ethoxylates (Isotridecyl) |
| TCE | Trichloroethylene |
| USEPA | United States Environmental Protection Agency |
| UV | Ultraviolet |
| VOCs | Volatile Organic Compounds |

# CHAPTER I

# INTRODUCTION

## 1.1 Statement of Problem

Trichloroethylene (TCE) is a nonflammable, colorless liquid with a somewhat sweet odor and a sweet, burning taste. In the past, TCE was used as a dry cleaning agent and for food extractions such as removal of caffeine from coffee. It was also used as analgesic and anesthetic agents, but it is now recognized as a potential human carcinogen (U.S. EPA, 1985). Currently, it is widely used as a solvent in the industrial degreasing of metals, with secondary solvent uses in adhesive paint and polyvinyl chloride production paint removers, typewriter correction fluids, and spot removers. In Thailand, Sukhapan (2007) reported the consumption of TCE in 2001- 2005 is about 5,797-7,841 ton/year. TCE is also used as a substitute for chlorofluorocarbon (CFC), a banned chemical related to greenhouse effect (TEI, 1998). Trichloroethylene is not thought to occur naturally in the environment. However, it has been found in groundwater sources and many surface waters as a result of improper handling, storage, manufacture, use, and disposal of the chemical (ASTDR, 2003). TCE is a typical pollutant of soil and groundwater, due to their low aqueous solubility and high affinity to soil (Li and Chen, 2008). For this reason, the concentration of TCE is strictly regulated since many people use groundwater for drinking.

In the past, several methods have been used to remove TCE from soil and groundwater such as soil vapor pressure (SVP), air stripping, and soil venting. However, the removal efficiencies in those methods are decrease with low concentrations of TCE and the methods are expensive for *in situ* treatment (Imamura *et al*., 1997). To solve these problems, surfactant have been considered as the alternative technologies for enhancing contaminant removal from media because these technologies use environmentally friendly surfactants as the separating agent

and have low energy requirement (Kimchuvanit *et al*., 2000). Surfactants can be used to vastly increase the solubility of the NAPL constituents in water and also lower the interfacial tension at the water–NAPL interface which, if sufficiently low, will result in mobilizing the NAPL (Fountain *et al.,* 1991).

Although, surfactant solutions may help in the washing of hydrophobic organic compounds from soils, many surfactants are not suitable for soil remediation due to their potential toxicity (Cort *et al*., 2002). Accordingly, cloud point extraction technique using non-ionic surfactant has been suggested for the remediation of contaminant soil and groundwater because it leave only small amount of surfactant in the environment (West and Harwell, 1992). Cloud point extraction (CPE) process is mainly based on the clouding phenomena of surfactants. When non-ionic surfactant is heated above a certain temperature known as the cloud point, the solution will separate into two coexisting phases. One is the surfactant-rich phase (coacervate phase), which contains most of surfactant molecules, whereas the other is the dilute phase, in which surfactant concentration is low and close to its critical micelle concentration (CMC). In general, the volume of surfactant rich phase is smaller than that surfactant dilute phase. Therefore, cloud point extraction process can significantly increase the concentration of pollutant by reducing the phase volume of the contaminant-containing solution (Li and Chen, 2008). The application of surfactant can also improve the microbial remediation of several pollutants in soil (Tsomides et al., 1995; Guha and Jaffe, 1996).

Bioremediation for TCE-contaminated soil is an attractive approach for in-situ treatment and many researches have been demonstrated that bacteria can degrade TCE either in soil and aqueous phases (Imamura *et al*, 1997). Since, TCE can be degraded by bacteria via aerobic co metabolism; bacteria required some substrate (primary substrate) to induce enzyme production. In 2008, Suttinun studied the ability of volatile essential oils to induce TCE degradation in 2 *Rhodococcus* strains. The result showed that cumene provided the highest effective for enzyme induction for TCE degradation. The use of immobilized microorganisms rather than free cells in biotransformation is advantageous as the process enhances the stability of the

biocatalyst and facilitates its recovery and reuse. Ushiyama *et al*. (1994) reported that immobilized bacteria have higher TCE degrading efficiency in wastewater than free cells. In 2009, Suttinun *et al.* indicated that cumin seeds containing cumene and cumin aldehyde could be used for *Rhodococcus* sp. L4 immobilization. The immobilized cells were able to degrade high TCE concentration and could be reused for TCE biodegradation after reactivation in mineral salt medium. Consequently, the *Rhodococcus* sp. L4 immobilization is one approach to develop an inoculum for increase TCE removal efficiency.

The previous study by Chuahom (2006) have shown that a combination of cloud point extraction technology and biodegradation can effectively remediate TCE contaminated soil. However, the properties of soil such as pH, soil types, cation exchange capacity (CEC) can affect the efficiencies of surfactant-enhanced remediation (Mulligan, 2001). Therefore, this study aims to apply the technique of combined cloud point extraction and bioremediation for remediate TCE in several types of soil including sandy clay loam, clay and sandy loam soil. The research optimized the conditions of cloud point extraction using Dehydol LS7 TH, a Thai produced surfactant, and developed immobilized inoculums for enhance the efficiency of TCE removal from various soil types.

## 1.2 **Objectives**

The main objective of this study was to optimize the combined cloud point extraction and bioremediation for clean-up trichloroethylene (TCE) in various soil types. The specific objectives were as follows:

1. To optimize cloud point extraction process to remove TCE from various soil types.

2. To determine an effective immobilization approach for producing *Rhodococcus* sp. L4 inoculum to use in TCE biodegradation.

3. To investigate the TCE removal efficiency of the combined cloud point extraction and bioremediation when applied to various soil types.

**1.3    Hypothesis:**

The optimal conditions of cloud point extraction using Dehydol LS7 TH and the encapsulation of *Rhodococcus* sp. L4 can enhance TCE removal from various contaminated soil.

**1.4    Scope of work**

The research was divided into three phases as follows:

Phase 1: Optimization of cloud point extraction.

Dehydol LS7 TH, a locally produced nonionic surfactant was utilized during cloud point extraction. Three types of soil with different soil texture including sandy clay loam, clay, and sandy loam were used as model soil sample since these soil types are often found in Thailand. The samples were collected from various uncontaminated-sites in Phatthalung, Karnchanaburee, and Suratthanee Provinces, respectively. TCE-contaminated soil was prepared from each type of soil. The surfactant solution with various concentrations was induced to cloud point extraction in aqueous solution by raising temperature above the cloud point. An optimal surfactant concentration that provides the highest the surfactant partitioning ratio in aqueous system was selected for future study. After that, sodium chloride (NaCl) was utilized as the electrolyte in order to enhance phase separation of Dehydol LS7 TH and TCE removal from the various contaminated soil types. Many studied have demonstrated that the addition of simple electrolytes can change the cloud point if the electrolyte concentration is greater than 0.1 M, but there is no significant effect at electrolyte concentrations less than 0.01 M. (Kimuvanit, 2000). Most simple anions such as sulfate, chloride, and carbonate typically depress the cloud point of nonionic surfactants because of their salting-out effect, with the effect of a given salt depending upon the hydrated radii of the ions ( Rosen, 2004).

Phase 2:  Immobilization of *Rhodococcus* sp. L4

Two types of immobilization methodology were used i.e. attachment and encapsulation. For attachment, cumin seeds were used as supporting material for immobilizing of *Rhodococcus* sp. L4. To further protect the cells, gellan gum gel was used for encapsulating cumin seed immobilized-*Rhodococcus* sp. L4. The ability of two immobilized bacteria to survive in the high surfactant concentration were studied and the efficiency of TCE degradation by those two immobilized bacteria was compared with that of killed-cell encapsulated in gellan gum and gellan gum bead. The immobilized inoculums that provided more bacteria survival in high surfactant concentration and more TCE degradation efficiency was selected to further study.

Phase 3: Combined process of cloud point extraction and biodegradation

The combination of the optimal cloud point extraction process using Dehydol LS7 TH and the selected immobilized *Rhodococcus* sp. L4 were performed to clean up TCE from various soil types. The TCE removal efficiency was determined from a decline of TCE concentrations over time.

# CHAPTER II

# BACKGROUND AND LITERATURE REVIEW

## 2.1    Trichloroethylene
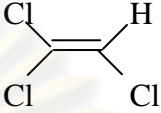
### 2.1.1 Introduction

Trichloroethylene (TCE), a dense non-aqueous phase liquid (DNAPL), is one of the common pollutants in groundwater, and could be a significant component of hazardous streams. TCE is a nonflammable, colorless liquid with a somewhat sweet odor and a sweet, burning taste. In the past, TCE was used as a dry cleaning agent and for food extractions such as removal of caffeine from coffee. It also had limited use as an analgesic and an anesthetic agent, but is no longer used for these purposes because it is now recognized as a potential human carcinogen (U.S. EPA, 1985). Currently, it is widely used as a solvent in the industrial degreasing of metals, with secondary solvent uses in adhesive paint and polyvinyl chloride production paint removers, typewriter correction fluids, and spot removers. Trichloroethylene is not thought to occur naturally in the environment. However, it has been found in groundwater sources and many surface waters as a result of the manufacture, use, and disposal of the chemical (ASTDR, 2003).

### 2.1.2 Properties

Trichloroethylene (TCE) is a man-made chlorinated solvent and also known by the names of Triclene, Vitran, Chlrinlen trichloroethene, ethylene trichloride, Trilene, Trichloran, Trichloren, Algylen, Trimar, Triline, Tri, Trethylene, Westrosol, Chlorylen, Gemalgene, and Germalgene among several others (ATSDR, 1997). The physico-chemical properties of TCE are presented in Table 2.1.

**Table 2.1** Physical and chemical properties of TCE

| Property | Characteristic |
|---|---|
| Structure | Cl—H / Cl—Cl |
| Chemical Formula | $C_2HCl_3$ |
| CAS number | 79-01-6 |
| Molecular weight (MW) | 134.4 |
| Color | Clear, colorless |
| Melting point | -87.1 $^o$ C |
| Boiling point | 86.7 $^o$ C |
| Density at 20$^o$ C | 1.465 g/ml |
| Odor threshold : Air | 100 ppm |
| Solubility: Water at 20$^o$C | 1.070 g/L |
|           25$^o$C | 1.366 g/L |
| Organic solvents | Miscible with many common organic solvents (such as ether, alcohol, and chloroform) |
| Partition coefficients: Log Kow | 2.42 |
|           Log Koc | 2.03-2.66 |
| Vapour pressure at 25$^o$C | 74 mm Hg |
| Henry's law constants:    at 25$^o$C | 0.011 atm-m$^3$/mol |

Source: Agency for Toxic Substances and Disease Registry (ATSDR), 2003.

### 2.1.3 TCE contaminated soil and groundwater

TCE is characterized as a DNAPL, thus it can easily invade the subsurface and difficult to remove. For these reasons, TCE tends to sink into soil surface and accumulate at the subsurface environments. TCE may be biotransformed into dichloroethylene and ultimately to a more potent carcinogen such as vinyl chlorine under anaerobic condition (Kneidel and Yang, 2003).

The contaminations of TCE in soil and groundwater have been reported in many countries for example;

The Environment Canada Agency (CEPA) reported TCE levels between 0.001 μg/L and 100 μg/L in the surface water, meanwhile the levels in Ontario waters generally below 1 μg/L. The levels in surface waters generally do not exceed 1 μg/L, unless there are direct releases into the water (1000 μg/L to almost $10^6$ μg/L) (CEPA, 1993).

In Japan, the Environment Agency of Japan reported that in 1994, from 232 cases of soil contamination, the major contaminants were organochlorine compounds such as trichloroethylene (TCE), tetrachloroethylene (PCE), and heavy metals (Environment Agency of Japan, 1995). TCE concentration in groundwater from the contaminated site ranged from 5.3 to 6.5 mg/l, while the environmental quality standards of TCE for all kinds of soil in 1994 were about 0.3 mg/l (Environment Agency of Japan, 1995).

Moreover, Kawamoto and Urashima (2006) reported that the illegal dumping cases in Japan have been increased annually from 1993 through 1998. TCE is one of the major pollutants in large-scale illegal dumping cases that found in Teshima Island, Kagawa and border between Amori and Iwate. The illegal dumping of waste can cause the serious environmental problem from the toxic substance that contaminated in dump waste. The large amount of waste (600,000 tones mixed with contaminant soil) from Teshima Island in Kagawa are being transported to Naoshima Island 5 km from Teshima Island for intermediated process.

In Thailand, chlorinated aliphatic compounds contaminated-soil and groundwater has been found on Lumpoon industrial estate area, northern of Thailand. Almost thousand ppm of TCE in groundwater at 2-3 meters below ground surface was reported (Malem *et al*., 2007). Therefore, TCE is a major environmental contaminant and may cause long term health effect in the future.

### 2.1.4 Health effects and related regulation status of trichloroethylene

The adverse health effect from TCE is depends on how much and how long TCE is exposed. Under poor ventilation, dizziness, headache, slowed reaction time, sleepiness, and facial numbness have been shown in person who inhale TCE vapor or use TCE-containing products. Concentrations causing these effects are higher than the allowable occupational exposure level (50 parts per million). Irritation of the eyes, nose, and throat also occur under these conditions. Due to concerning of TCE adverse effect on human health and environment, several regulation related with TCE contamination were set.

In the United State, Environmental Protection Agency (US.EPA) has set a drinking for TCE to 5 ppb.

In Thailand, according to the notification of National Environmental Board No.20, B.E. 2543 (2000), issued under the Enhancement and Convention of National Environment Quality Act. B.E. 2535 (1992), published in the Royal Government Gazette, Vol. 117 Special part 95 D, September 15, B.E. 2543 (2000), TCE level in groundwater should not exceed 5 ppb. Moreover, soil quality standard has been established and no more than 28 ppm and 61 ppm TCE should be found residential or agriculture soil and industrial soil, respectively (PCD, 2004).

The regulation of TCE in the work place also regulate by The Occupational Safety and Health Administration (OSHA). A TCE concentration of 100 ppm in the air is the occupational exposure limit for 8-hour days in a normal 40-hour workweek. OSHA also allows 15-minute exposure to be less than 300 ppm (ATSDR, 1997).

Moreover, the TCE exposure limits in many countries have been set and shown in Table 2.2.

**Table 2.2** TCE exposure limits in various countries

| Country | OEL (TWA)* | STEL** |
|---------|-----------|--------|
| Australia | 50 ppm | 200 ppm |
| Belgium | 50 ppm | 200 ppm |
| France | 75 ppm | 200 ppm |
| Germany | 50 ppm | 250 ppm |
| Japan | 50 ppm | - |
| Finland | 30 ppm | 45 ppm |
| Sweden | 10 ppm | 25 ppm |
| Thailand | 100 ppm | 200 ppm |
| Turkey | 100 ppm | - |

Source: International Program on Chemical Safety (IPCS, 2006)

*Occupational Exposure Limit (Time Weighted Average): 8 hours per day
**Short Term Exposure Limit (15 minutes)

**2.1.5 Current TCE remediation technology** (Oleszkiewicz and Elektorowicz, 1993)

Currently, there are several conventional and emerging alternative technologies to remediate TCE contaminated soil and groundwater, which have been implemented and demonstrated in contaminated site as shown below:

(a) Conventional cleanup technology

1) Pump and Treat systems

One of the most common technologies for remediating TCE in soil and groundwater is Pump and Treat (P&T) systems. Contaminated water is pumped from the ground and then, is treated using liquid treatment system above the ground. Although P&T systems can be easily installed and operated but the requirement for long term maintenance causes this technology to lose favor when compared to other alternatives.

2) Air sparging

Air sparging has more advantage over P&T systems in terms of without need to pump ground water to the surface for treatment. Compressed air would be injected into aquifer to increase oxygen levels available in the contaminant zone to accelerate biodegradation by aerobic indigenous microorganism. However, the implementation of this technology may be limited due to site geology. Moreover, it can not capture and treat contaminated groundwater effectively due to potential for the contaminated plume to spread in size before remediation occurs.

3) Carbon adsorption

Carbon adsorption is an effective way to treat off gases containing TCE in the final stage before venting out safely to atmosphere. Its effectiveness is depends on contaminant loading which increase with use. This technology usually would be use

followed by P&T system, thus effectiveness of this technology is depends on how much contaminant loading on carbon canister which increase with time.

4) Thermal oxidation

Similar with adsorption TCE vapor by activated carbon, thermal oxidation is an effective technology as the last step in the TCE contaminated - off gasses treatment. This alternative is quite easy and fast to implement in contaminated site. But in some circumstances, a alkaline scrubber may be require to be use coupled with oxidation thermally to remove acid vapor produced during TCE thermal oxidation.

(b) Emerging Technology

1) Reactive wall

Permeable reactive barrier would be installed in subsurface near the downstream of source zone. Polluted water usually is drive through the wall and then, the contaminant would be destructed within reactive wall. This technology has limit to apply with some specific site especially site consisting of rock geology or consisting of too deep aquifer.

2) Natural attenuation

After the source of contaminant was removed, regulatory agencies may allow to monitor the naturally occurrence of attenuation, normally around one to two years period. Installation of appropriate monitoring wells, periodically sampling for volatile organic compounds must be required. Effectiveness of this remedial technology is usually depends on site-specific condition. More benefit from choosing this passive alternative would be gained if it be applied with contaminated site where other active alternatives may not significantly decrease remediation time.

## 2.2 Surfactant Technology

### 2.2.1 Surfactant

Surfactant has two parts in molecule. A hydrophobic part (tail part) is a long chain of hydrocarbon; that acts as water hating group. The other is a hydrophilic part (head part) which has a polar group; this is water liking as shown in Figure 2.1. Consequently, the surfactant can dissolve either in water or oil and have the capability to solubilize water or oil to create homogeneous system (Uppgård, 2002).

In general, surfactants are divided into four classes: amphoteric, with zwitterionic head groups; anionic, with negatively charged head groups; cationic, with positively charged head groups; and nonionic, with uncharged hydrophilic head groups. Those with anionic head groups include long-chain fatty acids, sulfosuccinates, alkyl sulfates, phosphates, and sulfonates. Cationic surfactants may be protonated long-chain amines and long-chain quaternary ammonium compounds. The class of amphoteric surfactants is represented by betaines and certain lecithins, while nonionic surfactants include polyethylene oxide, alcohols, and other polar groups (Rosen, 2004).

Hydrophilic
head group

Hydrophobic tail
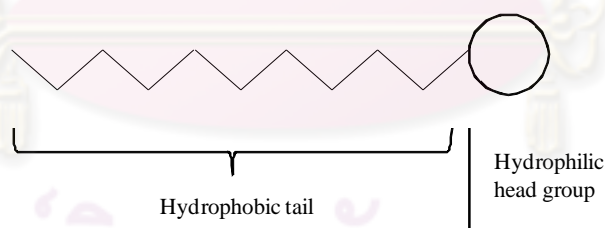
**Figure 2.1** Basic structure of a surfactant (Rosen, 2004)

Surfactants can play an important role in separation science. The unique tendency of surfactants to adsorb at interfaces and to form micelles in solution lead to separation ability called surfactant based separation technologies. The examples of surfactant-based separation techniques include cloud point extraction, surfactant-enhanced ultrafiltration, froth flotation, and foam fractionation. Moreover, these

techniques are utilized for many environmental applications such as in-situ or ex-situ remediation of contaminated soil, wastewater and groundwater clean-up, removal of ink to permit recycling of paper and plastic (Scamehorn and Harwell, 2000).

### 2.2.2 Principle of cloud point extraction (CPE)

Cloud point extraction (CPE) is based on the phase separation properties of aqueous nonionic surfactant solutions (Katsaounos *et al*., 2002). Cloud point extraction (CPE) process is mainly based on the clouding phenomena of surfactants, especially that of nonionic surfactants (Schott, 1997; Rosen, 2004). As the temperature of an aqueous nonionic surfactant solution is increased or some additives are added, the solution turns cloudy and phase separation occurs (Wang *et al.*, 2003). The solution may separate into two coexisting phases (Fig. 2.2). One is the surfactant-rich phase (coacervate phase), which contains most of surfactant molecules, whereas the other is the dilute phase, in which surfactant concentration is low and close to its critical micelle concentration (CMC). This temperature at which the phase separation occurs is called cloud point (Rosen, 2004).
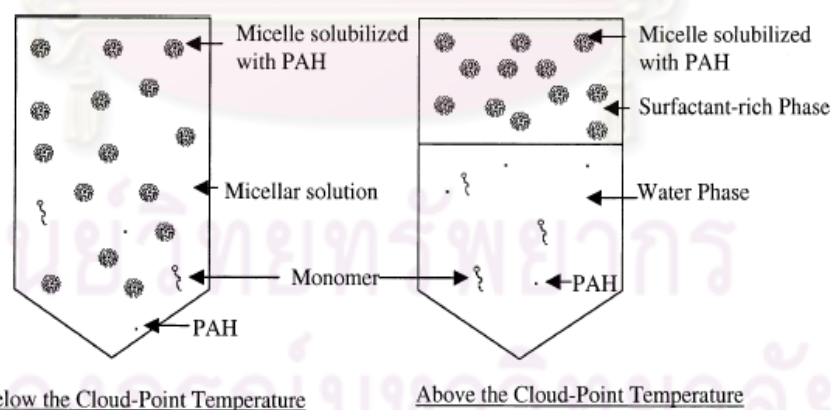


**Figure 2.2** Schematic description of phase equilibrium during CPE

(Li and Chen, 2003).

Any organic solutes in the water tend to solubilize in the micelles and concentrate in that coacervate phase (Kimchuwanit *et al.*, 2000). In general, the volume of surfactant-rich phase is smaller than that coexisting water phase. Therefore, the CPE process can significantly increase the concentration of extracts by reducing the phase volume of the contaminant-containing solution (Li and Chen, 2008). Consequently, the application of cloud point extraction can reduce the volume of washing solution generated in soil remediation process.

Moreover, the effect of salt on increasing the solubility of organic compound in water is often referred to as salting-in and the opposite behavior found on decreasing solubility is called salting-out.

a) Salting-out

Often the nonionic surfactant will still have a certain affinity to water. In order to decrease that affinity, an ionic salt like a NaCl solution is added to the water layer (Rosen, 2004). This will increase the ionic strength of the water layer. The increase in the ionic strength of the water layer will drive the non-polar hydrophobic species into the organic layer away from the ionic water layer. The ionic from added salt solution will attract the water molecules in an effort to the solvent ions. This releases the wter molecules from any salvation with the ether oxygen atoms. This results in a degrease of head size of he surfactant and subsequently in change in micellar shap. When the monomeric form of surfactant is salted out by the presence of an electrolyte, micellization is favoured and the cloud point is decreased.

b) Salting-in

At very low ionic strength, a phenomenon known as "salting-in" occurs. When some species are added into the surfactant solution and accompanied with the reduction in an ionic strength of the solution due to the interactions between hydrophilic part of the surfactant and added species, this result an increase of head size of the surfactant and the disordering of the water molecules in the solution. Thus, micellization are not favorable, consequently, the cloud point is increased. Hence, if the monomeric surfactant is salting-in, cloud point of the solution is risen up.

### 2.2.3 Application of cloud point extraction in the environment

Several types of non ionic surfactant have been applied to clean-up pollutants in the environment such as contaminated water and soil by cloud point extraction (Komaromy-Hiller and von Wandruszka, 1994; Kimchuwanit *et al.*, 2000; Chuahom, 2006).

