CHAPTER I
INTRODUCTION

1.1 Introduction

Trichloroethylene (TCE) is widely used in various industrial applications such as solvent to remove grease from metal parts, industrial dry-cleaning, printing, production of printing ink and paint, extraction process and textile printing. TCE enters the environment via an improper management such as storage, treatment facilities and disposal due to lack of knowledge and environmental concern of the manufacturers. Primary mechanism of TCE removal from surface water to the atmosphere is evaporation while photo oxidation, hydrolysis and biodegradation played insignificant role (CEPA, 1993). Half lives of TCE from surface water to the air are in the ranges of several days to several weeks. In soil, volatization is the principal process of TCE elimination. The mobility of TCE in soil depends on the organic carbon content which affects TCE sorption to the soil (CEPA, 1993).

TCE is a Dense Non-Aqueous Phase Liquids (DNAPLs) so it does not move with the groundwater flow but instead move downward by gravitation force through an aquifer until reaching an impermeable layer. Thus, DNAPLs can serve as a long-term source for dissolved contaminant plumes at many contamination sites (CEPA, 1993). Environmental Research and Training Center (ERTC) expects this situation would be the most important crisis in the future (ERTC, 2001). The contamination of TCE in environment is a serious problem because TCE is known to be a probably human carcinogenic substance (IARC, 1995). Therefore, the appropriate treatment technologies are required.

Variety of remediation technologies such as air stripping, carbon adsorption, soil venting and pump and treat have been applied to remove TCE from the contaminated areas. However, these technologies do not decompose TCE into a harmless substance and require the final disposal which increase operation cost. This limitation can be overcome by bioremediation. Microorganisms are the key to a successful bioremediation in which the contaminant will be metabolized as their energy source. By the activities of microorganisms, bioremediation can be used to treat contaminant until its concentration is in the acceptable level. In addition, bioremediation is cost-effective and non-complex to operate (Vidali, 2001).

Bioaugmentation and biostimulation are the promising bioremediation techniques for hazardous waste clean-up technology. Bioaugmentation is the technique that microorganisms capable of degrading the target contaminant are added into the contaminated matrix in order to enhance degradation efficiency (Vidali, 2001). Two types of microorganisms can be added into contaminated soil i.e., mixed culture such as those obtained from acclimatized activated sludge and pure culture. Main advantage of mixed culture as a key issue in field applications of bioremediation is their abilities to survive in a non-sterile environment (Murialdo et al., 2003) while the main advantage of pure culture is a convenient to monitor compared to mixed cultures. Successful bioaugmentation of TCE with pure culture was reported. For example, *Mycobacterium vaccae* JOB5 degraded up to 99% at the initial concentration of 20 uM of TCE in 24 hours (Wackett et al.,1989). *Burkholderia cepacia* G4, *Burkholderia pickettii* PKO1, and *Pseudomonas mendocina* KR1 degraded 36 to 67% of the TCE in 18 hours (Leahy et al., 1996). However, there is a limited information on using mixed culture in bioaugmentation of TCE. Thus, we are interested in examining a removal of TCE from soil by using mixed cultures obtained from acclimatized activated sludge compared to a pure culture of *Rhodococcus* sp P3.

Previous research had shown that a survival of free cell is low in natural condition (Kourkoutus et al., 2004). Immobilization is an attractive technique to solve this constraint because immobilized cultures tend to have a higher level of activity and more tolerant to environmental perturbations such as pH, temperature or toxicity of contaminants than free cells. Support materials could be synthetically made such as alginate, and polyvinyl alcohol and naturally available such as corncob and coir. Synthetic support materials are costly and difficult to be degraded due to a non-biodegradation characteristic. Therefore, there is an interest toward the use of natural materials such as agricultural residues in order to overcome this problem. Effective support materials using agricultural residues include coconut fiber (Pattanasupong et al., 2004), corncob powder (Labana et al., 2005), wheat straw and maple woodchips (Shin, et al., 2002) had been reported for bioremediation treatment. In this study, it is interesting to use corncob and coir to immobilize the inocula in order to increase their survivals as well as to complete and/or to rapidly degrade TCE.

Apart from bioaugmentation, indigenous microorganisms can be stimulated to degrade the contaminant by using organic materials without an addition of degraders or microorganisms (Vidali, 2001). This technique is known as biostimulation. Many organic materials were reported to be used in biostimulation of TCE by supplementing to the contaminated site as energy sources for indigenous microorganisms such as coconut charcoal (Cho et al., 1997), corn straw (Barahona et al., 2004) and compost (Olaniran et al., 2005). In this research, cassava pulp was used as a carbon source for stimulating microorganisms to degrade TCE in contaminated soil due to its high percentage, 68% dry wt, of starch (Sriroth et al., 2000) which can be used as a carbon source to stimulate indigenous microorganisms. In addition, it is a cost-effective material and can act as a bulking agent to improve air ventilation in soil microcosm when applied to the soil.

TCE cannot be used as energy and growth substrate by microorganisms, therefore, biodegradation of TCE needs a primary growth substrate to induce enzyme responsible for oxidation. Toluene and phenol are commonly used as primary growth substrates for TCE co-metabolism. However, they are hazardous compounds resulting in a need to find a less toxic growth substrate to replace toluene and phenol. Plant terpenes are of our interests because their structures are aromatic ring similar to toluene. The peels of orange, kaffir lime, lime, lemongrass etc. consist of terpenes which can be used as a primary substrate for TCE co-metabolism. A research by Suttinun et al. (2004) indicated that cumene, a plant terpene, could induce indigenous soil microorganisms to degrade 60 % of TCE at a concentration of 100 mg/kg soil. Thus, this study is interested in using plant terpenes i.