Kimchuwanit *et al.* (2000) studied the extraction of TCE from water using cloud point extraction. Octylphenoxypoly(ethyleneoxy)ethanol was used as a nonionic surfactant. The results showed that 91% of TCE was extracted into the surfactant-rich phase in one stage. TCE concentration in surfactant-rich phase can be over two orders of magnitude greater than in the surfactant-dilute phase. Increasing temperature, surfactant concentration, and adding of NaCl can improve the fraction of TCE extracted.

Trakultamupatam *et al.* (2002) applied CPE for removal of benzene, toluene, and ethylbenzene from wastewater. T-octylphenolpolyethoxylate was used as a nonionic surfactant for separating agent. The results were reported that the contaminants tend to solubilize into the micelles and concentrate in the coacervate phase. The concentration of the solutes in the coacervate phase increases as temperature, added electrolyte concentration, and degree of alkylation of the aromatic solutes increase.

For clean-up the contaminated soil, the selected surfactant should provide the coacervate phase above the dilute-phase and soil phase. Since, this phase will be easily removed out of the process.

In 1995, Komaromy-Hiller and von Wandruszka studied the decontamination of oil-polluted soil by cloud point extraction. They used Triton X-114 as a detergent for cloud point extraction and tested 2 soils that were different in organic carbon content. The experiment showed that 85-98 % of the oil present in the soil was found to enter the micellar phase of the separated liquid. When the concentration of Triton X-114 increased, the efficiencies of both the extraction into the washing liquid and oil migration from the aqueous phase to the detergent phase decreased. Moreover, they

found that the extraction efficiency decreased with increasing carbon content of the soil.

In 2007, Zhou and Zhu showed the effect of soil composition on the enhancing PAH desorption by Nonionic surfactants as Triton X-100 (TX100), Triton X-114 (TX114) and Triton X-305 (TX305). After the soil was equilibrated with PAH and surfactant, they found that the soil with relatively higher clay content reasonably adsorbed a greater amount of surfactant onto soil surface, which resulted in the lower surfactant effective concentration in aqueous phase. Moreover, this experiment showed that the surfactants were more effective in enhancing hydrophobic organic compounds (HOCs) desorption from the contaminated soil with relative lower clay content and higher organic carbon. However, it should be mentioned here that this previous study did not aim to carry out the cloud point extraction of PAH in their soil-surfactant-PAH system.

The cloud point of nonionic surfactant can be influenced by the addition of additive, such as electrolytes. These effects are respectively known as "salting-in" (the added electrolyte would reduce ionic strength of water layer and then bring nonionic species in organic layer more closer with water layer) and "salting-out" (the added electrolyte would increase ionic strength of water layer and then put nonionic species in organic layer far away from water layer). Up to the present time, there are a number of studies which focus on the effect of added electrolyte on cloud point extraction. Among those studies, Kimchuwanit *et al*. (2000) and Trakultamupatam *et al.* (2002) reported the occurrence of salting-out effect in the micellar solution of nonionic surfactants, in which the partition ratio increased from 10 to 25 at the concentrations of added NaCl between 0.0 and 0.6 M, respectively.

## 2.3 TCE bioremediation

The physical characteristics of TCE, i.e. limited water solubility and high sorption capacity is the main cause to make the common remedial action such as P&T system or reactive wall to become an inappropriate alternative. Moreover, these common remediation technologies usually have high maintenance cost. With these reasons, bioremediation could become an attractive emerging technology for in-situ remediation of TCE-contaminated soil and groundwater.

### 2.3.1   Co-metabolism of TCE

TCE can be degraded by bacteria such as phenol degraders (Ayoubi and Harker, 1998), toluene degraders (Landa *et al.,* 1994; Suttinun, 2003), ammonium oxidizing bacteria (Hyman *et al*., 1995), propane degraders and methanotrophs (Deane Little *et al*., 1988) via co-metabolism under aerobic condition.  Cometabolism describes the ability of microorganisms to transform non-growth supporting substrates, naturally in the presence of a growth supporting substrate (Arp *et al*., 2001). For example, in the process of degrading methane, some bacteria can degrade hazardous chlorinated solvents that they would otherwise be unable to attack. 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.

The toluene-degrading enzyme shown in Figure 2.3 was an example of TCE co-metabolism. 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).
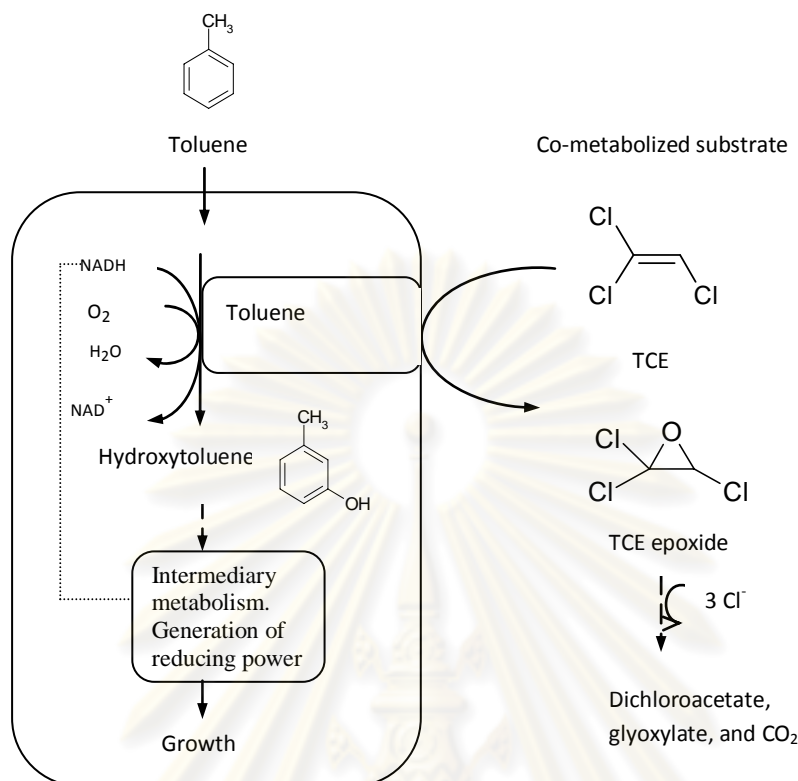
**Figure 2.3** Example of TCE co-metabolic pathway by toluene monooxygenase enzyme. The microorganism utilizes toluene as primary substrate while oxidizes TCE to an epoxide and later to $CO_2$. (Source: www. wiley-vch.de/books/biotech/pdf/v11b_aero.pdf)

Several researches using cell suspensions showed that high concentrations of TCE are toxic to cells. For example, Ayoubi and Harker (1998) found that TCE degradation by *P. putida* F1 at TCE concentration of 80 μM (10 ppm) give the highest rate degrading and the rate dropped rapidly at the concentration higher than 300 μM (40 ppm). Finally, at TCE concentration 320 μM (42 ppm), the degradation by *P. putida* F1 no longer occurred. In the same experiment, the toxicity of methane-induced, *Methylosinus trichosporium* OB3b was apparent at a concentration of 70 μM TCE (9.22 ppm). The rate of TCE degradation of *P. putida* F1 and *M. trichosporium* were sustained only 20 and 60 min, respectively.

In Suttinun *et al.* (2008), they studied the ability of terpenes, the main component in volatile essential oils of plant, to induced TCE degradation in 2 bacterial strains. Selected terpenes including cumene, limonene, carvone and pinene were used for studied. The result showed that cumene provided the highest effective for enzyme induction for TCE degradation. Moreover, Suttinun *et al.* (2008) showed the TCE removal efficiency from cumin seeds (the plant that rich in cumene and cumin aldehyde) as a supporting material for immobilizing *Rhodococcus sp* L4. The immobilized-bacteria were capable of degrading higher concentrations of TCE than free cells. Therefore, the immobilization is an attractive approach to apply the bacteria for remediating sites with high concentration of TCE.

### 2.3.2   Bioremediation using immobilized bacteria

Immobilization is a general term that describes the artificial immobilization of cells to the bedding material. Up to now, there are several methods to effectively immobilize cells. Some of those methods, which were earlier reviewed by Pilkington *et al.* (1998) and Cohen (2001), was selected to present here as below ;

1)  Attachment or adsorption on solid carrier surfaces

Cell immobilization on a solid carrier is carried out by electrostatic forces or by covalent binding between the cell membrane and the carrier. Examples of solid carriers used in this type of immobilization are cellulosic materials, inorganic etc.

2) Entrapment or encapsulation within a porous matrix

This technique consists of trapping microbes within polymer matrix. This method can be an effective way to maintain high cell viability and high resistance to toxic compounds.

| Attachment or Adsorption on solid carrier surfaces | Entrapment within polymer |
|---|---|
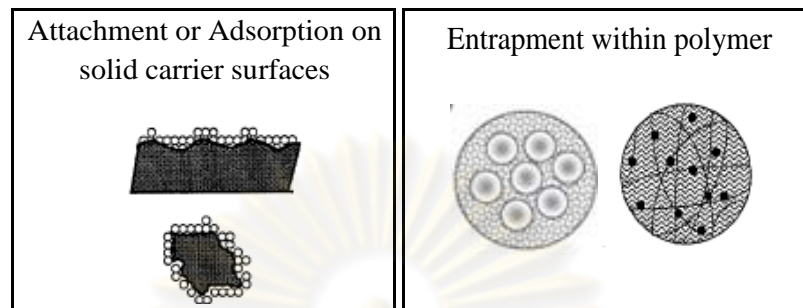
**Figure 2.4**   Basic methods for cell immobilization (Pilkington *et al*., 1998)

3) Membrane separation

Porous membranes act being barrier to separate the cells from the liquid outside. This membranes (usually is ultrafiltration membranes) will let the substrate and growth media to penetrate to the cells. One problem, which was oftenly found for this method, is membrane fouling due to clogging and covering the membrane pores with biofilm.

4) Covalent bonding and covalent crosslinking

This immobilized method is the developing of covalent bonds between reactive groups at the cell's surface and different ligands on the bedding material. Exposing to toxic reactive group of the cells is still the most concerning problem for applying this method.

This study focused on only two immobilization methods which are attachment and encapsulation (Figure 2). The encapsulation of microorganisms in gel-matrix has been used for in situ bioremediation of contaminated soil and groundwater; however it is still in the early development stage (Cassidy, 1996). Gel-encapsulated cells provide a number of advantages over free cells or other immobilization methods to remediate TCE- contaminated soil. Some of advantage and limitation of using encapsulation for contaminated soil application were shown on table 2.3.

**Table 2.3 Advantage and limitation of using encapsulation for contaminated soil application (Cassidy, 1996)**

| Advantages | Limitation |
|---|---|
| Beads are non-toxic, biodegradable, and non-polluting | Gas and solute diffusion may be restricted |
| Provides protection from biotic and abiotic environment stresses leading to increase microbial survival | Reduced oxygen consumption rates of encapsulated cells may occur |
| Slow cell release with reduced cell movement through soil from water flow-induced transport | Cell morphological or metabolic alternations may have a detrimental effect |
| Can be produced in large quantities, stored for extended periods as dried beads and used with existing mechanical application equipment | Effects of changes in water activity may limit bacteria capability for biodegradation of pollutant |

Several previous studies focused on the application of gel-entrapped or encapsulated cells for aromatic and aliphatic compound degradation. In 1994, Uchiyama *et al.* studied the TCE degradation by entrapped TCE-degrading methanotroph, *Methylocystis* sp. strain M in different matrices. Cells immobilized in Ca-alginate, z-carrageenan, and agarose showed higher or almost the same degradation activity in comparison with that of free cells, while low activity was observed in the cells immobilized in photocrosslinkable resin, polyurethane, and polyelectrolyte complex. In repeated use, only the agarose-immobilized cells were not damaged and retained about 40 % of the initial TCE degradation activity among the cells immobilized in those three agents.

Wang *et al.* (2007) investigated the degradation of PAHs and carbazole by immobilizing *Sphingomonas* sp. XLDN2-5 cells in four kinds of polymer. Gellan gum gel was selected as an optimal immobilization support material that gave the lowest carbazole remaining than other materials. In addition, when the mixture of gellan gum gel and $Fe_3O_4$ nanoparticles served as an immobilized support, the result showed that the magnetically immobilized cells presented higher carbazole degradation activity than nonmagnetically immobilized cells and free cells.

The application of gellan gum for encapsulation of viable cells requires lower concentration of both gel and gelling agent when compare to k-carrageenan, agar, and alginate (Nilsson *et al.*, 1983). Unlike some other ion-sensitive gelling polysaccharides such as alginate, the reactivity between gellan gum and ions is non-specific and gels can be formed with a wide variety of cations including alkaline and alkaline-earth cations. For a given gellan gum concentration however, divalent calcium and magnesium ions have been used at substantially lower levels than monovalent sodium and potassium ions to achieve gelation leading to strong gels (Moslemy *et al.*, 2002).

Inhibition effect from toxic substance as salt and surfactant on microbial activity is reported widely. It has been observed high saline or high chloride wastewater can inhibit aerobic bacteria activity in petroleum industrial wastewater treatment system (Lefebvre and Moletta, 2006). Anionic surfactant such as linear sodium dodecylbenzene sulfonate (LAS) can suppressed the activity of activated sludge even at very low concentration (Temmink and Klapwijk, 2004; Gutiérrez et al, 2002). However, as stated above, significant higher toxic tolerance capacity of immobilized cells compare with free cells is extensively accepted. Protective effect of immobilized cells has been demonstrated that it can help to effectively maintain the microbial activity in immobilized bioreactor even under stress condition from an existing of toxicant (Scott, 1987).

**2.4 Example and principle of combined technology for soil remediation**

As stated above, bioremediation have significantly contributed and implemented to remediate TCE contaminated soil. However, there are some limitations for direct applying bioremediation to remove TCE from contaminated soil such as low bioavailability and high toxic load for degrading microorganisms. The capabilities of nonionic surfactant to solubilize and then concentrate TCE can reduce the toxic load and thereby enhance microbial degradation. Thus, it is worth to use cloud point extraction in combination with bioremediation. This study proposed a sequential treatment, which start with surfactant flushing (solubilize and desorb TCE out of contaminated soil), next, TCE extraction (concentrate and separate the contaminated streams which is either TCE rich or TCE dilute streams) and last step, bioremediation (degrade TCE in the dilute stream).

The proposed three-stage combined treatment or sequential treatment would effectively enhance TCE removal by biological process. In economic point of view, the separate treatment of a concentrated TCE stream (high contaminant load but low hydraulic load) and a dilute TCE stream (low contaminant load but high hydraulic load) would be less expensive than the treatment of large volume of surfactant washed solution. Moreover, the three-stage combined treatment could possibly apply with any organic pollutant with high hydrophobicity. For our knowledge, there are limited studies dedicated to the combination between organic compound extraction step (cloud point extraction), surfactant flushing step and/or bioremediation. Some of those studies were reviewed and shown below:

The combination of micellar solubilization and cloud point extraction techniques was demonstrated to recover phenanthrene from spiked sand by Li and Chen (2008). Nonionic surfactant, Tergitol 15-S-7 was used to decontaminate phenanthrene from spiked sand samples. It was observed that the presence of surfactant decreased the mass-transfer coefficient of phenanthrene from sand surface to surfactant solutions. Cloud-point extraction can concentrate the phenanthrene solubilized in the washing solutions and thereby minimize the amount of wastewater.

The extraction was carried out, subsequently, at room temperature by adding sodium sulfate to suppress the cloud-point low enough to induce phase-separation of the surfactant-rich phase with a minimal phase volume from the coexisting water phase. Recoveries higher than 93% were achieved in the combined process of micellar solubilization and cloud-point extraction on ultimate removal of immobilized phenanthrene sorbed on sands. The results showed that this combined process is efficient in recovering phenanthrene sorbed and immobilized on sands from contaminated sites, and produces only minimal amount of wastewater, i.e. less than 3% of its original volume.

Chuahom (2006) studied the removal of TCE-contaminated soil by a sequential surfactant-based separation and bioremediation. In the experiment, SURFONIC TDA6, a non-ionic surfactant that provided the lowest growth-inhibition of *Rhodococcus* sp. L4, was used for cloud point extraction process to remove TCE from soil. The study used sandy clay loam soil collected from Chiang Mai province, Thailand. The optimal conditions of SURFONIC TDA6 for cloud point extraction on this soil were determined to provide a good efficiency for TCE extraction. The initial concentration, the contact time between surfactant and soil, and the equilibrium time were 70 mM, 1 hr, and 72 hr, respectively. For the biodegradation process, free cells of *Rhodococcus* sp. L4 was used to degrade TCE in soil. The result showed the efficiencies of TCE removal by cloud point extraction process were about 74 and 59 % for initial TCE concentrations of 100 and 300 ppm, respectively. For only biodegradation process, the result showed TCE degraded by *Rhodococcus* sp. L4 was about 74 and 57% for the same concentrations of TCE. The efficiency for TCE removal was enhanced when combined the bioremediation after cloud point extraction process. About 94% for the initial TCE concentrations of 100 and 300 ppm was removed.

However, the composition of soil such as soil pH, soil types, cation exchange capacity (CEC) can affect the efficiencies for surfactant-enhanced remediation (Mulligan, 2001). Consequently, the application of combined techniques should be optimized to achieve the high TCE removal efficiency from different soil types. Therefore, this study aimed to improve the technique of combined cloud point extraction and bioaugmentation for remediate TCE in various soil samples. The research optimized the conditions of cloud point extraction and developed the immobilized inoculums to facilitate TCE removal from various soil types.
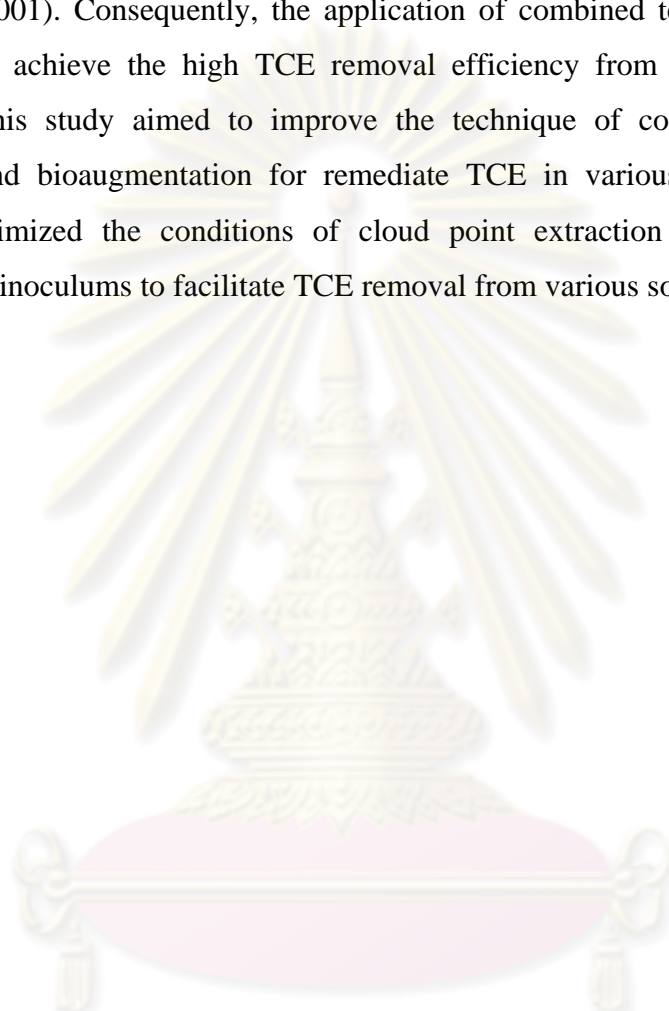
# CHAPTER III

# METHODOLOGY

## 3.1 Research overview

The research was divided into three phases including the optimal condition cloud point extraction process for TCE removal on three types of soil, the determination an effective immobilization approach for producing *Rhodococcus* sp. L4 inoculums to use in TCE biodegradation, and the investigation of TCE removal efficiency in the combined cloud point extraction and bioremediation when applied to various soil types. The results from the first and second phases were applied in the last phase. Flowchart of the research was illustrated in Figure 3.1. In the preliminary study, SURFONIC TDA 6 was used to confirm Chuahom (2006) results and determine the effect of soil types on cloud point extraction. Due to the limited supply of SURFONIC TDA 6, this study used Dehydol LS7 TH that is manufactured in Thailand for cloud point extraction.

**Preliminary study using SURFONIC TDA6**

- Effect of initial TCE concentration on remediation sandy clay loam

- Effect of soil types on CPE extraction

Optimal cloud point extraction for TCE removal

1. Phase separation of DEHYDOL LS7 TH in aqueous system

2. Effect of CPE on TCE removal

3. Effect of electrolyte addition on TCE removal

Effectiveness of immobilized inoculums for TCE biodegradation

1. Develop *Rhodococcus* sp. L4-immobilized on supporting material
2. Effect of surfactant dilute-phase on bacteria remaining
3. TCE degradation by immobilized inoculums bacteria
4. Effect of increased TCE concentration on TCE degradation

Optimal condition of cloud point extraction

A selected immobilized bacteria

TCE removal efficiency of the combined cloud point extraction and bioremediation on various soil types

**Figure 3.1** Flow chart of the research.

**3.2 Materials**

**3.2.1 Soil samples**

Three soil samples including sandy clay loam, clay, and sandy loam soil were collected from uncontaminated areas in Phatthalung, Karnchanaburee, and Suratthanee Province, Thailand, respectively. The soil were air-dried and sieved by passaging 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 3.1. The TCE contaminated soils were prepared by adding 1.91 µL TCE solution into 22 mL headspace vials that containing 2.8 g soil sample to get the initial TCE concentration of 1000 ppm. The spiked soil was left overnight to provide the homogenous distribution of TCE.

**Table 3.1** Properties of soil samples

| Source | Soil texture | % Sand | % Silt | % Clay | % Organic Matter | CEC (cmol/kg) |
|---|---|---|---|---|---|---|
| Phatthalung | Sandy Clay Loam | 51.8 | 15.8 | 32.4 | 1.06 | 5.2 |
| Karnchanaburee | Clay | 39.8 | 19.6 | 40.6 | 3.47 | 14.7 |
| Suratthanee | Sandy Loam | 70.0 | 19.2 | 10.8 | 1.09 | 3.3 |

Source: Development of Soil and Water Analysis Subgroup, Agricultural Chemistry Research Group, Department of Agricultural.

### 3.2.2 Surfactant

DEHYDOL    LS7 TH was used as a nonionic surfactant to induce a phase separation, in which the coacervate phase is presented on top of the aqueous solution. This surfactant was kindly provided by Thai Ethoxylate Ltd, Rayong, Thailand. The physical and chemical properties of DEHYDOL LS7 TH were shown in Table 3.2

**Table 3.2** Physical and chemical properties of DEHYDOL LS7 TH

| Property | Characteristic |
|---|---|
| Surfactant class | Fatty Alcohol C12-14, (7) Ethoxylates |
| Odor | Specific |
| Color | White to pale yellow |
| Density (g/cm$^3$) at 70$^o$ C | 0.949 |
| pH (1% Aq) | 6.0-7.0 |
| Cloud point(1% Aq) | 52$^o$C-58$^o$C |
| HLB | 12.1 |
| Solubility at 20$^o$C | Soluble, may forms a gel at mid ranges |
| CMC at 25°C | 0.20 mM |
| Biodegradation | Readily and rapidly degradable |

**Source:** Thai Ethoxylate Ltd., Rayong, Thailand.

### 3.2.3   Bacteria

*Rhodococcus* sp. L4 was used as inoculum for TCE biodegradation. It was isolated earlier by Ekawan Luepromchai from petroleum contaminated soil collected in Bangkok using enrichment culture technique. The bacterium is maintained by providing toluene as the sole carbon source. The bacterium was deposited at the Microbiological Resources Center, Thailand Institute of Scientific and Technological Research (TISTR). The partial 16S rRNA gene sequences of *Rhodococcus* sp. L4 was reported by GenBank as EF527237.

The culture medium was a mineral salt medium (MSM) with details in Appendix A. The method was described by Focht (1994) where all chemicals were in analytical reagent grade and obtained from Merck.

### 3.2.4   Chemicals

TCE with 99.5% purity were purchased from Fluka Chemical Industrial. Toluene with 99.5% purity was purchased from Merck Ltd. NaCl and $CaCl_2$ at the analytical grade was purchased from Ajax FineChem. Gerlite gellan gum used as material for encapsulation was purchased from Sigma-Aldrich. Seeds of cumin were obtained in one batch from its distributor (Nguan Soon, Bangkok.).

**3.3 Procedure**

    **3.3.1 Optimal cloud point extraction for TCE removal**

    **1) Initial surfactant concentration of DEHYDOL LS7 TH for cloud point extraction in aqueous phase solution**

        To obtain the highest surfactant partition ratio for cloud point extraction process, the initial concentrations of DEHYDOL LS7 TH were varied at 30, 50, 70, 90, and 110 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 1 hr. Then, 20 mL of surfactant solution was added to 22 mL screw cap vial and was heated in water bath at the temperature 60$^{\text{o}}$C. In this study, 60°C was used because it was over the cloud point temperature of this surfactant. After phase separation, the surfactant concentration in surfactant rich-phase that present on the top and surfactant dilute-phases was investigated by Iodine-Iodide method (Baleux, 1972). The optimal initial surfactant concentration in aqueous solution was defined as a concentration that provided the highest surfactant partition ratio. The surfactant partition ratio defined as the ratio of surfactant concentration in rich-phase to that of the surfactant concentration in dilute-phase.

    **2) Effect of cloud point extraction on TCE removal from various contaminated soil types**

        The contaminated soils were prepared as in section 3.2.1 to get the final initial TCE concentration of 1000 ppm. The initial concentration of DEHYDOL LS7 TH from previous study was added into 22 mL head space vial until full in vial to avoid head space. During cloud point extraction, the samples were stirred for 2 hr to get the equilibrium contact between soil and surfactant. The condition was applied from Chuahom (2006). Then, the sample was centrifuged at 2000 rpm for 10 min to enhance the phase separation between soil and surfactant solution. The sample was

later placed in water bath at 60°C for 96 hr to obtain equilibrium phase separation (Chuahom, 2006). After the equilibrium time, 100 µL of surfactant rich-phase solution was sampled for TCE analysis. Then, the entire surfactant-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, 2 mL of the dilute phase solution was sampled for TCE analysis. The remaining TCE in soils was also analyzed. TCE concentrations in all phases were analyzed by head space gas chromatograph with FID detector. The concentration of TCE was determined using external calibration curve prepared for each phase.

**3) Effects of electrolyte addition on cloud point extraction for TCE removal in various soil types**

To improve the cloud point extraction for TCE removal, the addition of NaCl as an electrolyte was used in this study. NaCl concentrations in the surfactant solution were varied at 0.2, 0.4 and 0.6 M as in Trakultamupatam *et al*. (2002). The surfactant solution was prepared by mixing a stock of 1 M NaCl with 110 mM DEHYDOL LS7 TH in a 250 mL volumetric flask to achieve the surfactant concentration of 90 mM and NaCl at various concentrations. Then, the same cloud point extraction procedure was applied as previously described in section 3.3.1(2). Triplicate tests were carried out for this experiment. At the end of this study, the lowest NaCl concentration that provided the increasing of phase separation of surfactant and TCE removal efficiency was selected to further study.

**3.3.2 Effectiveness of immobilized inoculums for TCE biodegradation**

**1) Inoculum preparation**

- Free cells

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. 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.

- Immobilized cells

• Cell attachment: the immobilization method was performed as described by Suttinun (2008). Briefly, 2 g of sterilized cumin seeds (size 500 μm - 1 mm) were mixed with 100 ml cell suspensions of *Rhodococcus* sp L4 before incubated at 130 rpm and room temperature for 4 days. Subsequently, the immobilized cultures were washed with mineral salt (MS) medium and filtrated through sterilized filter paper for removing unattached cells. The attached bacteria on cumin seeds were air dried in a sterile hood before used as inoculum.

• Cell Encapsulation: The method was adapted from Wang *et al.* (2007). The dry gellan gum powder 1 g was dissolved with 100 mL DI water and autoclaved at 121 °C for 15 min. After cooling, gellan gum solution and cumin seed-immobilized *Rhodococcus* sp. L4 prepared as above were mixed at a ratio of cumin seed- immobilized *Rhodococcus* sp. L4 wet weight to dry gellan gum powder 3 % [wt/wt]. Then, the beads were formed by extruding the mixture through a syringe into 0.2 M of cooling $CaCl_2$ and letting it solidify for 2 hr. Finally, the beads were filtrated

through sterile filter paper for separating the beads from solution and then placed into a sterilized flask.

### 2) Effect of dilute-phase surfactant on bacteria remaining

Three bacteria inoculums including free cells, attached cells and encapsulated cells that prepared in previous experiment were used to test the effect of the remaining surfactant in the surfactant dilute-phase on bacterial survival. The concentration of surfactant dilute-phase was prepared at the concentration of 4, 8, and 12 mM. These surfactant concentrations were the concentrations that remained in the surfactant dilute-phase after cloud point extraction.

For free cells, 2 mL of the surfactant-dilute phase solution was added into the 22 mL vial that contained 2 mL of 2.5 OD bacteria inoculums. For immobilized cells, 2 mL of the surfactant-dilute phase solution was added into the 22 mL vial that contained either 0.02 g attached cells (Sutthinun *et al.,* 2008) or 0.3 g encapsulated cells (the amount that used in biodegradation test). The study was done in triplicate. The initial amounts of bacteria in each inoculum were determined at time zero after added surfactant solution. After incubation on orbital shaker at 200 rpm, room temperature for 96 hr the amounts of bacteria on cumin seeds immobilization and bacteria in cell encapsulated were determined by dilution plate count method on toluene-MS agar. The efficiency of bacteria remaining was determined from the remaining bacteria in each inoculums at 96 hr compared with amount of bacteria at the time zero.

### 3) TCE removal by immobilized *Rhodococcus* sp. L4 in the presence of surfactant

The immobilized inoculums, either 0.02 g attached cells or 0.3 g encapsulated cells, were placed into 22 ml headspace vials containing 2 ml of the 4 mM of surfactant solutions. TCE were added to provide the initial TCE concentration at 10 ppm. The samples were incubated at 130 rpm, room temperature. The efficiency of TCE degradation was determined from the concentration of TCE remaining by headspace HS-GC. Control treatments were bottles containing gellan gum bead

without cells and killed-immobilized cells. The killed-immobilized cells were prepared by adding 1 mL of 10 M of $H_2SO_4$ into 250 mL Erlenmeyer flask containing the cells in 100 mL MSM. The acid stopped the reaction of bacteria attached on cumin seeds.

**4) Effect of increased TCE concentrations and NaCl on TCE degradation of the encapsulated *Rhodococcus* sp. L4**

The effect of increased TCE concentration on the TCE degradation of cumin seed-*Rhodococcus* sp. L4 encapsulated in gellan gum was studied. The experiment was performed by placed 0.3 g of encapsulated cells into 22 ml headspace vials containing 2 ml of the 4 mM of surfactant solutions. TCE were added to provide the initial TCE concentration of 10, 20 30 40 or 50 ppm. The samples were incubated at 130 rpm, room temperature. The efficiency of TCE degradation was determined from the concentration of TCE remaining by headspace HS-GC after incubation time.

Moreover, the effect of salt on TCE degradation were performed as same as the above experiment. It was different in the presence of NaCl 0.2 M in the 4 mM of surfactant solution. The solution of 0.2 M NaCl with 4 mM of surfactant were preformed by mixing stock of 1 M NaCl and 10 mM of surfactant into 100 mL volumetric flask and adjusted the volume to 100 mL.

**5) Characteristic of encapsulated bacteria**

The characteristic of cumin seeds-*Rhodococcus* sp. L4 encapsulated in gellan gum were analyzed by scanning electron microscope (SEM). The sample was fixed with 1% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 for 2 hr. The specimens were rinsed 10 min in phosphate buffer for twice and rinsed 3 times in distilled water for 10 min. Then, the samples were dehydrated by a series of 30, 50, 70, and 95% ethanol and dried by critical point dryer. The samples were put on a stub then coated

with gold before SEM analysis. The analysis was performed by the Scientific and Technological Research Equipment Centre, Chulalongkorn University.

### 3.3.3 Effectiveness of the combined process of cloud point extraction and bioaugmentation of TCE on various soil types

The effectiveness of combined cloud point extraction and biodegradation were performed to completely remove TCE from sandy clay loam, clay and sandy loam soils. Firstly, the cloud point extraction process was performed to remove TCE from soil contaminated with 1000 ppm TCE. The optimal initial concentration of DEHYDOL LS7 TH with or without NaCl was added in to the vial containing soil sample. Then, the samples were stirred for 2 hr to get the equilibrium contact between soil and surfactant. Then, the sample was centrifuged at 2000 rpm for 10 min to enhance the phase separation between soil and surfactant solution. The sample was later placed in water bath at 60°C for 96 hr to obtain the equilibrium phase separation. After the equilibrium time, 100 μL of surfactant 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, 2 mL of the dilute phase solution was sampled for TCE analysis. The remaining TCE in soils was also analyzed. TCE concentrations in all phases were analyzed by head space gas chromatograph with FID detector. The concentration of TCE was determined using external standard of each phase.

Another 2 mL of the surfactant dilute-phase were transferred to another vial containing 0.3 g of encapsulated cells and capped immediately with Teflon-lined silicone septum. The remaining surfactant-dilute phase was separated out to leaving only soil in vial. Then, 2 mL of bacterial inoculums (2.5 OD) were added in to each vial. The samples in surfactant-dilute phase and soil after were incubated at 130 rpm, room temperature for 48 hr. After incubating time, the remaining TCE concentration was determined by Head space-GC. The effectiveness of TCE remediation was determined from the amount of TCE remaining in aqueous phase after incubating time by compared with TCE remaining after cloud point extraction. The control of this

treatment was a treatment without bacterial inoculums. The effectiveness of TCE remediation in soil was determined from the amount of TCE remaining in soil after incubating time compared with initial TCE 1000 ppm.
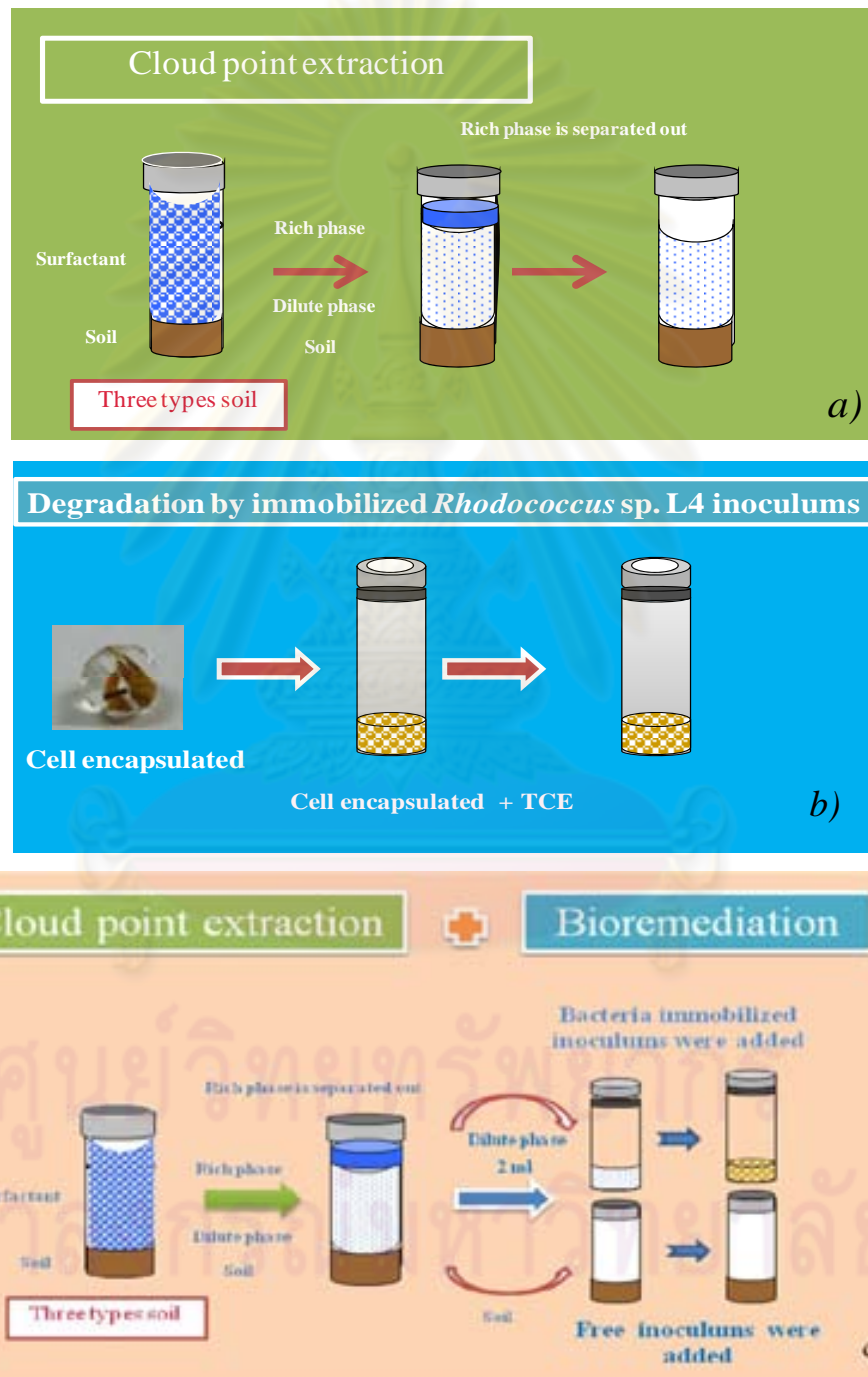


**Figure 3.2** Brief procedure of TCE removal by three treatment types: (a) Cloud point extraction, (b) Degradation by immobilized *Rhodococcus* sp.*L4*., and (c) combined cloud point extraction bioremediation for clean up TCE in various soil types

## 3.4 Analytical methods

### 3.4.1 Determination of surfactant concentration

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

### 3.4.2 Bacterial number

The number of bacteria in immobilized on attachment and encapsulation were quantified by plate count technique. For attachment; the immobilized cells were enumerated by resuspending in MS medium and leave to rehydrate for 2 min. The suspensions were sonicated for another 2 min and shaken vigorously on a vortex mixer for 3 min. The process was repeated twice. The suspensions were centrifuged to collect the cells. Aliquots containing 0.1 ml were plated on agar plate and incubated at room temperature in a glass box supplied with toluene as the sole carbon and energy source for 1 week. Then, bacterial colonies were counted and the results were averaged. For encapsulation immobilized cell; the experiment was done as same as attachment but the beads were grinded before sonicated.

### 3.4.3 TCE concentration

The amount of TCE was analyzed by PerkinElmer TurboMatrix Automated Headspace Sampler with the Clarus 500 Gas Chromatography equipped with a flame ionization detector (Headspace GC-FID) and a HP-5 (5% Phynyl 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 $^{\circ}$C, detector temperature 250 $^{\circ}$C, oven temperature 90$^{\circ}$C isothermal (3 min). The carrier gas was helium with gas flow rate of 40 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. The retention time of TCE was 1.24 min. External standard quantitative calibrations were performed for the analysis of TCE. TCE standard curves for surfactant-rich, surfactant-dilute, and soil phases were shown in Appendix A.

### 1) 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, 200 mM. TCE concentrations were added into the surfactant rich-phase solutions to make different known concentrations of 50-200 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.

### 2) Standard curve of TCE in the surfactant-dilute phase

The TCE concentrations at 10 - 100 ppm were prepared in the volumetric flasks using deionized water as solvent because the existing of surfactant at low concentration did not alter the measured TCE concentration. The standards were mixed for 30 minutes and only 2 mL of aqueous solution was transferred to 22 mL aluminum cap vials and the concentration of TCE was determined by GC.

### 3) Standard curve of TCE in soil

TCE in three type's soil of experimental set of cloud point, cloud point after bioaugmentation used the same standard curve. Uncontaminated soils were prepared at the same amount (2.8 g) as sample in the vials. Water will be added into vial fully, cap the vial and then stirred these soil for 2 hr and take them into the water bath for equilibrate at 60$^{o}$C for 96 hrs. After that, water solution was separated out. $H_2SO_4$ at 10 M were drop as doing in samples. TCE at the initial concentration 20- 100 ppm

were spiked to the soils and then shaken for 2 hours. The concentration of TCE was determined by GC.

## CHAPTER IV

## RESULTS AND DISCUSSION

## 4.1 Preliminary Study

### 4.1.1 Effect of initial TCE concentrations on the remediation of sandy clay loam from Chiang Mai

Chuahom (2006) studied the efficiency of natural attenuation, cloud point extraction, bioaugmentation and the combined process of cloud point extraction and bioaugmentation for clean-up TCE in sandy clay loam soil from Chiang Mai. The study used SURFONIC TDA6 (non-ionic surfactant) as a separating agent and the initial TCE concentration was 100 ppm. The result showed that the combined process provided the highest TCE removal efficiency from soil than the individual method. Meanwhile, the effects of high TCE concentrations on TCE removal efficiency of each method have not been studied.

In this preliminary study, the efficiency of natural attenuation, bioaugmentation, cloud point extraction and the combined process of cloud point extraction and bioaugmentation was investigated on sandy clay loam soil (2.8 g) that spiked with TCE to give an initial concentration of either 200, 500 or 1000 ppm. For natural attenuation process, it was performed by adding 5 mL of mineral salt medium directly into the contaminated soil. The TCE remaining were analyzed after samples were incubated in orbital-shaker at room temperature for 96 hr. Bioaugmentation process was performed by adding 5 mL of 1.0 OD bacterial inoculums directly into the contaminated soil at various TCE concentrations. After that cumene in N,N dimethylformamide was added to make the final concentration of 25 mg/L to maintain the enzyme induction. The TCE remaining were analyzed after samples were incubated in orbital-shaker at room temperature for 96 hr. For cloud point extraction, SURFONIC TDA6 at the initial concentration 70 mM was added into the 22 mL vial

containing the contaminated soil to almost full, stirred for 1 hr, and equilibrated for phase separation in a water-bath at 60°C for 72 hr. After the phase separation, the remaining TCE in soil was analyzed. The combined process was demonstrated by adding bacteria inoculums into the soil after cloud point extraction for remediating the remaining TCE in the system. The TCE removal efficiency of each treatment was determined from the remaining TCE in soil after each process achieved (Table 4.1).

**Table 4.1** Effects of initial TCE concentrations on the efficiency of different treatment methods.

| Initial TCE (mg/kg) | TCE remaining in soil (mg/kg) | | | |
|---|---|---|---|---|
| | Natural attenuation | Cloud point extraction | Bioaugmentation | Cloud point extraction and Bioaugmentation |
| 200 | 102.2 ± 8.7 | 42.7 ± 3.2 | 51.3 ± 2.4 | 8.6 ± 0.5 |
| 500 | 333.7 ± 7.7 | 141.4 ± 2.2 | 229.6 ± 11.6 | 23.4 ± 0.8 |
| 1000 | 784.5 ± 3.2 | 252.9 ± 3.9 | 740.4 ± 42.5 | 79.3 ± 0.9 |

The increasing of initial TCE concentrations caused the increase in remaining TCE in all treatments. Meanwhile, the effects of TCE concentrations were minor for cloud point extraction and combined technique in which the removal efficiencies of these techniques were nearly the same in all TCE concentrations. The TCE removal efficiency for cloud point extraction and combined process was about 72-79 % and 92-96%, respectively. For biodegradation treatment, the TCE removal efficiency was decreased significantly from 74% to 56% at the initial TCE concentrations of 200 to 1,000 ppm, respectively. This may cause from the low bioavailability of TCE or the toxic effects of high TCE concentrations.

The result was corresponded with the study of Mu and Scow (1994). They found that as the initial TCE concentration increased, the number of toluene/TCE

degraders and the rate of toluene degradation decreased, and consequently no TCE degradation occurred. The results indicated that immobilization technique should be used to protect the adding TCE-degrading bacteria from high TCE concentration. Moreover, the combined technique showed the highest TCE removal efficiency when compared to other treatment. When using the combined technique, the cloud point extraction process was the main process to achieve a high TCE removal efficiency. Therefore, the cloud point extraction process was tested with various soils type in further study.

**4.1.2 Effects of soil types on cloud point extraction using SURFONIC TDA6**

The effectiveness of surfactant remediation for organic contaminant removal might be limited by physical and chemical properties of soil (Mulligan *et al.*, 2000). In this study, three types of soil including sandy clay loam soil from Phatthalung, clay soil from Karnchaburee and sandy loam soil from Suratthanee were used to study the effect of soil types on cloud point extraction process. SURFONIC TDA6 was used as a separating agent in this study. The initial surfactant concentration of SURFONIC TDA6 was followed the experiment of Chuahom (2006). After phase separation occurred, the surfactant partition ratio of surfactant concentration in the surfactant-rich phase to that of in the dilute phase was used to determine the efficiency of cloud point extraction. The higher surfactant partition ratio provided the higher amount of surfactant presents in the rich-phase and also leaving the lower amount of surfactant in dilute-phase. The surfactant partition ratios of SURFONIC TDA6 surfactant in three soils were shown in Table 4.2.

**Table 4.2** Effect of soil types on surfactant partition ratio of SURFONIC TDA6

| Soil types | Surfactant concentration (mM) | | Surfactant partition ratio | Source |
|---|---|---|---|---|
| | Rich-phase | Dilute-phase | | |
| Sandy clay loam* | 345.37 ± 32.94 | 2.78 ± 0.08 | 124.1 | Chiang Mai |
| Sandy clay loam | 257.52±37.82 | 3.37±0.10 | 76.2 | Phatthalung |
| Clay | 121.99±14.29 | 2.53±0.08 | 48.2 | Karnchanaburee |
| Sandy loam | 167.01±7.20 | 3.77± 0.16 | 44.2 | Suratthanee |

*Data from (Chuahom, 2006)

When compared between soil types, the surfactant partition ratio of each surfactant was different. The surfactant partition ratio of SURFONIC TDA6 in sandy clay loam soil from Chiang Mai, sandy clay loam soil from Phatthalung, clay soil from Karnchanaburee and Sandy clay soil from Suratthanee were 124.1, 76.2, 48.2 and 44.2, respectively. The order of surfactant partition ratios from low to high was sandy loam < clay < sandy clay loam from Phatthalung < sandy clay loam from Chiang Mai. Meanwhile, the order of clay contents from high to low was clay (40.6%) > sandy clay loam from Phatthalung (32.4%) > sandy clay loam from Chiang Mai (20%) > sandy loam (10.8%). It indicated that soil with higher clay content had lowered surfactant partition ratios. It was corresponded with the result of Mulligan *et al*. (2001) which suggested that surfactant might adsorb on clay fractions and thereby reducing their availability. However, this trend was not observed with sandy loam soil. Therefore, other soil components in sandy loam may influence the phase separation of SURFONIC TDA6. To apply the cloud point extraction on various soil types, the process was optimized in the following research. Due to the limit quantity of SURFONIC TDA6, another non-ionic surfactant, DEHYDOL LS7 TH was used as separating agent.

## 4.2 Optimization of cloud point extraction process using DEHYDOL LS7 TH

### 4.2.1 Phase separation of DEHYDOL LS7 TH in aqueous systems

The application of cloud point extraction requires an optimal initial concentration of the surfactant. When the concentration of surfactant is too low, high amount of pollutants will remain in soil, while using too high surfactant concentration will lead to a high surfactant remaining in the effluent (Kimchuwanit *et al.,* 2000; Wang *et al.,* 2002). In addition, the high concentration of surfactant in dilute-phase may cause the toxicity to the microorganisms in the environment (Rothmel *et al.,* 1998) Therefore, the initial surfactant concentration was determined with the purpose to use the lowest amount of surfactant for cloud point extraction.

The initial surfactant concentrations of DEHYDOL LS7 TH ranging from 30–110 mM were prepared. The phase separation of surfactant was induced by raising temperature to $60^{\circ}C$ in water bath. After phase separation, the surfactant concentration in surfactant dilute-phase and surfactant rich-phase were analyzed using the Iodine-Iodide method (Baleux, 1972). The results were shown in Table 4.3. The surfactant concentration of DEHYDOL LS7 TH in the surfactant dilute-phase drastically increased with the increasing initial surfactant concentration, whereas the surfactant concentration in surfactant rich-phase was slightly increased. Additionally, the surfactant partition ratio that defined as the ratio of the concentration of surfactant rich-phase to that of the concentration of surfactant dilute-phase was increased with the increasing surfactant concentration until it reached the maximum value at the initial surfactant concentration of 90 mM. At this concentration, most of micellar tend to solubilize in surfactant rich-phase and leaving small amount in surfactant dilute-phase. For this result, the initial surfactant concentration at 90 mM was selected for the next study.

**Table 4.3** Effect of initial DEHYDOL LS7 TH concentrations on the amounts of remaining surfactant in dilute-phase, rich-phase and the surfactant partition ratio in aqueous system

| Initial surfactant concentration (mM) | Remaining surfactant concentration (mM) | | Surfactant partitioning ratio |
|---|---|---|---|
| | Dilute phase | Rich phase | |
| 30 | 4.34 ± 0.32 | 38.28 ± 8.37 | 9.7 ± 0.8 |
| 50 | 8.43 ± 0.42 | 123.78 ± 11.32 | 14.7 ± 0.7 |
| 70 | 10.33 ± 1.12 | 154.97 ± 8.24 | 15.1 ± 1.2 |
| 90 | 10.42 ± 0.85 | 198.41 ±18.40 | 19.0 ± 0.8 |
| 110 | 32.28 ± 6.45 | 194.15 ± 8.02 | 6.1 ± 1.3 |

### 4.2.2 Effect of soil types on cloud point extraction of TCE

The initial surfactant concentration of DEHYDOL LS7 TH at 90 mM was used in cloud point extraction for TCE removal from three types of soil. This experiment were performed by mixed 2.8 g of 1000 ppm TCE contaminated soil with 90 mM of DEHYDOL LS7 TH for 2 hrs, centrifuged for separation of soil and surfactant solution, and then equilibrated in water bath for 96 hr. After equilibrium time, TCE concentration in surfactant rich-phase, surfactant dilute-phase and soil were determined by HS-GC with FID detector. Additionally, the surfactant concentration in surfactant rich-phase and surfactant dilute-phase were analyzed using the Iodine-Iodide method (Baleux, 1972). The surfactant concentrations in surfactant dilute-phase and surfactant rich-phase were different for each soil type (Table 4.4).

**Table 4.4** Effect of soil types on remaining surfactant concentration, surfactant partition ratio, and remaining TCE in various soils.

| Soil Type | Surfactant remaining concentration (mM) | | Surfactant partition ratio | % TCE remaining in soil |
|---|---|---|---|---|
| | Rich phase | Dilute phase | | |
| Sandy clay loam | $171.98 \pm 3.58$ | $11.77 \pm 1.02$ | 14.6 | $2.6 \pm 0.0$ |
| Clay | NA | | | |
| Sandy loam | $126.32 \pm 9.07$ | $11.79 \pm 0.99$ | 10.7 | $2.8 \pm 0.2$ |

NA: Not available since the phase separation was not developed well.

From the experiment, the phase separation of clay soil by DEHYDOL LS7 TH was not clearly developed. This might be resulted from the sorption of surfactant on clay fractions of this soil and thereby reduced their availability to phase separation in cloud point extraction process. Hence, the % TCE remaining in clay soil could not be determined. The results confirmed our preliminary study with SURFONIC TDA 6 that different soil samples affected surfactant partitioning ratios.

Although, fraction of clay content in sandy clay loam soil was higher than that of sandy loam soil but the surfactant partition ratio in sandy clay loam soil was higher than sandy loam soil. This result showed that the soil properties other than the fraction of clay content might also affect on surfactant partition ratio. Surfactant may also adsorb on organic part of soil and thereby reducing the surfactant partition ratio (Komaromy-Hiller and von Wandruszka, 1995). Consequently, sandy clay loam soil, which has slightly lower organic matter content than sandy loam soil, might sorb less surfactant than sandy loam soil and thereby give a higher surfactant partition ratio.

The amounts of TCE remaining in both soil samples were almost similar (Table 4.4). The percent TCE remaining in sandy clay loam soil and sandy loam soil were 2.6 and 2.8 %, respectively. It was suggested that the amount of DEHYDOL LS7 TH was sufficient for the extraction of TCE from soil. The percent recovery of

TCE was from this experiment was shown in appendix D-7. The % TCE recovery was determined from the mass of TCE remaining in surfactant rich-phase, surfactant dilute-phase, and soil after cloud point extraction compared with the initial TCE (2.8 mg). The % TCE recovery in sandy clay loam soil and sandy loam soil were 55.3 and 87.94. The loss of TCE may be due to the high volatilization during the sampling. Moreover, the % TCE recovery in sandy clay loam soil was lower than sandy loam soil, it might be cause from the interaction mechanism between some component of each soil and TCE.

The efficiency of TCE removal from sandy clay loam by using either SURFONIC TDA6 or DEHYDOL LS7 TH was compared from their molar solubilization ratios (MSR). The molar solubilization ratio was calculated as a ratio of the remaining TCE concentration (M) in their surfactant solution including surfactant dilute-phase and surfactant rich-phase after cloud point extraction (TCE remaining in SURFONIC TDA6 was 0.0057 M and DEHYDOL LS7 TH was 0.0072 M) per the surfactant concentration (molar) subtracts with theirs CMC (SURFONIC TDA6 was 0.07 M and DEHYDOL LS7 TH was 0.089 M). The MSR values of both surfactant were the same (0.1), thus indicated that the efficiencies of both surfactant were not difference. The low amount of TCE in soil after extraction by DEHYDOL LS7 TH was therefore due to the use of high surfactant concentration in this experiment.

### 4.2.3 Effects of electrolyte addition on TCE removal by cloud point extraction

The cloud point of nonionic surfactant can be influenced by the addition of electrolytes such as NaCl. Kimchuwanit *et al*. (2000) reported the occurrence of salting-out effect in the micellar solution of nonionic surfactants, in which the partition ratio increased from 10 to 25 at the concentrations of added NaCl between 0.2 and 0.6 M, respectively. In the previous experiment, the cloud point extraction process using DEHYDOL LS7 TH gave an unstable phase separation, in which the surfactant rich-phase quickly mixed with the surfactant dilute-phase when the solution was not at the cloud point temperature. Moreover, the surfactant was not fully separated when applied to clay soil. Therefore, this experiment aimed to enhance phase separation of DEHYDOL LS7 TH in various soil types by adding NaCl from

0.2 to 0.6 M. The effect of NaCl concentrations on the surfactant partition ratio and the percentage of remaining TCE were shown in Table 4.5

**Table 4.5** Effects of NaCl concentrations on the surfactant partition ratio and TCE remaining in three types of soils.

| Soil types | NaCl concentration (M) | Surfactant remaining concentration (mM) | | Surfactant partition ratio | % TCE remaining in soil |
|---|---|---|---|---|---|
| | | Rich phase | Dilute phase | | |
| Sandy clay loam | 0.2 | 271.58 ± 8.9 | 2.93 ± 0.16 | 92.7 | 2.3 ± 0.1 |
| | 0.4 | 320.12 ± 9.8 | 2.34 ± 0.03 | 136.8 | 2.2 ± 0.2 |
| | 0.6 | 366.51 ± 2.9 | 2.15 ± 0.03 | 170.1 | 2.1 ± 0.0 |
| Clay | 0.2 | 379.15 ± 3.4 | 2.56 ± 1.01 | 147.9 | 3.2 ± 0.3 |
| | 0.4 | 486.58 ±12.86 | 1.96 ± 0.18 | 247.2 | 2.6 ± 0.1 |
| | 0.6 | 364.72 ± 2.70 | 0.97 ± 0.05 | 376.1 | 2.7 ± 0.3 |
| Sandy loam | 0.2 | 262.92 ± 2.65 | 2.39 ± 0.08 | 110.0 | 3.0 ± 0.0 |
| | 0.4 | 276.82 ± 8.57 | 2.35 ± 1.58 | 117.8 | 2.5 ± 0.1 |
| | 0.6 | 316.93 ± 5.38 | 1.07 ± 0.12 | 296.2 | 2.5 ± 0.1 |

In all soil types, the concentration of surfactant in rich-phase increased with an increase in NaCl concentrations, while the concentration of surfactant in surfactant dilute-phase decreased. Consequently, the partition ratio of surfactant in each soil types also increased with increasing NaCl concentrations. Moreover, the phase separation of DEHYDOL LS7 TH with adding NaCl in all concentrations was more stable than without adding salt. The salt ions probably attracted the water molecules in an effort to the solvent ions and released the water molecules from any solvation with the ether oxygen atoms. This result  in a decrease of head size of the surfactant and

subsequently changed the micellar shape. When the monomeric form of surfactant is salted out by the presence of an electrolyte, micellization is favored and the cloud point is decreased (Rosen, 2004).

The % mass recovery of DEHYDOL LS7 TH surfactant in the presence of NaCl from three types of soils was demonstrated in APPENDIX B-12, B-13, and B-14. The order of % surfactant recoveries from high to low was sandy loam > sandy clay loam > clay soil. As a result, it can indicate that surfactant sorbed more on soil with high clay content. The result was corresponded with Mulligan (2001) who reported that non ionic surfactant can adsorb on clay fraction and thereby reduce their availability.

Moreover, the effect of NaCl concentrations on TCE partition ratio was shown in Figure 4.1.
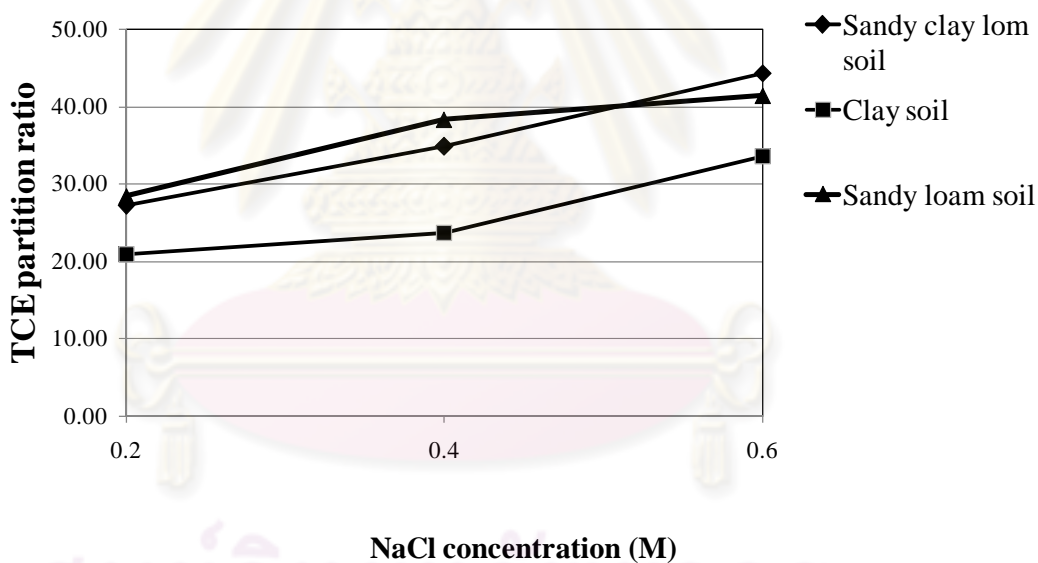


**Figure 4.1** Effects of NaCl concentrations on the TCE partition ratio from three types of soil.

The TCE partition ratio was defined as the ratio of TCE concentrations in surfactant rich-phase to that of TCE concentration in surfactant dilute-phase. The higher TCE partition ratio means that TCE tends to solubilize in surfactant rich-phase more than surfactant dilute-phase. From the Figure 4.1, the TCE partition ratio substantially increases with increasing NaCl concentrations. The result corresponded with Table 4.5, which showed the increased NaCl concentrations led to the increased surfactant concentrations in the surfactant rich-phase. Moreover, the TCE partition

ratio in three types of soil from high to low were sandy loam soil, sandy clay loam soil and clay soil, respectively. The % TCE recovery of sandy clay loam soil, clay soil and sandy loam soil with the presence of NaCl from 0.2 to 0.6 M was about 58-64%, 69-78% and 67-89%, respectively (Appendix B-7, B-8 and B-9). The loss of TCE may cause from the volatization of TCE during sampling sample in the experiment and it might be cause from the interaction mechanism between some component of each soil and TCE that caused the % TCE recovery in each soil were difference..

From the table 4.5 the percentage of TCE remaining in three types of soil was slightly decreased with the increased NaCl concentrations. For that reason, the lowest NaCl concentration at 0.2 M was selected for the following experiment (section 4.5). This added electrolyte effects agreed with the study of Trakulamupatum *et al.* (2002). They studied the effect of NaCl on cloud point extraction of benzene. The result showed that the benzene partition ratio increased with increasing NaCl concentrations. Nevertheless, the fraction of extracted benzene was not much affected by the increasing NaCl concentrations.

## 4.3 Immobilization of *Rhodococus sp.* L4 inoculums for TCE degradation

Suttinun *et al.* (2008) indicated that cumin seeds containing cumene and cumin aldehyde can be used as support material for *Rhodococcus* sp. L4 immobilization. The cumin seeds can provide the continuous enzyme induction in *Rhodococcus* sp. L4 and can protect the bacteria from the high TCE concentration. However, the limitation of cell attachment is that the bacteria may contact with the environment directly. Therefore, the bacteria may be suffered from the environmental stress and toxic pollutant (Suttinun, 2008). To improve bacteria capability for TCE removal, encapsulation process was applied for producing immobilized *Rhodococcus* sp. L4 inoculum to use in TCE biodegradation. In this study, gellan gum gel was used to encapsulate the cumin seeds containing *Rhodococcus* sp. L4 cells. After mixed cumin seed-*Rhodococcus* sp. L4 with the gellan gum solution and extrude it into 0.2

M CaCl$_2$ solution for 2 hr, the beads were formed and the characteristic of cumin seed-*Rhodococcus* sp. L4 encapsulated in gellan gum were illustrated in section 4.3.1.

**4.3.1 Characterizations of the cumin seed-*Rhodococcus* sp. L4 encapsulated in gellan gum**

The characteristic of cumin seed-*Rhodococcus* sp. L4 encapsulated in gellan gum was shown in Figure 4.2. Exterior view of gellan gum bead was clear and the *Rhodococcus* sp. L4 attached on cumin seeds could be seen inside the bead. Scanning electron microscope (SEM) image of gellan gum bead shown small pores distributed all over inside the bead (Figure 4.3 (A)). It was suggested that growth supporting mineral and TCE could transport through these micro pores. The cross section of bead after encapsulated the cumin-attached bacteria was shown in Figure 4.3 (B). Some of cumin seeds were placed at the middle or closed to the surface of the beads. Figure 4.3 (C) showed that *Rhodococcus* sp. L4 grown on the surface of cumin seeds. According to Suttinun *et al*. (2008), the bacteria could use essential oil components in cumin seeds as substrate for their growth. On the surface of the seeds, a bunch of cells were surrounded by exopolysaccharides (Figure 4.3 (D)). The results were corresponded to Suttinun *et al*. (2008), which explained that *Rhodococcus* sp. L4 produce extracellular polysaccharides during their attachment. This exopolymeric substance could minimize the leakage of cells during exposing with toxic substance.
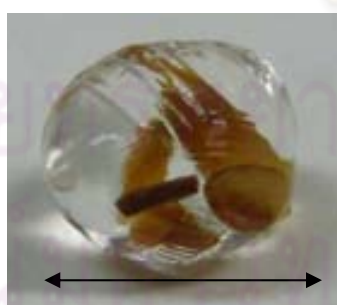


0.5 mm

**Figure 4.2** Cumin seed-*Rhodococcus* sp. L4 encapsulated in gellan gum
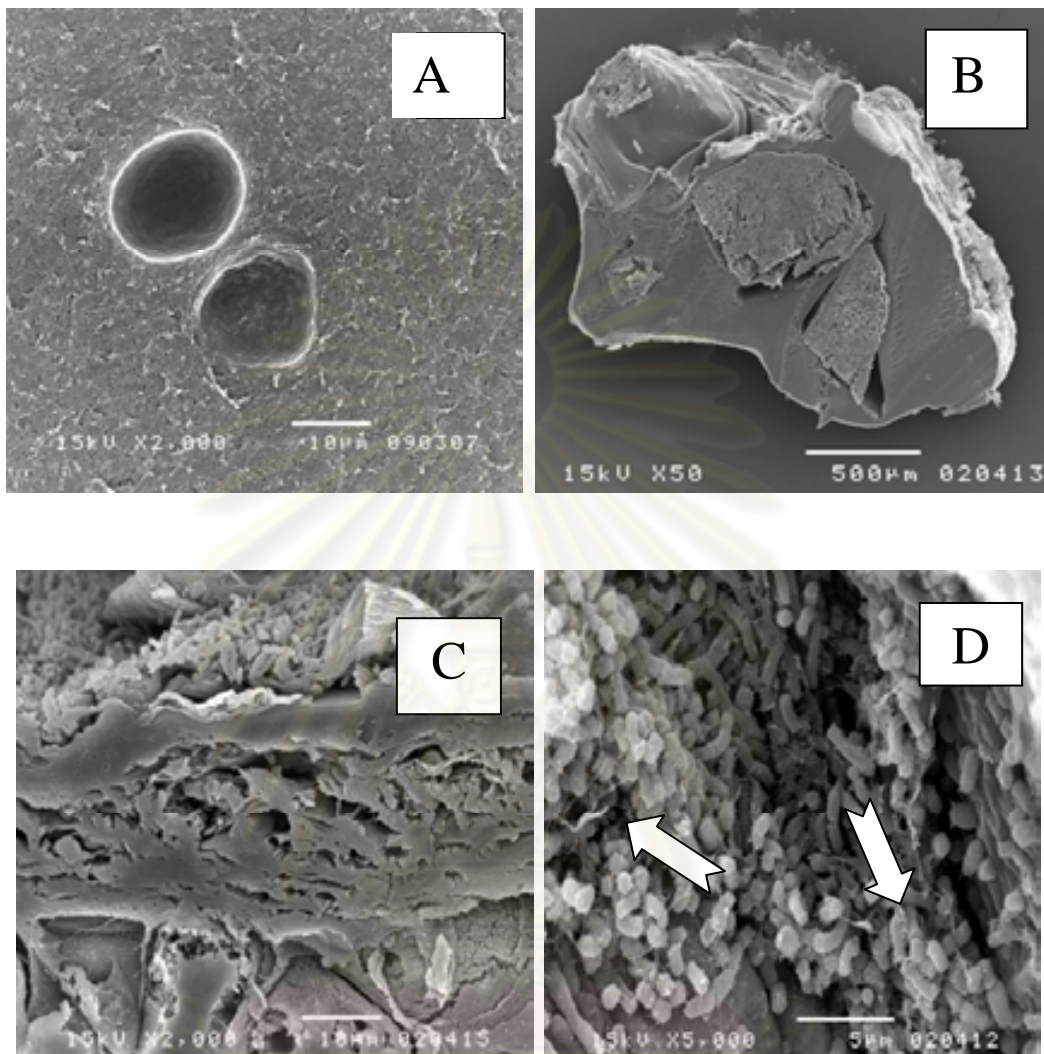
**Figure 4.3** SEM photographs show cross section of gellan gum bead without cells at 2000x (A), cross section of bead after encapsulated the cumin-attached bacteria at 50x (B), and bacteria cells between gel and cumin seeds (C). Bacteria cells between gel and cumin seeds were surrounded by exopolysaccharide as point by arrows (D).

**4.3.2 Effect of dilute-phase surfactant on *Rhodococcus* sp. L4**

After cloud point extraction process, some amount of surfactant was left in the dilute phase that may be toxic to *Rhodococcus* sp. L4. From 4.2.1, the concentration of DEHYDOL LS7 TH remaining in surfactant dilute-phase was about 4 mM to 10 mM. Generally, toxic effect from surfactant can significantly suppress the microbial activity (Quintero *et al.*, 2005). Therefore, the toxicity of remaining surfactant concentration at various concentrations was performed on the inoculums of *Rhodococcus* sp. L4. The experiment performed by incubated 3 types of *Rhodococcus* sp. L4 inoculum including free cells, cumin seeds-attached cells and encapsulated cells for 4 days in the presence of 4 mM – 12 mM surfactant.

In all concentrations, *Rhodococcus* sp. L4 encapsulated in gellan gum had more % bacteria remaining in surfactant dilute-phase than the attached cells and free cells (Figure 4.4). For free cells, no bacterium was found in all surfactant concentrations after 4 days of incubation. The effect may cause by 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 can bury in the lipid layer of the bacterial liposome (Cserhati, 1991). The used of encapsulation technique provides an increased of cell densities while protecting them from the exterior biotic and abiotic stresses (Mosley, 2002). The result confirmed that the encapsulation of *Rhodococcus* sp. L4 by gellan gum can protect cells from the high concentration of surfactant.
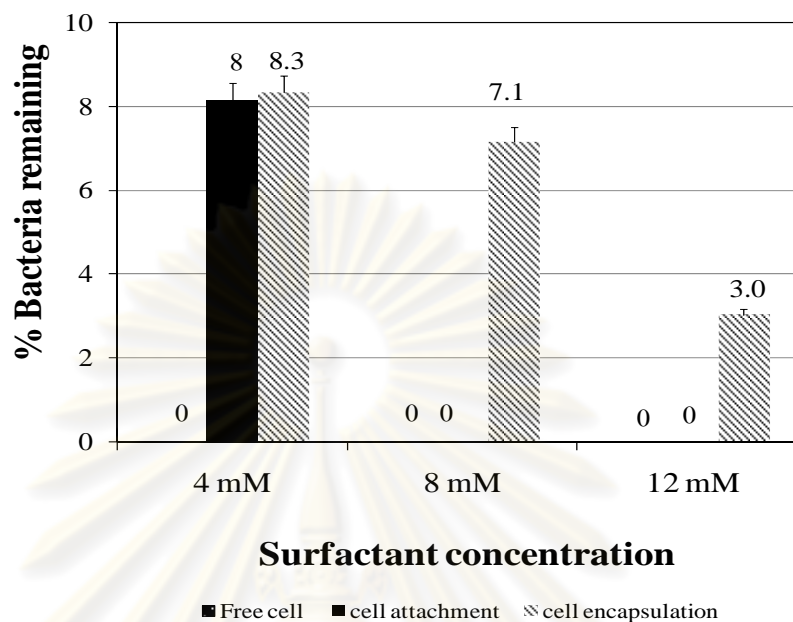
**Figure 4.4** Effect of surfactant concentrations on viability of *Rhodococcus* sp. L4 in different type of cells.

### 4.3.3 TCE removal by immobilized *Rhodococcus* sp. L4 in the presence of surfactant solution and NaCl

At the beginning, the efficiency of the immobilized inoculums on TCE degradation was determined with 4 mM Dehydol LS7 TH. The result showed that the encapsulated cells provided the lowest TCE remaining after incubated for 48 hr. The TCE remaining by encapsulated cells after 48 hr was 3.44 ppm from the initial TCE concentration 8.09 ppm (Figure 4.5), while the % TCE remaining was about 40.45%. For the attached cells, the TCE remaining after 48 hr was 6.38 ppm from the initial TCE concentration 7.58 ppm, consequently the % TCE remaining of this inoculums was 84.25 %. For control treatment, gellan gum bead without cells and killed-cell encapsulated in gellan gum were used. TCE concentrations in the control treatment were increased after incubating. This may result from the sorption of TCE on the control gel beads at the beginning of the study and its desorption to the liquid medium after incubation. The sorption of TCE by gel beads may facilitate TCE degradation by the bacteria inside the gel. The higher TCE degradation by the encapsulated cells was also due to the higher bacteria survival in surfactant dilute-phase than the attached

cells and free cells (figure 4.4). The result confirmed that the encapsulation of bacteria by gellan gum can protect cells from the high concentration of surfactant and environment stress.
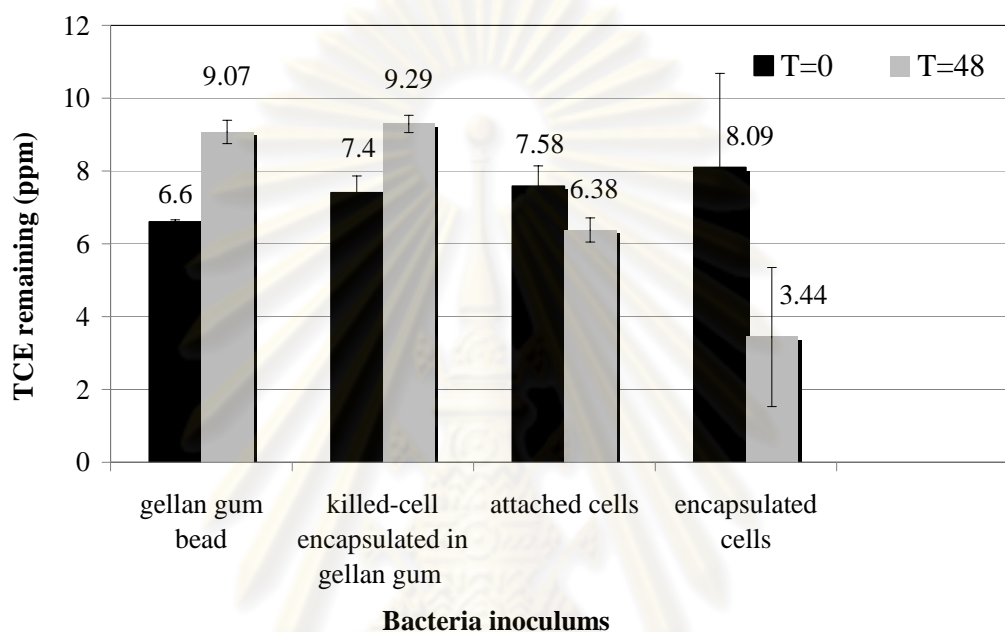


**Figure 4.5** TCE remaining by different types of immobilized cells at initial TCE concentration 10 ppm and 4 mM of Dehydol LS7 TH

The efficiency of *Rhodococcus* sp. L4 encapsulated in gellan gum was further tested with increasing TCE concentrations and adding NaCl. The control study was performed by killed-cells encapsulated in gellan gum beads. The amount of remaining TCE in the control treatment after 48 hr incubation was higher than that of time zero (figure 4.6), which was similar to the previous study. It was indicated that TCE was not loss by abiotic process. Moreover, TCE was adsorbed on the control gel beads at the beginning of the study, and then it had been desorbed to the liquid medium after incubation. For cumin seed-*Rhodococcus* sp. L4 encapsulated in gellan gum, % TCE remaining in the treatment without NaCl and with adding NaCl were increased with the increasing TCE concentrations from 10 ppm to 50 ppm (Figure 4.6). At the initial TCE concentration of 10 ppm, the % TCE remaining was the lowest. When compared with that of free cells from Suttinun *et al.* (2008), it was found that % TCE remaining in the treatment of free cells (63%) was much higher than that of the encapsulated

cells found in this study (48.62±1.27 %). These differences may be because much higher amount of bacteria (more dense) in the encapsulated cells compared with those in free cells.
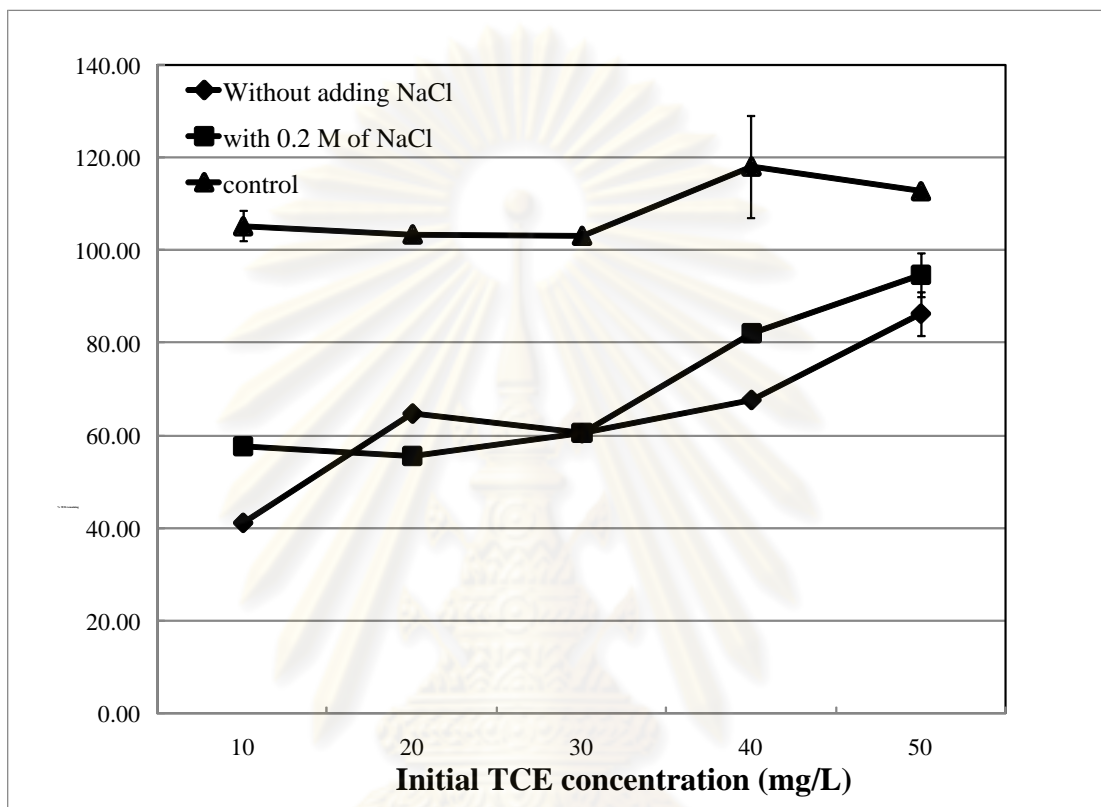


**Figure 4.6** Effect of initial TCE concentrations and NaCl on TCE removal by cumin seeds-*Rhodococcus* sp. L4 encapsulated in gellan gum. Control study was performed by encapsulated killed-cells.

The effect of high TCE concentration on its degradation has been reported. Ayoubi and Harker (1998) found that TCE degradation by *P. putida* F1 at 80 μM TCE (10 ppm) was the highest and the rate dropped rapidly at the concentration higher than 300 μM (40 ppm). Finally, at 320 μM TCE (42 ppm), the degradation by *P. putida* F1 was no longer occurred. In the same experiment, the toxicity of methane-induced, *Methylosinus trichosporium* OB3b was apparent at a concentration of 70 μM TCE (9.22 ppm).

In the presence of 0.2 M NaCl, % TCE remaining at the initial TCE concentration of 10 to 30 ppm was about 57-60 %, whereas % TCE remaining was more than 80 % at the initial TCE concentration of 40 – 50 ppm. The effect of NaCl

on TCE degradation of cumin seeds-*Rhodococus* sp. L4 was similar to the condition without NaCl. From this study, the cells encapsulated in gellan gum could degrade high concentration of TCE in the presence of both NaCl and surfactant. It can indicate that the encapsulated cells were resistant to various toxic compounds. The application of the encapsulated cells in the process after cloud point extraction that contained high amount of TCE, NaCl and some amount of surfactant would be feasible.

## 4.4 Combined process of water flushing and bioaugmentation.

The study of combining water flushing and bioaugmentation for TCE removal from soil and aqueous solution were demonstrated to compare its efficiency with the combined cloud point extraction and bioaugmentation process in section 4.5. The experiment was performed by adding DI water into vial containing 1000 ppm TCE contaminated soil. Then, mixed with magnetic stirrer for 2 hr and centrifuged to separate soil and aqueous solution. After that, those vials were incubated in a water bath at 60 $^{o}$C for 96 hr. The adjusted temperature and time were chosen to simulate the condition used during cloud point extraction. Then, 2 mL of aqueous solution were separated to another vial containing cumin seed-*Rhodococcus* sp. L4 encapsulated in gellan gum. Free cells of *Rhodococcus* sp. L4 were added into the remaining soil. The sample was shaken for 48 hr. The TCE remaining in soil and aqueous solution were illustrated in Table 4.6.

**Table 4.6** TCE remaining for the solution of various soil types by water flushing method compared with the combination of water flushing and bioaugmentation method.

| Soil types | TCE remaining | | | |
| --- | --- | --- | --- | --- |
| | Water flushing | | Combination of water flushing and bioaugmentation | |
| | Soil (mg/kg) | Aqueous (mg/L) | Soil (mg/kg) | Aqueous (mg/L) |
| Sandy clay loam | 94.6 ± 7.2 | 74.6 ± 6.3 | 88.1 ± 1.0 | 64.6 ± 4.9 |
| Clay | 79.5 ± 6.6 | 100.8 ± 8.6 | 71.8 ± 6.9 | 91.1 ± 11.7 |
| Sandy loam | 83.9 ± 7.9 | 73.9 ± 1. 5 | 82.0± 11.0 | 70.7 ± 0.9 |

TCE remaining in soil phase after water flushing of sandy clay loam, clay, and sandy loam soil were 94.6 ± 7.2, 79.5 ± 6.6 and 83.9 ± 7.9 mg/kg, respectively. While the TCE remaining in aqueous phase were 74.6 ± 6.3, 100.8 ± 8.6 and 73.9 ± 1.5 mg/L for the solution of sandy clay loam, clay and sandy loam soil, respectively. The % TCE recovery from water flushing was shown in Appendix D-4. The % TCE recovery was determined from the mass of TCE remaining in aqueous solution and soil after cloud point extraction compared with the initial TCE (2.8 mg). The % mass of TCE recovery in sandy clay loam soil, clay soil and sandy loam soil were 61.3, 79.7, and 87.5 %, respectively. The % TCE recovery in each soil was difference, it might be caused from some of the soil composition interact with TCE, therefore TCE loss from each soil was difference.

After augmenting the encapsulated cells into the aqueous phase and the free cells into the soil phase, the TCE remaining in both soil and aqueous phases were slightly decreased. It was probably due to the high concentration of TCE after water

flushing, which was toxic to bacteria. The TCE removal efficiencies in all soil types were not much different. Therefore, cloud point extraction is an attractive approach to induce phase separation and to reduce TCE concentration in surfactant dilute-phase (aqueous phase) and soil.

## 4.5 Combined process of cloud point extraction and bioaugmentation on the TCE contaminated in various soil types

### 4.5.1 Combined process in the absence of NaCl solution

For combined process, bioaugmentation was combined as a post treatment after cloud point extraction process for improving the efficiency of TCE removal from various soil types. After phase separation, the coacervate phase and some portions of surfactant-dilute phase solution were separated out to ensure no contamination of surfactant coacervate phase. Then, 2 mL of surfactant dilute-phase were separated in to another vial and cumin seed-immobilized *Rhodococcus* sp. L4 encapsulated in gellan gum was added. TCE biodegradation was taken place under aerobic conditions by incubating on an orbital shaker for 48 hr. TCE removal efficiency of bioaugmentation after cloud point extraction process was determined from the remaining TCE concentration in surfactant dilute-phase after incubation compared with TCE remaining at time zero. The control treatment was a treatment without bioaugmentation.

**Table 4.7** TCE remaining in each phase after cloud point extraction in the absence of NaCl

| Type of soil | TCE remaining after cloud point extraction | | | % TCE removal from soil |
|---|---|---|---|---|
| | Rich phase (mg/L) | dilute phase (mg/L) | Soil (mg/kg) | |
| Sandy clay loam | 126.0 ± 10.9 | 66.8 ± 1.4 | 26.3 ± 0.3 | 98.0 ± 0.3 |
| Clay | NA | | | |
| Sandy loam | 135.0 ± 6.9 | 114.0 ± 8.3 | 27.7 ± 1.7 | 97.0 ± 0.6 |

Only sandy clay loam soil and sandy loam soil were conducted in this experiment because the phase separation of cloud point extraction in clay soil was not occurred in the absence of NaCl. The efficiency of TCE removal from soil after cloud point extraction was about 96-97%. Since, the TCE and surfactant concentrations in surfactant rich-phase were high. This phase should be treated by chemical or physical processes such as air stripping or vacuum extraction process to regenerate the surfactant and TCE. Only the solution in surfactant dilute-phase was remediated by bioaugmentation. The TCE removal efficiency in surfactant dilute-phase after remediation by bioaugmentation was shown in Figure 4.7. The efficiencies of TCE removal by combined process in surfactant dilute-phase from sandy clay loam soil and sandy loam soil after incubating were about 30 % and 36 %, respectively. Meanwhile, the amounts of remaining TCE after combined treatment in the surfactant dilute-phase 46 and 72 ppm for sandy clay loam and sandy loam soil, respectively (Table 4.10). The high amount of remaining TCE in sandy loam soil was due to the high amount of TCE after cloud point in this soil. From the control treatment without adding bacteria immobilized inoculums, the TCE removal may cause from the biotic loss such as the TCE volatilization during sampling and incubating the sample. The combination of cloud point extraction and bioremediation with the absence of NaCl can remove TCE from surfactant dilute-phase after cloud point extraction. Although,

the remaining TCE in effluent after remediation was still high, TCE in this effluent may be remediated with natural attenuation afterward.
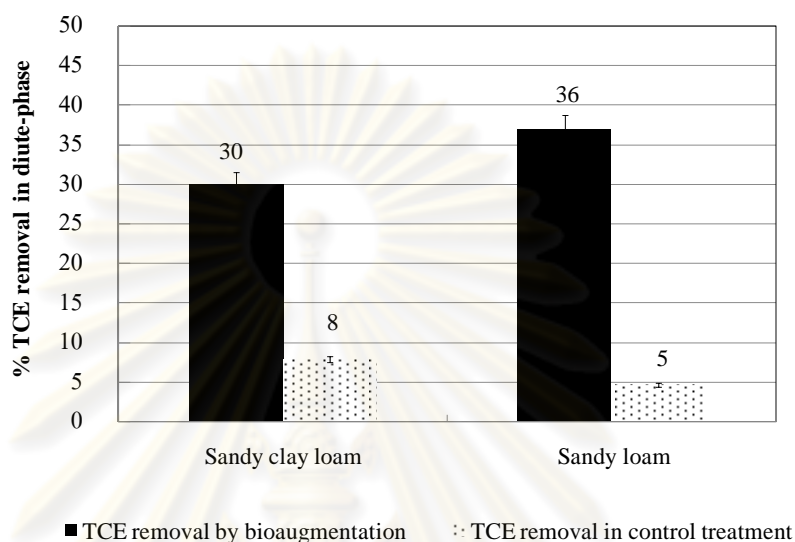


**Figure 4.7** The removal of TCE from dilute-phase surfactant after the combined cloud point extraction and bioaugmentation in the absence of NaCl.

### 4.5.2 Combined process in the presence of NaCl solution

From the previous experiment, the TCE in surfactant dilute-phase remained at the high concentration. Therefore, cloud point extraction process with adding 0.2 M NaCl was used to improve the phase separation of surfactant before remediated with the bioaugmentation method. After phase separation, the coacervate phase and some portions of surfactant-dilute phase solution were separated out to ensure no contamination of surfactant coacervate phase. Then, 2 mL of surfactant dilute-phase were separated in to another vial and cumin seed-immobilized *Rhodococcus* sp. L4 encapsulated in gellan gum was added. In this study, free cells (2.5 OD) were added into soil to remove the remaining TCE. The vials were incubated, sampled, and analyzed as in 4.5.1. The amount of remaining TCE after cloud point extraction in the presence with 0.2 M of NaCl was shown in Table 4.8

**Table 4.8** TCE remaining in each phase after cloud point extraction in the
      presence of NaCl.

| Type of soil | TCE remaining after cloud point extraction | | |
|---|---|---|---|
| | Rich phase (mg/L) | dilute phase (mg/L) | Soil (mg/kg) |
| Sandy clay loam | $296.8 \pm 13.0$ | $12.4 \pm 2.1$ | $29.9 \pm 1.0$ |
| Clay | $433.4 \pm 18.2$ | $21.3 \pm 2.8$ | $32.4 \pm 0.6$ |
| Sandy loam | $357.9 \pm 30.4$ | $16.9 \pm 2.6$ | $34.2 \pm 1.1$ |

In all three soil samples, the amounts of remaining TCE in surfactant dilute-
phase after cloud point extraction in the presence of NaCl were lower than after water
flushing and cloud point extraction without adding NaCl. Most of TCE was
solubilized in the surfactant rich-phase. The % TCE recovery and mass balance of
TCE in surfactant rich-phase, surfactant dilute-phase, and soil was demonstrated in
Appendix D-13. The % TCE recovery from sandy clay loam soil, clay soil and sandy
loam soil were 58.71 %, 86.09 % and 71.25 %, respectively. The % TCE recovery in
each soil was difference, it might be caused from some of soil composition in each
soil interact with TCE ,therefore TCE loss from each soil was difference.

The TCE removal efficiency of the combined process from soil and surfactant
dilute-phase were shown in Figure 4.8 and 4.9.
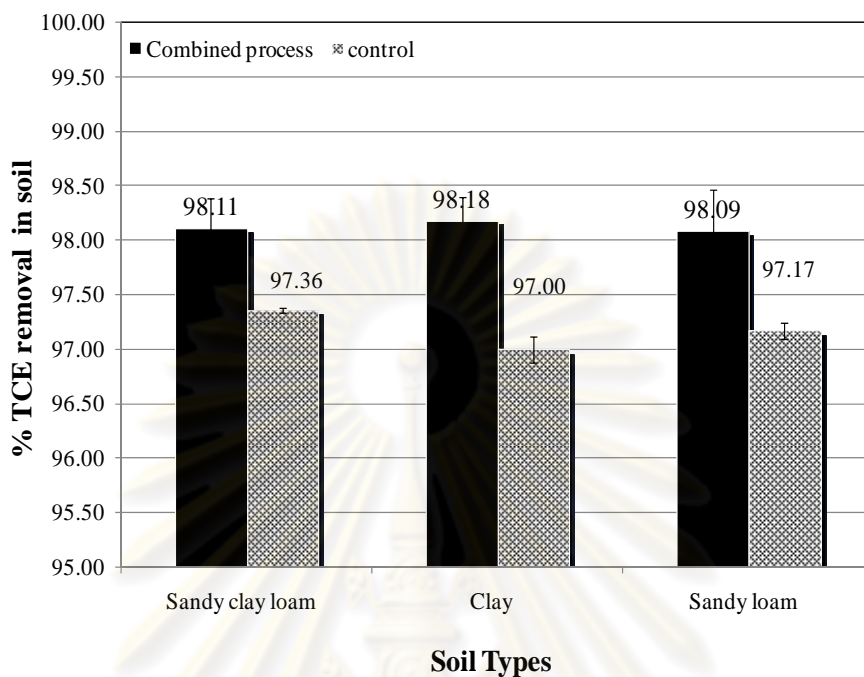
**Figure 4.8** The removal of TCE from soil after the combined cloud point extraction and bioaugmentation in the presence of NaCl.
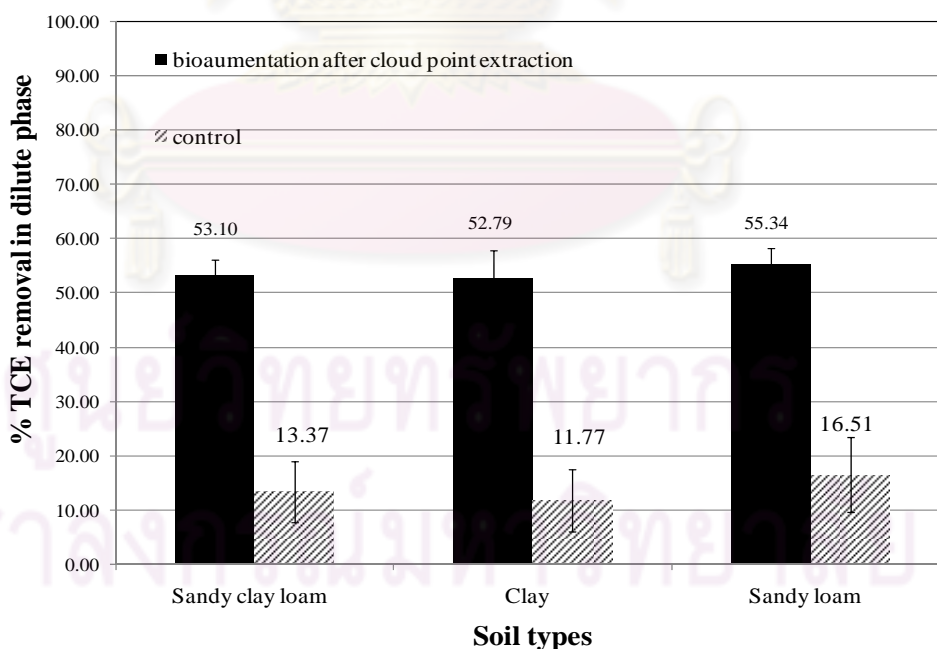


**Figure 4.9** The removal of TCE from dilute-phase surfactant after the combined cloud point extraction and bioaugmentation in the presence of NaCl.

TCE removal efficiencies in three types of soil were about 98 % (Figure 4.8) and the amounts of remaining TCE in soil were about 18-19 mg/kg (Table 4.9). While, the removal of TCE from surfactant dilute-phase after the combined cloud point extraction and bioaugmentation was about 52-55% (Figure 4.9) and TCE remaining in the effluent was about 6.29 – 10.69 mg/L (Table 4.9). The effectiveness of this combined process was consistent with the study on combination process of surfactant and bioremediation by Rothmel *et al.* (2009). They used the anionic surfactant Steol CS-330 that foam injected into TCE-DNAPL-contaminated sand columns to enhance the mobilization of TCE-DNAPLs. Injection of foam followed by artificial groundwater (AGW) and then by foam again resulted in flushing of 75% of the initial TCE-DNAPL. The residual TCE was dispersed within the column at concentration levels compatible with biodegradation. After adding the TCE-degrading bacterial strain ENV 435 simultaneously with the second pulse of foam, they reported 95-99% degradation of the residual TCE.

## 4.6 Comparison of TCE removal efficiency

TCE removal efficiency from three types of soils was determined by comparing the amount of remaining TCE in soil and aqueous solution after remediated with difference treatments. After remediated the 1000 ppm TCE contaminated soils by water flushing, cloud point extraction without NaCl and cloud point extraction with NaCl, the TCE remaining in soil and aqueous solution from three types of soils was shown in Table 4.9

**Table 4.9** The amounts of TCE in soil and aqueous solution after remediating with different treatment methods

| Soil types | TCE remaining after treatment | | | | | |
|---|---|---|---|---|---|---|
| | Water flushing | | Cloud point extraction without NaCl | | Cloud point extraction with NaCl | |
| | Soil (mg/kg) | Aqueous (mg/L) | Soil (mg/kg) | Aqueous (mg/L) | Soil (mg/kg) | Aqueous (mg/L) |
| Sandy clay loam | $94.6 \pm 7.2$ | $74.6 \pm 6.3$ | $26.3 \pm 0.3$ | $66.8 \pm 1.4$ | $29.9 \pm 1.0$ | $12.4 \pm 2.1$ |
| Clay | $79.5 \pm 6.6$ | $100.8 \pm 8.6$ | NA | | $32.4 \pm 0.6$ | $21.3 \pm 2.8$ |
| Sandy loam | $83.9 \pm 7.9$ | $73.9 \pm 1.5$ | $27.7 \pm 1.7$ | $114.0 \pm 8.3$ | $34.2 \pm 1.1$ | $16.9 \pm 2.6$ |

The amounts of remaining TCE in soil after cloud point extraction were nearly the same, either with or without NaCl. The treatment provided less amount of TCE remaining in soil than the remediation by water flushing. The results indicated that surfactant enhanced the solubility of TCE from soil and left a small amount of TCE in soil. In all three types of soil, the amounts of remaining TCE in surfactant dilute-phase were lowered to 12-21 mg/L when NaCl was presence. On the other hand,

cloud point extraction without NaCl and water flushing left high amount of TCE (about 66 -114 mg/L) in the aqueous solutions.

When bioaugmentation were conducted to remediate the residual TCE, the amounts of remaining TCE were further decreased (Table 4.10). The presence of NaCl provided the lowest TCE remaining (18-19 mg/kg) in all soil samples after the combined cloud point extraction and bioaugmentation with *Rhodococcus* sp. L4 inoculums. The amounts of remaining TCE by bioaugmentation after water flushing were highest at 71-88 mg/kg. When compared the combination process with the individual water flushing and cloud point extraction, the results found that the combination process provided the highest TCE removal efficiency in all three soil types. The remaining TCE in soil could be further remediated by the natural attenuation or another technology to lower TCE in soil. Similarly, the amounts of remaining TCE in aqueous solution after combined process were the lowest when compared with the individual treatment. Therefore, the application of bioremediation after cloud point extraction is an attractive technique for the removal of residual TCE in aqueous solution. When TCE concentrations are high, the immobilized technique could be applied to improve the TCE removal efficiency. From this experiment, the encapsulated cumin seed-*Rhodococcus* sp. L4 in gellan gum beads significantly increased the TCE degradation efficiency. The immobilization of bacteria in gellan gum was simple to operate.

**Table 4.10** Amounts of TCE in soil and aqueous phases after combined treatment

| Soil types | TCE remaining in combined process treatment | | | | | |
|---|---|---|---|---|---|---|
| | Bioaugmentation after water flushing | | Bioaugmentation after Cloud point extraction without NaCl | | Bioaugmentation after Cloud point extraction with NaCl | |
| | Soil (mg/kg) | Aqueous (mg/L) | Soil (mg/kg) | Aqueous (mg/L) | Soil (mg/kg) | Aqueous (mg/L) |
| Sandy clay loam | 88.1 ± 1.0 | 64.6 ± 4.9 | ND | 46.7 ± 3.3 | 18.9 ± 2.7 | 6.3 ± 0.2 |
| Clay | 71.8 ± 6.9 | 91.1± 11.7 | ND | NA | 18.2 ± 2.2 | 10.7 ± 0.7 |
| Sandy loam | 82.0± 11.0 | 70.7 ± 0.9 | ND | 72.0 ± 8.1 | 19.1 ±3.8 | 7.1 ± 0.6 |

ND: Not determined.

NA: Not available since Dehydol LS7 TH was not separated well.

# CHAPTER V

# CONCLUSIONS AND SUGGESTIONS

## 5.1 Conclusions

The contamination of trichloroethylene (TCE) in soil and groundwater become an important environmental problem because of their toxicity and persistence. This study aims to apply the technique of combined cloud point extraction and bioremediation to remove TCE from several types of soil including sandy clay loam, clay and sandy loam soil. In this study, DEHYDOL LS7 TH, a nonionic surfactant was used in cloud point extraction process. The surfactant partition ratio from sandy clay loam and sandy loam were 14.61 and 10.71, respectively. Meanwhile, this surfactant could not separate well in clay soil. It was indicated that soil types can affect the phase separation. However the different in TCE removal efficiency from each soil types was minor and about 3% of the initial TCE was remained in those soil samples. After that, the addition of sodium chloride (NaCl) was utilized to enhance phase separation and increased the surfactant partition ratio of DEHYDOL LS7 TH in all three types of soil. The maximum surfactant partition ratios from sandy clay loam, sandy loam, and clay were 170.1, 296.2, and 376.1, respectively. The addition of NaCl also increased TCE partition ratio, in which only small amount of TCE was remained in the dilute phase surfactant. TCE removal efficiencies of this condition in all soil types were similar to the cloud point extraction without adding NaCl.

To produce bacteria inoculums for bioremediation, two types of immobilization methodology were used i.e. attachment and encapsulation. For attachment, cumin seeds were used as supporting material for immobilizing *Rhodococcus* sp. L4. To further protect the cells, gellan gum gel was used for encapsulating the cumin seed immobilized-*Rhodococcus* sp. L4. The used of

encapsulation technique provides an isolated microenvironment to the bacteria while protecting them from the exterior biotic and abiotic stresses (Mosley, 2003). The cumin seed- *Rhodococcus* sp. L4 encapsulated in gellan gum could tolerate high surfactant concentrations than the attached cells and free cell inoculums. TCE removal efficiency of cumin seed-*Rhodococcus* sp. L4 encapsulated in gellan gum at the initial TCE concentration 10 ppm was about 60% while the efficiency of cumin seed-*Rhodococcus* sp. L4 was only 26%. Moreover, the encapsulated cells were still effective at high TCE concentrations and in the presence of NaCl in the system.

The combination of cloud point extraction process using DEHYDOL LS7 TH in presence of NaCl and bioaugmentation with *Rhodococcus* sp. L4 inoculum were later performed to clean up 1,000 ppm TCE in sandy clay loam, clay and sandy loam soil. After cloud point extraction, the residual TCE in soil was degraded by free *Rhodococcus* sp. L4 inoculums and the residual TCE in surfactant dilute-phase was degraded by cumin seed-*Rhodococcus* sp. L4 encapsulated in gellan gum. The amounts of TCE after the combined treatment were decreased to only 18-19 mg/kg in all three soil types and to 6-10 mg/L in the dilute phase surfactant. Therefore, the effects of soil properties on this combined technique were minimal. The success of this combined technique was mainly due to the removal of large TCE fractions from soil by cloud point extraction in the presence of NaCl and the degradation of the remaining TCE by *Rhodococcus* sp. L4 inoculums. The results suggested that the combination of these techniques could be effectively applied for TCE removal from various soil types.

**5.2 Suggestions and recommendations for further work**

There are many factors affecting the efficiency of cloud point extraction and bioremediation methods for TCE removal from various soil types. Actually, the difference in soil properties can affect the removal efficiency of surfactant-enhanced remediation such as pH, CEC, clay content, particle size, permeability (Mulligan, 2001). Especially, high content of clay and organic carbon could lead to the sorption of nonionic surfactant on soil (Paria, 2008). Meanwhile, the effects of soil properties on cloud point extraction were not found in this study. This may be due to the use of only three types of soil. The relationship between properties of soil and the partition ratio of surfactant in cloud point extraction process should be concerned and determined to improve its efficiency when apply to other soil types. In addition, more soil samples with difference soil properties should be studied. Other surfactants that produce in Thailand may be studied in the future as the alternatives of DEHYDOL LS7 TH. The surfactant with surfactant rich-phase in the bottom of solution may be applied by flushing the soil with surfactant first, removing the flushed surfactant out from soil, and then inducing the phase separation. In addition, the adsorption of TCE and surfactant on each soil types should be studied to know the phenomena of TCE and surfactant interactions with each soil type.

To improve bioremediation process, many types of materials for immobilizing cells are available, thus they should be studied in the future as the alternatives of gellan gum. The material may be the natural materials, inorganic materials or synthetic materials that will give an effective protection to the bacteria inoculums. Moreover, future study on reactivation process is required for effective reuse of the encapsulated cells. The combined cloud point extraction and bioremediation technique should be tested with various contaminated soil types as well as soil with various pollutants. These technique may be conducted as both in-situ and ex-situ remediation. Accordingly, the surfactant solution could be injected to the contaminated site to solubilize the pollutant and the flushed solution will be pumped out and induced the phase separation by cloud point extraction process in a tank or reactor. The surfactant and TCE in rich-phase may be reused by another technique

such as air stripping or vacuum TCE out of the surfactant. At the same time, the immobilized cells should be added to the surfactant-dilute phase to remove the residual TCE and then the solution could be pumped back into the site. The immobilized cells could be added into the contaminated soil after surfactant flushing to remove the residual TCE in soil.

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**APPENDICES**

**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 solution | 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. CMC of DEHYDOL LS7 TH
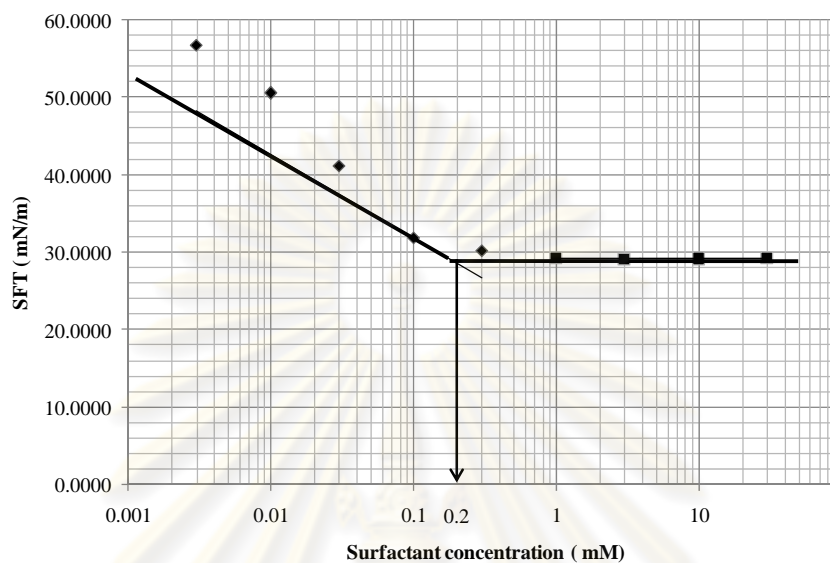


**Figure A-1** Critical Micelle Concentration of DEHYDOL LS7 TH

## 3. Standard curve of TCE in rich phase
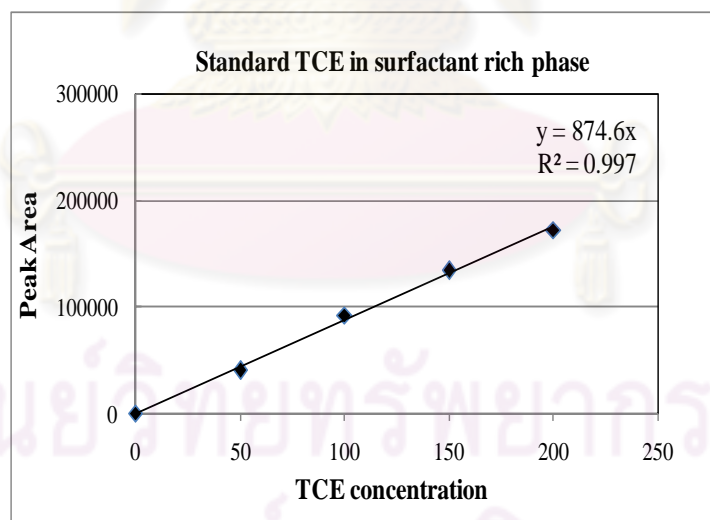


**Figure A-2** Standard curve of TCE in rich-phase   50- 200 ppm of TCE concentration

in soil; Surfactant concentration in rich phase was 200 mM

**4. Standard curve of TCE in Sandy clay loam soil**
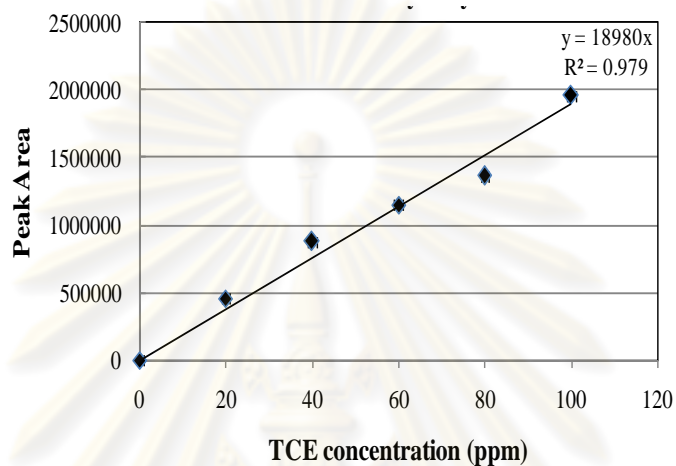


**Figure A-3** Standard curve of TCE in Sandy clay loam soil for contaminated soil remediation by surfactant extraction and bioremediation

**5. Standard curve of TCE in Clay soil**
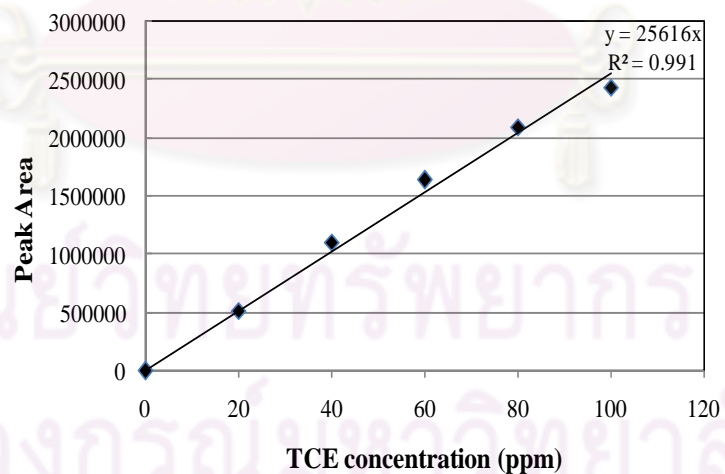


**Figure A-4** Standard curve of TCE in Clay soil for contaminated soil remediation by surfactant extraction and bioremediation

**6. Standard curve of TCE in Sandy loam soil**
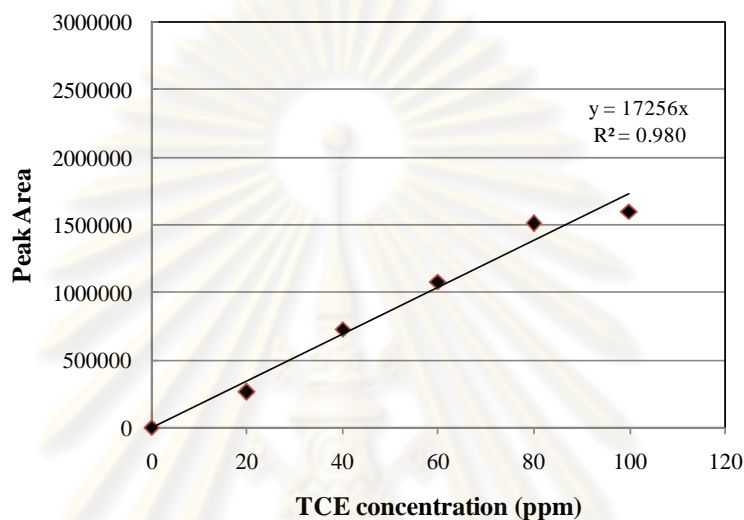


**Figure A-5** Standard curve of TCE in Sandy loam soil for contaminated soil remediation by surfactant extraction and bioremediation

**7. Standard curve of TCE in surfactant dilute-phase after cloud point extraction**
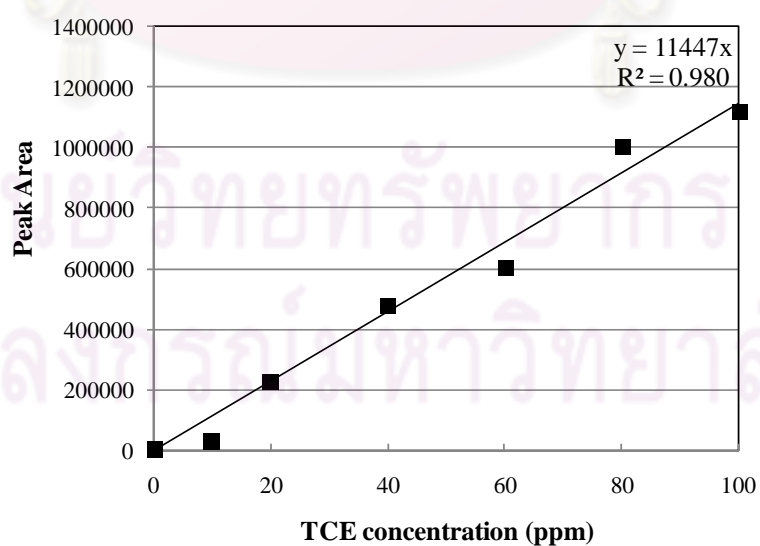


**Figure A-6** Standard curve of TCE in the surfactant-dilute phase

**8. Standard curve of surfactant concentration**
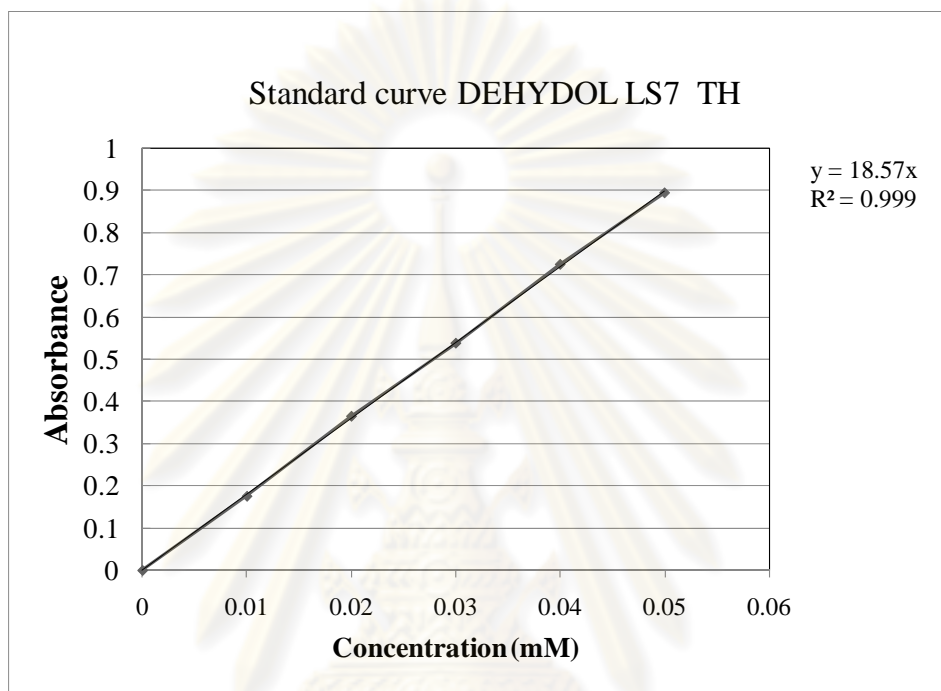


**Figure A-7** Standard curve of remaining surfactant concentration in dilute phase

**APPENDIX B**

## 1. Effect of initial DEHYDOL LS7 TH concentrations on the amounts of remaining surfactant

**Table B-1** The surfactant remaining in surfactant rich phase

| surfactant Conc. (mM) | vial (triplicates) | Absorbance Surfactant-rich phase | | | Abs x dilute factor Surfactant-rich phase | | | Concentration (mM) Surfactant-rich phase | | | AVG | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 (500x) | 1 | 0.0740 | 0.0848 | 0.0572 | 740.0 | 848.0 | 572.0 | 39.8 | 45.7 | 30.8 | | |
| | 2 | 0.0664 | 0.1013 | 0.0548 | 664.0 | 1013.0 | 548.0 | 35.8 | 54.6 | 29.5 | | |
| | 3 | 0.0702 | 0.0751 | NA | 702.0 | 751.0 | NA | 37.8 | 40.4 | | | |
| | Avg | 0.0702 | 0.0871 | 0.0560 | 702.0 | 870.7 | NA | 37.8 | 46.9 | 30.2 | 38.3 | 8.4 |
| 50 (500x) | 1 | 0.2254 | 0.2615 | 0.2133 | 2254.0 | 2615.0 | 2133.0 | 121.4 | 140.8 | 114.9 | | |
| | 2 | 0.2177 | 0.2599 | 0.2135 | 2177.0 | 2599.0 | 2135.0 | 117.2 | 140.0 | 115.0 | | |
| | 3 | 0.2232 | 0.2396 | 0.2147 | 2232.0 | 2396.0 | 2147.0 | 120.2 | 129.0 | 115.6 | | |
| | Avg | 0.2221 | 0.2537 | 0.2138 | 2221.0 | 2536.7 | 2138.3 | 119.6 | 136.6 | 115.1 | 123.8 | 11.3 |
| 70 (500x) | 1 | 0.2863 | 0.3127 | 0.2918 | 2863.0 | 3127.0 | 2918.0 | 154.2 | 168.4 | 157.1 | | |
| | 2 | 0.2791 | 0.3005 | 0.2599 | 2791.0 | 3005.0 | 2599.0 | 150.3 | 161.8 | 140.0 | | |
| | 3 | 0.2684 | 0.3007 | 0.2883 | 2684.0 | 3007.0 | 2883.0 | 144.5 | 161.9 | 155.3 | | |
| | Avg | 0.2779 | 0.3046 | 0.2800 | 2779.3 | 3046.3 | 2800.0 | 149.7 | 164.0 | 150.8 | 154.8 | 8.0 |
| 90 (500x) | 1 | 0.3468 | 0.3339 | 0.4102 | 3468.0 | 3339.0 | 4102.0 | 186.8 | 179.8 | 220.9 | | |
| | 2 | 0.3607 | 0.3470 | 0.4018 | 3607.0 | 3470.0 | 4018.0 | 194.2 | 186.9 | 216.4 | | |
| | 3 | 0.3547 | 0.3506 | 0.4060 | 3547.0 | 3506.0 | 4060.0 | 191.0 | 188.8 | 218.6 | | |
| | Avg | 0.3541 | 0.3438 | 0.4060 | 3540.7 | 3438.3 | 4060.0 | 190.7 | 185.2 | 218.6 | 198.2 | 17.9 |
| 110 (500x) | 1 | 0.3442 | 0.3846 | 0.3616 | 3442.0 | 3846.0 | 3616.0 | 185.4 | 207.1 | 194.7 | | |
| | 2 | 0.3403 | 0.3566 | 0.3865 | 3403.0 | 3566.0 | 3865.0 | 183.3 | 192.0 | 208.1 | | |
| | 3 | 0.3492 | 0.3478 | 0.3485 | 3492.0 | 3478.0 | 3485.0 | 188.0 | 187.3 | 187.7 | | |
| | Avg | 0.3446 | 0.3630 | 0.3655 | 3445.7 | 3630.0 | 3655.3 | 185.6 | 195.5 | 196.8 | 192.6 | 6.2 |

**Table B-2** The surfactant remaining in surfactant dilute-phase

| surfactant conc.(mM) | vial (triplicates) | Absorbance | | | Abs x dilute factor | | | Concentration (mM) | | | Average | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Surfactant-dilute phase | | | Surfactant-dilute phase | | | Surfactant-dilute phase | | | | |
| 30 (500x) | 1 | 0.1851 | 0.1390 | 0.1851 | 92.55 | 69.50 | 92.55 | 4.98 | 3.74 | 3.74 | | |
| | 2 | 0.1685 | 0.1508 | 0.1685 | 84.25 | 75.40 | 84.25 | 4.54 | 4.54 | 4.06 | | |
| | 3 | 0.1549 | 0.1683 | 0.1549 | 77.45 | 84.15 | 77.45 | 4.17 | 4.17 | 4.53 | | |
| | Avg | 0.1695 | 0.1527 | 0.1695 | 84.75 | 76.35 | 84.75 | 4.56 | 4.56 | 4.11 | 4.3 | 0.3 |
| 50 (500x) | 1 | 0.2974 | 0.3114 | 0.2974 | 148.70 | 155.70 | 148.70 | 8.01 | 8.01 | 8.38 | | |
| | 2 | 0.3225 | 0.3157 | 0.3225 | 161.25 | 157.85 | 161.25 | 8.68 | 8.68 | 8.50 | | |
| | 3 | 0.3733 | 0.2898 | 0.3733 | 186.65 | 144.90 | 186.65 | 10.05 | 10.05 | 7.80 | | |
| | Avg | 0.3311 | 0.3056 | 0.3311 | 165.53 | 152.82 | 165.53 | 8.91 | 8.91 | 8.23 | 8.4 | 0.4 |
| 70 (500x) | 1 | 0.4288 | 0.3217 | 0.4288 | 214.40 | 160.85 | 214.40 | 11.55 | 11.55 | 8.66 | | |
| | 2 | 0.3912 | 0.3506 | 0.3912 | 195.60 | 175.30 | 195.60 | 10.53 | 10.53 | 9.44 | | |
| | 3 | 0.4319 | 0.3398 | 0.4319 | 215.95 | 169.90 | 215.95 | 11.63 | 11.63 | 9.15 | | |
| | Avg | 0.4173 | 0.3374 | 0.4173 | 208.65 | 168.68 | 208.65 | 11.24 | 11.24 | 9.08 | 10.3 | 1.1 |
| 90 (500x) | 1 | 0.3865 | 0.3323 | 0.3865 | 193.25 | 166.15 | 193.25 | 10.41 | 10.75 | 9.24 | | |
| | 2 | 0.3920 | 0.3817 | 0.3920 | 190.85 | 196.00 | 196.00 | 10.28 | 10.61 | 10.90 | | |
| | 3 | 0.3852 | 0.3510 | 0.3852 | 175.50 | 192.60 | 192.60 | 9.45 | 9.76 | 10.71 | | |
| | Avg | 0.3879 | 0.3550 | 0.3879 | 186.53 | 184.92 | 193.95 | 10.04 | 10.37 | 10.28 | 10.4 | 0.9 |
| 110 (500x) | 1 | 1.461 | 1.147 | 1.4604 | 730.3 | 573.3 | 730.2 | 39.32 | 30.87 | 39.32 | | |
| | 2 | 1.453 | 0.977 | 1.4800 | 726.6 | 488.3 | 740.0 | 39.13 | 26.29 | 39.85 | | |
| | 3 | 1.411 | 0.995 | 1.4862 | 705.5 | 497.7 | 743.1 | 37.99 | 26.80 | 40.02 | | |
| | Avg | 1.442 | 1.039 | 1.4755 | 720.78 | 519.73 | 737.77 | 38.81 | 27.99 | 39.73 | 32.3 | 6.5 |

**2. Effect of cloud point extraction on the remaining surfactant concentration in various soil types**

**Table B-3** Surfactant concentration and partition ratio of DEHYDOL LS7 TH in various soil types

| soil | Rich phase | | | Dilute phase | | | Surfactant Partition ratio |
|---|---|---|---|---|---|---|---|
| | Absorbance | Conc. | x10000 | Absorbance | Conc. | x500 | |
| Sandy clay loam | 0.31690 | 0.0175 | 175.37 | 0.4130 | 0.0229 | 11.43 | 15.35 |
| | 0.31140 | 0.0172 | 172.33 | 0.4670 | 0.0258 | 12.92 | 13.34 |
| | 0.30400 | 0.0168 | 168.23 | 0.3965 | 0.0219 | 10.97 | 15.33 |
| | | AVG | 171.98 | | AVG | 11.77 | 14.61 |
| | | SD | 3.58 | | SD | 1.020 | 1.157 |
| Sandy loam | 0.2126 | 0.0118 | 117.65 | 0.4653 | 0.0257 | 12.87 | 9.14 |
| | 0.2269 | 0.0126 | 125.57 | 0.4189 | 0.0232 | 11.59 | 10.83 |
| | 0.2453 | 0.0136 | 135.75 | 0.3943 | 0.0218 | 10.91 | 12.44 |
| | | AVG | 126.32 | | AVG | 11.79 | 10.71 |
| | | SD | 9.07 | | SD | 0.99 | 0.99 |

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### 3. Effect of electrolyte addition on TCE removal by using cloud point extraction

**Table B- 4** TCE remaining in soil, surfactant rich-phase, surfactant dilute phase after cloud point extraction from sandy clay loam soil

| Conc. NaCl | Soil | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Peak area | | | concentration | | | | |
| | sample 1 | sample 2 | sample 3 | sample 1 | sample 2 | sample 3 | AVG | SD |
| 0.2 | 426090.39 | 433775.88 | 448135.84 | 22.45 | 22.85 | 23.61 | 22.97 | 0.59 |
| 0.4 | 427213.44 | 426359.45 | 420633.97 | 22.51 | 22.46 | 22.16 | 22.38 | 0.19 |
| 0.6 | 406420.20 | 400864.10 | vial break | 21.41 | 21.12 | NA | 21.27 | 0.21 |
| Control | 495194.38 | 505475.18 | 498695.81 | 26.09 | 26.63 | 26.27 | 26.33 | 0.28 |
| Conc. NaCl | Rich phase | | | | | | | |
| | Peak area | | | concentration | | | | |
| | sample 1 | sample 2 | sample 3 | sample 1 | sample 2 | sample 3 | AVG | SD |
| 0.2 | 249545.49 | 235180.25 | 248156.69 | 285.33 | 268.90 | 283.74 | 279.32 | 9.06 |
| 0.4 | 290563.27 | 281449.35 | 286862.59 | 332.22 | 321.80 | 327.99 | 327.34 | 5.24 |
| 0.6 | 322811.56 | 313993.89 | vial break | 369.10 | 359.01 | NA | 364.06 | 7.13 |
| control | 100566.22 | 99620.34 | 110421.19 | 114.99 | 113.90 | 126.25 | 118.38 | 6.84 |
| Conc. NaCl | Dilute phase | | | | | | | |
| | Peak area | | | concentration | | | | |
| | sample 1 | sample 2 | sample 3 | sample 1 | sample 2 | sample 3 | AVG | SD |
| 0.2 | 91731.83 | 122896.09 | 138752.72 | 4.83 | 6.48 | 7.31 | 6.21 | 1.26 |
| 0.4 | 107605.48 | 114125.88 | 101332.61 | 5.67 | 6.01 | 5.34 | 5.67 | 0.34 |
| 0.6 | 93297.09 | 91007.73 | 98044.52 | 4.92 | 4.79 | 5.17 | 4.96 | 0.19 |
| control | 491068.35 | 494784.00 | 464432.87 | 25.87 | 26.07 | 24.47 | 25.47 | 0.87 |

**Table B- 5** TCE remaining in soil, rich-phase, dilute phase after cloud point extraction from clay soil

| Conc. NaCl | Soil | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Peak area | | | concentration | | | | |
| | sample 1 | sample 2 | sample 3 | sample 1 | sample 2 | sample 3 | AVG | SD |
| 0.2 | 759946.48 | 893134.10 | 802349.26 | 29.67 | 34.87 | 31.32 | 31.95 | 2.66 |
| 0.4 | 706182.76 | 638585.03 | 686702.65 | 27.57 | 24.93 | 26.81 | 26.43 | 1.36 |
| 0.6 | 734243.52 | 641415.65 | vial break | 28.66 | 25.04 | NA | 26.85 | 2.56 |
| Control | The phase separation not well | | | | | | | |
| concentration of NaCl | Rich phase | | | | | | | |
| | Peak area | | | concentration | | | | |
| | sample 1 | sample 2 | sample 3 | sample 1 | sample 2 | sample 3 | AVG | SD |
| 0.2 | 296749.78 | 268754.98 | 298208.38 | 339.29 | 307.28 | 340.96 | 329.18 | 18.9 |
| 0.4 | 315641.00 | 314085.81 | 290998.99 | 360.89 | 359.11 | 332.72 | 350.91 | 15.7 |
| 0.6 | 416888.46 | 335212.17 | 284486.36 | 476.66 | 383.27 | 325.27 | 395.07 | 76.3 |
| control | The phase separation not well | | | | | | | |
| concentration of NaCl | Dilute phase | | | | | | | |
| | Peak area | | | concentration | | | | |
| | samle 1 | sample 2 | sample 3 | sample 1 | sample 2 | sample 3 | AVG | SD |
| 0.2 | 182799.09 | 182053.07 | 176662.88 | 15.96 | 15.9 | 15.43 | 15.76 | 0.29 |
| 0.4 | 163244.68 | 176327.88 | 169707.09 | 14.26 | 15.40 | 14.82 | 14.83 | 0.57 |
| 0.6 | 133682.00 | 135858.17 | | 11.67 | 11.86 | NA | 7.84 | 6.79 |

**Table B-6** TCE remaining in soil surfactant rich-phase, surfactant dilute phase after cloud point extraction from sandy loam soil

| concentration of NaCl | soil phase | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Peak area | | | concontration | | | | |
| | samle 1 | sample 2 | sample 3 | samle 1 | sample 2 | sample 3 | av | sd |
| 0.2 | 523966.63 | 523737.91 | 523122.36 | 30.36 | 30.35 | 30.32 | 30.34 | 0.03 |
| 0.4 | 426716.97 | 438517.85 | vial break | 24.73 | 25.41 | vial break | 25.07 | 0.48 |
| 0.6 | 433642.18 | 417500.49 | vial break | 25.13 | 24.19 | vial break | 24.66 | 0.66 |
| Control | 481284.30 | 505927.24 | 446389.17 | 27.89 | 29.32 | 25.87 | 27.69 | 1.73 |
| concentration of NaCl | Rich phase | | | | | | | |
| | Peak area | | | concontration | | | | |
| | samle 1 | sample 2 | sample 3 | samle 1 | sample 2 | sample 3 | av | sd |
| 0.2 | 315116.18 | 292967.76 | 326800.47 | 360.30 | 334.97 | 373.66 | 356.31 | 19.65 |
| 0.4 | 310600.61 | 323756.05 | 334579.89 | 355.13 | 370.18 | 382.55 | 369.29 | 13.73 |
| 0.6 | 335544.76 | 341684.83 | 338390.89 | 383.66 | 390.68 | 386.91 | 387.08 | 3.51 |
| control | 108974.30 | 126570.19 | 118760.59 | 124.60 | 144.72 | 135.79 | 135.04 | 10.08 |
| concentration of NaCl | dilute phase | | | | | | | |
| | Peak area | | | concontration | | | | |
| | samle 1 | sample 2 | sample 3 | samle 1 | sample 2 | sample 3 | av | sd |
| 0.2 | 125142.45 | 159331.14 | 147140.54 | 10.93 | 13.92 | 12.85 | 12.57 | 1.51 |
| 0.4 | 108053.43 | 107162.29 | 116098.78 | 9.44 | 9.36 | 10.14 | 9.65 | 0.43 |
| 0.6 | 111819.37 | 102178.83 | vial break | 9.77 | 8.93 | NA | 9.35 | 0.60 |
| control | 1169314.74 | 1278509.67 | 1323508.65 | 102.15 | 111.69 | 115.62 | 109.82 | 6.93 |

**Mass balance of TCE from cloud point extraction using DEHYDOL LS7 TH with adding NaCl**

**Table B-7**  Mass balance and % recovery of TCE in  sandy clay loam soil

| concentration of NaCl | Sandy clay loam soil | | | | %recovery |
|---|---|---|---|---|---|
| | mass of TCE  (mg) | | | | |
| | rich | dilute phase | soil | total mass | |
| 0.2 | 1.59 | 0.14 | 0.06 | 1.79 | 64.09 |
| 0.4 | 1.47 | 0.13 | 0.06 | 1.67 | 59.65 |
| 0.6 | 1.46 | 0.12 | 0.06 | 1.64 | 58.57 |

**Table B-8** Mass balance and % recovery of TCE in clay   soil

| concentration of NaCl | Clay soil | | | | %recovery |
|---|---|---|---|---|---|
| | mass of TCE  (mg) | | | | |
| | rich | dilute phase | soil | total mass | |
| 0.2 | 1.88 | 0.22 | 0.09 | 2.19 | 78.09 |
| 0.4 | 1.65 | 0.22 | 0.07 | 1.95 | 69.49 |
| 0.6 | 1.70 | 0.17 | 0.07 | 1.94 | 69.33 |

**Table B-9**  Mass balance and % recovery of TCE in sandy   loam soil

| concentration of NaCl | Sandy loam soil | | | | %recovery |
|---|---|---|---|---|---|
| | mass of TCE  (mg) | | | | |
| | rich | dilute phase | soil | total mass | |
| 0.2 | 2.24 | 0.18 | 0.08 | 2.51 | 89.49 |
| 0.4 | 1.96 | 0.15 | 0.07 | 2.18 | 77.85 |
| 0.6 | 1.66 | 0.16 | 0.07 | 1.89 | 67.49 |

**4. Effect of NaCl concentrations on the TCE partition ratio from various soil types.**

**Table B-10** Surfactant concentration in surfactant rich phase of DEHYDOL LS7 TH in various soil types

| Sandy clay loam | Rich phase | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Absorbance | | | Concentration | | | dilution factor x10000 | | | | |
| | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | AVG | SD |
| 0.2 | 0.4674 | 0.4956 | 0.4946 | 0.026 | 0.028 | 0.028 | 261.26 | 277.03 | 276.47 | 271.59 | 8.94 |
| 0.4 | 0.5805 | 0.5525 | 0.5851 | 0.032 | 0.031 | 0.033 | 324.48 | 308.83 | 327.05 | 320.12 | 9.86 |
| 0.6 | 0.6497 | 0.659 | 0.6584 | 0.036 | 0.037 | 0.037 | 363.16 | 368.36 | 368.03 | 366.52 | 2.91 |
| Control no salt | 0.2522 | 0.2230 | 0.2089 | 0.014 | 0.012 | 0.012 | 140.97 | 124.65 | 116.77 | 127.46 | 12.34 |
| Clay | Absorbance | | | Concentration | | | dilution factor x10000 | | | | |
| | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | AVG | SD |
| 0.2 | 0.6715 | 0.6837 | 0.6797 | 0.038 | 0.038 | 0.038 | 375.35 | 382.17 | 379.93 | 379.15 | 3.48 |
| 0.4 | 0.8606 | 0.8541 | 0.8968 | 0.048 | 0.048 | 0.050 | 481.05 | 477.42 | 501.29 | 486.58 | 12.86 |
| 0.6 | 0.6471 | 0.6568 | 0.6536 | 0.036 | 0.037 | 0.037 | 361.71 | 367.13 | 365.34 | 364.73 | 2.76 |
| Control no salt | The phase separation not well | | | | | | | | | | |
| Sandy loam | Absorbance | | | Concentration | | | dilution factor x10000 | | | | |
| | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | AVG | SD |
| 0.2 | 0.4866 | 0.4800 | 0.4445 | 0.027 | 0.027 | 0.025 | 272.00 | 268.31 | 248.46 | 262.92 | 2.65 |
| 0.4 | 0.5095 | 0.4790 | 0.4972 | 0.028 | 0.027 | 0.028 | 284.80 | 267.75 | 277.92 | 276.82 | 8.57 |
| 0.6 | 0.5601 | 0.5780 | 0.5629 | 0.031 | 0.032 | 0.031 | 313.08 | 323.09 | 314.65 | 316.94 | 5.38 |
| Control no salt | 0.2040 | 0.1811 | 0.1548 | 0.011 | 0.010 | 0.009 | 114.03 | 101.23 | 86.53 | 100.60 | 13.76 |

**Table B-11** Surfactant concentration in surfactant dilute-phase of DEHYDOL LS7 TH in various soil types

| Sandy clay loam | Dilute phase | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Absorbance | | | Concentration | | | dilution factor x100 | | | | |
| | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | AVG | SD |
| 0.2 | 0.5108 | 0.5073 | 0.5583 | 0.029 | 0.028 | 0.031 | 2.86 | 2.84 | 3.12 | 2.94 | 0.16 |
| 0.4 | 0.4191 | 0.4132 | 0.4261 | 0.023 | 0.023 | 0.024 | 2.34 | 2.31 | 2.38 | 2.34 | 0.04 |
| 0.6 | 0.3891 | 0.3785 | 0.3892 | 0.022 | 0.021 | 0.022 | 2.17 | 2.12 | 2.18 | 2.15 | 0.03 |
| Control no salt | 0.2882 | 0.3095 | 0.2855 | 0.016 | 0.017 | 0.016 | 1.61 | 1.73 | 1.60 | 1.65 | 0.07 |
| Clay | Absorbance | | | Concentration | | | dilution factor x500 | | | | |
| | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | AVG | SD |
| 0.2 | 0.13380 | 0.06940 | 0.07190 | 0.0075 | 0.0039 | 0.0040 | 3.74 | 1.94 | 2.01 | 2.56 | 1.02 |
| 0.4 | 0.07070 | 0.06360 | 0.07700 | 0.0040 | 0.0036 | 0.0043 | 1.98 | 1.78 | 2.15 | 1.97 | 0.19 |
| 0.6 | 0.03270 | 0.03520 | 0.03620 | 0.0018 | 0.0020 | 0.0020 | 0.91 | 0.98 | 1.01 | 0.97 | 0.05 |
| Control no salt | The phase separation not well | | | | | | | | | | |
| Sandy loam | Absorbance | | | Concentration | | | dilution factor x500 | | | | |
| | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | Sample 1 | Sample 2 | Sample3 | AVG | SD |
| 0.2 | 0.0843 | 0.0836 | 0.0892 | 0.0047 | 0.0047 | 0.0050 | 2.36 | 2.34 | 2.49 | 2.39 | 0.09 |
| 0.4 | 0.1480 | 0.0645 | 0.0401 | 0.0083 | 0.0036 | 0.0022 | 4.14 | 1.80 | 1.12 | 2.35 | 1.58 |
| 0.6 | 0.0361 | 0.0357 | 0.0437 | 0.0020 | 0.0020 | 0.0024 | 1.01 | 1.00 | 1.22 | 1.07 | 0.12 |
| Control no salt | 0.4653 | 0.4189 | 0.3943 | 0.0260 | 0.0234 | 0.0220 | 13.00 | 234.15 | 220.40 | 155.85 | 123.90 |

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**Table B-12** Mass balance and % recovery of surfactant in sandy clay loam soil

| concentration of NaCl | Sandy clay loam soil | | | | |
| | mass of surfactant (mg) | | | Initial total mass of surfactant at 90 mM (mg) | % mass recovery in solution |
| | Rich phase | Dilute phase | total mass in solution | | |
| 0.2 | 0.64 | 0.02 | 0.66 | 0.815 | 81.197 |
| 0.4 | 0.61 | 0.02 | 0.63 | 0.815 | 77.141 |
| 0.6 | 0.55 | 0.02 | 0.57 | 0.815 | 69.876 |

**Table B-13** Mass balance and % recovery of surfactant in clay  soil

| concentration of NaCl | Clay soil | | | | |
| | mass of surfactant (mg) | | | Initial total mass of surfactant at 90 mM (mg) | % mass recovery in solution |
| | Rich phase | Dilute phase | total mass in solution | | |
| 0.2 | 0.61 | 0.02 | 0.63 | 0.86 | 73.69 |
| 0.4 | 0.71 | 0.02 | 0.73 | 0.86 | 84.79 |
| 0.6 | 0.51 | 0.01 | 0.52 | 0.86 | 60.95 |

**Table B-14** Mass balance and % recovery of surfactant in   sandy loam soil

| concentration of NaCl | Sandy loam soil | | | | |
| | mass of surfactant (mg) | | | Initial total mass of surfactant at 90 mM (mg) | % mass recovery in solution |
| | Rich phase | Dilute phase | total mass in solution | | |
| 0.2 | 0.83 | 0.02 | 0.85 | 0.92 | 92.04 |
| 0.4 | 0.74 | 0.02 | 0.76 | 0.92 | 81.93 |
| 0.6 | 0.75 | 0.01 | 0.76 | 0.92 | 82.05 |

**APPENDIX C**

**1. The average size of cell immobilization on gellan gum bead**

**Table C- 1** The sizes of beads were measured the diameter by average 50 beads.

| Bead size (cm.) | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | AVG | SD |
| 0.5 | 0.5 | 0.6 | 0.6 | 0.5 | | |
| 0.5 | 0.5 | 0.5 | 0.6 | 0.6 | | |
| 0.5 | 0.5 | 0.6 | 0.6 | 0.5 | | |
| 0.6 | 0.5 | 0.5 | 0.5 | 0.6 | | |
| 0.5 | 0.5 | 0.6 | 0.6 | 0.5 | 0.56 | 0.050 |
| 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | | |
| 0.5 | 0.5 | 0.6 | 0.6 | 0.5 | | |
| 0.5 | 0.5 | 0.6 | 0.6 | 0.5 | | |
| 0.5 | 0.5 | 0.5 | 0.6 | 0.6 | | |
| 0.5 | 0.5 | 0.6 | 0.6 | 0.5 | | |

## 2. Effect of increase TCE concentration on the encapsulated cell

**Table C-2** Peak area of TCE, % TCE remaining and % TCE degradation of
encapsulated cell with increase initial TCE concentration without adding NaCl

| Initial TCE conc. (ppm) | Time (day) | peak area | % TCE reaming | AVG | SD | % TCE degradation | AVG | SD |
|---|---|---|---|---|---|---|---|---|
| 10 | 0 | 182318.81 | 106.00 | 100.17 | 8.24 | | | |
| | | 171999.36 | 94.34 | | | | | |
| | | | | | | | | |
| | 1 | 108756.47 | 59.65 | 60.46 | 1.14 | 40.35 | 39.54 | 1.14 |
| | | 105366.67 | 61.26 | | | 38.74 | | |
| | | vial break | | | | | | |
| | 2 | 87570.79 | 48.03 | 48.55 | 0.73 | 51.97 | 51.45 | 0.73 |
| | | 84392.27 | 49.07 | | | 50.93 | | |
| | | vial break | | | | | | |
| 20 | 0 | 336717.32 | 98.17 | 100.02 | 2.61 | | | |
| | | 342979.29 | 101.86 | | | | | |
| | | 340857.71 | | | | | | |
| | 1 | 239514.30 | 71.13 | 70.43 | 0.99 | 28.87 | 29.57 | 0.99 |
| | | 239158.45 | 69.73 | | | 30.27 | | |
| | | vial break | | | | | | |
| | 2 | 216590.83 | 64.32 | 64.85 | 0.47 | 35.68 | 35.22 | 0.64 |
| | | 223743.80 | 65.24 | | | 34.76 | | |
| | | 221543.66 | 65.00 | | | | | |

**Table C-2** (cont.)

| Initial TCE conc. (ppm) | Time (day) | peak area | % TCE reaming | AVG | SD | % degradation | AVG | SD |
|---|---|---|---|---|---|---|---|---|
| 30 | 0 | 646341.90 | 100.90 | 99.96 | 0.89 | | | |
| | | 640602.03 | 99.11 | | | | | |
| | | 645575.91 | 99.88 | | | | | |
| | 1 | 428288.94 | 66.26 | 68.58 | 3.27 | 33.74 | 31.42 | 3.27 |
| | | 454132.11 | 70.89 | | | 29.11 | | |
| | | vial break | | | | vial break | | |
| | 2 | 384925.82 | 59.55 | 60.52 | 1.37 | 40.45 | 39.48 | 1.37 |
| | | 393883.91 | 61.49 | | | 38.51 | | |
| | | vial break | | | | vial break | | |
| 40 | 0 | 1202521.88 | 106.14 | 102.00 | 6.74 | | | |
| | | 1132998.92 | 94.22 | | | | | |
| | | 1270357.78 | 105.64 | | | | | |
| | 1 | 1059899.91 | 88.14 | 87.50 | 1.54 | 11.86 | 11.62 | 0.34 |
| | | 1004020.53 | 88.62 | | | 11.38 | | |
| | | 1089297.12 | 85.75 | | | vial break | | |
| | 2 | 980197.86 | 81.51 | 81.78 | 0.37 | 18.49 | 18.22 | 0.37 |
| | | 929498.95 | 82.04 | | | 17.96 | | |
| | | vial break | vial break | | | vial break | | |

**Table C- 2** (cont.)

| Initial TCE conc. (ppm) | Time (day) | peak area | % TCE remaining | AVG | SD | % TCE degradation | AVG | SD |
|---|---|---|---|---|---|---|---|---|
| 50 | 0 | 1576946.98 | 95.19 | 100.44 | 11.79 | | | |
| | | 1656616.98 | 113.95 | | | | | |
| | | 1453789.98 | 92.19 | | | | | |
| | 1 | 1513298.15 | 95.96 | 93.97 | 2.82 | 4.04 | 6.03 | 2.82 |
| | | 1523668.67 | 91.97 | | | 8.03 | | |
| | | vial break | | | | vial break | | |
| | 2 | 1519350.82 | 96.35 | 97.15 | 4.71 | 3.65 | 5.39 | 2.45 |
| | | 1538696.89 | 92.88 | | | 7.12 | | |
| | | 1485885.04 | 102.21 | | | vial break | | |

**Table C-3** Peak area of TCE, % TCE remaining and % TCE degradation of
encapsulated cell with increase initial TCE concentration with NaCl 0.2 M

| Initial TCE conc. (ppm) | Time (day) | peak area | % TCE reaming | AVG | SD | % degradation | AVG | SD |
|---|---|---|---|---|---|---|---|---|
| 10 | 0 | 257662.14 | 97.60 | | | | | |
| | | 263990.08 | 102.31 | | | | | |
| | | 258024.04 | 100.14 | 100.01 | 2.35 | | | |
| | 1 | 175376.80 | 68.06 | | | 31.94 | | |
| | | 174414.14 | 66.07 | | | 33.93 | 32.43 | 1.33 |
| | | 176968.55 | 68.59 | 67.57 | 1.3 | 31.41 | | |
| | 2 | 140339.69 | 54.47 | | | 45.53 | | |
| | | 151669.16 | 57.45 | | | 42.55 | 42.45 | 3.14 |
| | | 156732.23 | 60.74 | 57.55 | 3.13 | 39.26 | | |
| 20 | 0 | 593475.33 | 107.63 | | | | | |
| | | 551383.65 | 99.32 | | | | | |
| | | 555155.50 | 93.54 | 100.16 | 7.0 | | | |
| | 1 | 431072.79 | 72.64 | | | 27.36 | | |
| | | 434542.12 | 78.81 | | | 21.19 | | |
| | | 428969.95 | 77.27 | 76.23 | 3.2 | 22.73 | 23.76 | 3.21 |
| | 2 | 319772.02 | 53.88 | | | 46.12 | | |
| | | 312540.59 | 56.68 | | | 43.32 | | |
| | | 311338.42 | 56.08 | 55.54 | 1.4 | 43.92 | 44.45 | 1.47 |

**Table C-3** (cont.)

| Initial TCE conc. (ppm) | Time (day) | peak area | % TCE reaming | AVG | SD | % degradation | AVG | SD |
|---|---|---|---|---|---|---|---|---|
| 30 | 0 | 835935.31 | 101.72 | | | | | |
| | | 821793.77 | 97.31 | 100.02 | | | | |
| | | 844537.58 | 101.03 | | 2.37 | | | |
| | 1 | 650078.84 | 77.77 | | | 22.23 | | |
| | | 646057.31 | 78.62 | | | 21.38 | | |
| | | 624637.80 | 73.96 | 76.78 | 2.48 | 26.04 | 23.22 | 2.48 |
| | 2 | 506670.10 | 60.61 | | | 39.39 | | |
| | | 502092.85 | 61.10 | | | 38.90 | | |
| | | 506816.16 | 60.01 | 60.57 | 0.54 | 39.99 | 39.43 | 0.54 |
| 40 | 0 | 937455.35 | 98.26 | | | | | |
| | | 954045.59 | 98.51 | 100.03 | | | | |
| | | 968488.56 | 103.31 | | 2.85 | | | |
| | 1 | 802554.70 | 85.61 | | | 14.39 | | |
| | | 817696.14 | 85.71 | | | 14.29 | | |
| | | 801762.15 | 82.78 | 84.70 | 1.66 | 17.22 | 15.30 | 1.66 |
| | 2 | 801454.26 | 85.49 | | | 14.51 | | |
| | | 780907.22 | 81.85 | | | 18.15 | | |
| | | 767623.95 | 79.26 | 82.20 | 3.13 | 20.74 | 17.80 | 3.13 |
| 50 | 0 | 1421350.87 | 99.58 | | | | | |
| | | 1427205.03 | 96.67 | 100.04 | | | | |
| | | 1476298.09 | 103.86 | | 3.61 | | | |
| | 1 | 1397158.76 | 98.29 | | | 1.70 | | |
| | | 1386067.80 | 97.11 | | | 2.88 | | |
| | | 1371320.91 | 92.88 | 96.10 | 2.84 | 7.11 | 3.90 | 2.84 |
| | 2 | 1371554.58 | 96.49 | | | 3.50 | | |
| | | 1358097.02 | 95.15 | | | 4.84 | | |
| | | 1369216.17 | 92.74 | 94.80 | 1.90 | 7.25 | 5.20 | 1.90 |

**Table C – 3** Peak area of TCE, % TCE remaining and % TCE degradation of encapsulated cell with increase initial TCE concentration with killed cell encapsulated in gellan gum

| Initial TCE conc. (ppm) | Time (day) | peak area | % TCE reaming | AVG | SD |
|---|---|---|---|---|---|
| 10 | 0 | 291980.35 | 104.12 | 100.08 | 5.71 |
| | | 280432.87 | 96.05 | | |
| | | | | | |
| | 2 | 300339.69 | 102.86 | 105.22 | 3.33 |
| | | 301669.16 | 107.57 | | |
| | | | | | |
| 20 | 0 | 496712.72 | 100.68 | 100.00 | 0.96 |
| | | 493369.28 | 99.33 | | |
| | | | | | |
| | 2 | 512540.59 | 103.19 | 103.45 | 0.37 |
| | | 511641.91 | 103.70 | | |
| | | | | | |
| 30 | 0 | 875935.31 | 100.48 | 100.00 | 0.67 |
| | | 871793.77 | 99.53 | | |
| | | | | | |
| | 2 | 900309.47 | 102.78 | 103.14 | 0.51 |
| | | 902290.08 | 103.50 | | |
| | | | | | |
| 40 | 0 | 1086083.64 | 105.25 | 100.13 | 7.24 |
| | | 1031930.06 | 95.01 | | |
| | | | | | |
| | 2 | 1198485.30 | 110.35 | 118.12 | 10.99 |
| | | 1299161.22 | 125.90 | | |
| | | | | | |
| 50 | 0 | 1540641.40 | 99.56 | 100.00 | 0.62 |
| | | 1547416.37 | 100.44 | | |
| | | | | | |
| | 2 | 1735571.44 | 112.65 | 112.78 | 0.19 |
| | | 1747275.92 | 112.92 | | |
| | | | | | |

**APPENDIX D**

## 1. Water flushing Process

**Table D-1**  TCE remaining in soil and aqueous solution in water flushing process in Sandy clay loam soil

| | Sandy clay loam | | | |
|---|---|---|---|---|
| | TCE concentration (mg/kg) | | | |
| | Soil T=0 | aqueous  T = 0 | Soil T = 48 | aqueous T= 48 |
| | 86.31 | 72.44 | 87.73 | 61.87 |
| | 99.24 | 81.71 | 89.26 | 70.28 |
| | 98.25 | 69.66 | 87.26 | 61.71 |
| AVG | 94.60 | 74.60 | 88.08 | 64.62 |
| SD | 7.20 | 6.31 | 1.04 | 4.90 |

**Table D-2** TCE remaining in soil and aqueous solution in water flushing process Clay soil

| | Clay | | | |
|---|---|---|---|---|
| | TCE concentration (mg/kg) | | | |
| | Soil T=0 | aqueous T = 0 | Soil T = 48 | aqueous T= 48 |
| | 72.79 | 91.93 | 76.86 | 79.24 |
| | 86.05 | 109.03 | 64.04 | 102.58 |
| | 79.50 | 101.41 | 74.63 | 91.36 |
| AVG | 79.45 | 100.79 | 71.84 | 91.06 |
| SD | 6.63 | 8.57 | 6.85 | 11.68 |

**Table D-3** TCE remaining in soil and aqueous solution in water flushing process Sandy loam soil

| | Sandy loam | | | |
|---|---|---|---|---|
| | TCE concentration (mg/kg) | | | |
| | Soil T=0 | aqueous T = 0 | Soil T = 48 | aqueous T= 48 |
| | 74.77 | 72.26 | 70.09 | 70.40 |
| | 88.82 | 74.40 | 88.36 | 69.96 |
| | 88.16 | 75.04 | 87.41 | 71.60 |
| AVG | 83.91 | 73.90 | 81.95 | 70.65 |
| SD | 7.93 | 1.45 | 10.28 | 0.85 |

**Table D-4** Mass balance and % recovery of TCE from water flushing

| soil | mass balance from water flushing | | | |
|---|---|---|---|---|
| | dilute phase (mg) | Soil (mg) | total mass (mg) | %mass recovery |
| sandy clay loam soil | 1.45 ±0.05 | 0.26 ±0.02 | 1.72 ±0.05 | 61.36 ± 1.78 |
| clay soil | 2.01 ± 0.31 | 0.22 ±0.22 | 2.23 ± 0.30 | 79.73 ± 10.80 |
| sandy loam soil | 2.27 ± 0.01 | 0.23 ±0.02 | 2.51 ± 0.01 | 87.53 ± 0.49 |

## 2. Cloud point extraction

**2.1** TCE remaining in surfactant rich-phase, dilute-phase and soil after cloud point extraction process without adding NaCl

**Table D-5** TCE remaining after cloud point extraction without adding NaCl in Sandy clay loam soil

| | sandy clay loam | | |
|---|---|---|---|
| | TCE remaining (mg/kg) | | |
| | dilute phase (mg/l) | rich phase (mg/l) | Soil (mg/kg) |
| | 42.90 | 91.21 | 26.09 |
| | 33.11 | 86.26 | 26.63 |
| | 34.20 | 90.45 | 26.78 |
| AVG | 36.74 | 89.31 | 26.33 |
| SD | 5.36 | 2.67 | 0 |

**Table D-6**  TCE remaining after cloud point extraction without adding NaCl in

Sandy loam soil

| sandy loam | | |
|---|---|---|
| TCE remaining | | |
| dilute phase (mg/l) | rich phase (mg/l) | Soil (mg/kg) |
| 121.03 | 119.29 | 27.89 |
| 124.45 | 207.45 | 29.31 |
| 109.61 | 221.61 | 25.86 |
| AVG 118.36 | 182.79 | 27.69 |
| SD 7.77 | 55.44 | 1.73 |

**Table D-7**  Mass balance and % recovery of TCE from cloud point extraction without

adding NaCl

| soil | mass balance from cloud point extraction | | | | % TCE recovery |
|---|---|---|---|---|---|
| | rich phase (mg) | dilute phase (mg) | soil (mg) | total mass | |
| sandy clay loam soil | 0.68 | 0.8 | 0.07 | 1.55 | 55.33 |
| clay soil | phase separation not separate well | | | | |
| sandy loam soil | 1.14 | 1.25 | 0.08 | 2.46 | 87.94 |

**2.2** TCE remaining in surfactant dilute-phase in combined bioremediation after cloud point extraction without NaCl

**Table D-8** TCE remaining combined bioremediation after cloud point extraction without NaCl Sandy clay loam soil

Sandy clay loam

| | TCE remaining (mg/l) | | % TCE removal efficiency |
|---|---|---|---|
| | dilute phase T = 0 | dilute phase T= 96 | |
| | 65.81 | 44.43 | 32.49 |
| | 67.75 | 49.06 | 27.58 |
| AVG | 66.78 | 46.74 | 30.04 |
| SD | 1.37 | 3.28 | 3.47 |

**Table D-9** TCE remaining combined bioremediation after cloud point extraction without NaCl in Sandy loam soil

Sandy loam

| | TCE remaining (mg/l) | | % TCE removal efficiency |
|---|---|---|---|
| | dilute phase T = 0 | dilute phase T= 96 | |
| | 97.09 | 62.60 | 35.52 |
| | 133.42 | 75.79 | 43.20 |
| | 111.51 | 77.46 | 30.54 |
| AVG | 114.01 | 71.95 | 36.42 |
| SD | 18.30 | 8.14 | 6.38 |

## 3. Combined cloud point extraction and bioremediation process

3.1 TCE remaining in surfactant rich-phase, surfactant dilute-phase and soil after cloud point extraction in the presence of NaCl

**Table D-10** TCE remaining after cloud point extraction of Sandy clay loam  soil

Sandy Clay loam

| | TCE remaining after cloud point extraction | | |
|---|---|---|---|
| | rich phase (mg/l) | dilute phase (mg/l) | Soil (mg/kg) |
| | 292.94 | 14.66 | 30.57 |
| | 311.28 | 10.59 | 29.67 |
| | 286.05 | 12.02 | 29.69 |
| Av | 296.76 | 12.42 | 29.98 |
| SD | 13.04 | 2.06 | 0.52 |

**Table D-11** TCE remaining after cloud point extraction of Clay soil

Clay

| | TCE remaining cloud point extraction | | |
|---|---|---|---|
| | rich phase (mg/l) | dilute phase (mg/l) | Soil (mg/kg) |
| | 414.07 | 18.62 | 32.48 |
| | 434.23 | 24.17 | 32.73 |
| | 451.76 | 21.14 | 31.68 |
| AV | 433.35 | 21.31 | 32.40 |
| SD | 18.86 | 2.78 | 0.60 |

**Table D-12** TCE remaining after cloud point extraction of Sandy loam soil

Sandy loam

| | TCE remaining cloud point extraction | | |
|---|---|---|---|
| | rich phase (mg/l) | dilute phase (mg/l) | Soil (mg/kg) |
| | 377.64 | 19.43 | 27.54 |
| | 322.92 | 16.95 | 32.60 |
| | 373.21 | 14.29 | 34.42 |
| AV | 357.92 | 16.89 | 31.52 |
| SD | 30.39 | 2.57 | 3.56 |

**Table D-13** Mass balance and % recovery of TCE from cloud point extraction in the presence of NaCl

| | mass balance from cloud point extraction in the presence of NaCl | | | | |
|---|---|---|---|---|---|
| soil | rich phase (mg) | dilute phase (mg) | soil (mg) | total mass | % TCE recovery |
| sandy clay loam | 1.39 ±0.06 | 0.17 ±0.02 | 0.08 ± 0.001 | 1.64 ± 0.05 | 58.71±1.82 |
| clay | 2.03 ± 0.08 | 0.28 ± 0.03 | 0.09 ±0.001 | 2.41 ± 0.11 | 86.09 ±3.95 |
| sandy loam | 1.68 ±0.14 | 0.22 ± 0.03 | 0.08 ±0.009 | 1.99 ±0.14 | 71.25 ± 5.12 |

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## 3. Combined cloud point extraction and bioremediation from various soil types

3.1 TCE remaining in combined process surfactant rich-phase, surfactant
dilute-phase and soil after cloud point extraction in the presence with NaCl

**Table D-14** TCE remaining in combined cloud point extraction and bioremediation
in Sandy clay loam soil in the presence of NaCl

| | Sandy Clay loam soil | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TCE conc. After cloud point extraction (mg/l) | | TCE remaining in combined process | | | | % TCE degradation | |
| | | | Bioremediation After Cloud point | | | | | |
| | dilute phase | rich phase | Soil T=0 (mg/kg) | dilute phase T = 0 (mg/l) | Soil T = 96 (mg/kg) | dilute phase T= 48 | soil | dilute phase |
| | 14.66 | 292.94 | 28.48 | 13.31 | 16.92 | 6.51 | 98.31 | 51.10 |
| | 10.59 | 311.28 | 28.17 | 12.94 | 17.66 | 6.23 | 98.23 | 51.84 |
| | 12.02 | 286.05 | 29.80 | 14.05 | 21.99 | 6.12 | 97.80 | 56.47 |
| Av | 12.42 | 296.76 | 28.82 | 13.43 | 18.86 | 6.29 | 98.11 | 53.14 |
| SD | 2.06 | 13.04 | 0.86 | 0.57 | 2.74 | 0.20 | 0.27 | 2.91 |

**Table D-15** TCE remaining in combined cloud point extraction and bioremediation in
Clay soil in the presence of NaCl

| | Clay soil | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TCE conc. After cloud point extraction (mg/l) | | TCE remaining in combined process | | | | % TCE degradation | |
| | | | Bioremediation After Cloud point | | | | | |
| | dilute phase | rich phase | Soil T=0 (mg/kg) | dilute phase T = 0 (mg/l) | Soil T = 96 (mg/kg) | dilute phase T= 48 | soil | dilute phase |
| | 18.62 | 414.07 | 30.48 | 23.81 | 17.39 | 9.85 | 98.26 | 58.65 |
| | 24.17 | 434.23 | 33.73 | 21.94 | 16.51 | 11.03 | 98.35 | 49.74 |
| | 21.14 | 451.76 | 29.68 | 22.36 | 20.65 | 11.18 | 97.94 | 49.99 |
| AV | 21.31 | 433.35 | 31.30 | 22.71 | 18.18 | 10.69 | 98.18 | 52.79 |
| SD | 2.78 | 18.86 | 2.15 | 0.98 | 2.18 | 0.73 | 0.22 | 5.07 |

**Table D-16** TCE remaining in combined cloud point extraction and bioremediation in Sandy loam soil in the presence of NaCl

| | Sandy loam | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TCE conc. After cloud point extraction (mg/l) | | TCE remaining in combined process | | | | % TCE degradation | |
| | | | Bioremediation After Cloud point | | | | | |
| | dilute phase | rich phase | Soil T=0 (mg/kg) | dilute phase T = 0 (mg/l) | Soil T = 96 (mg/kg) | dilute phase T= 48 | soil | dilute phase |
| | 19.43 | 377.64 | 27.54 | 16.09 | 18.22 | 7.72 | 98.18 | 52.03 |
| | 16.95 | 322.92 | 32.60 | 15.16 | 23.31 | 6.48 | 97.67 | 57.25 |
| | 14.29 | 373.21 | 34.42 | 16.04 | 15.78 | 6.94 | 98.42 | 56.75 |
| AV | 16.89 | 357.92 | 31.52 | 15.76 | 19.11 | 7.05 | 98.09 | 55.34 |
| SD | 2.57 | 30.39 | 3.56 | 0.52 | 3.84 | 0.62 | 0.38 | 2.88 |

# BIOGRAPHY

Miss Witchaya Kaewtip was born on July 19, 1984 in Phatthalung, Thailand. She attended Stree Phatthalung School in Phatthalung and graduated in 2002. She graduated with a second class honors in Bachelor degree of Science in Environmental Science and Technology from 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 Center of Excellence for Environmental and Hazardous Waste Management (NCE-EHWM), Graduate School, Chulalongkorn University, Bangkok, Thailand since 2006-2008.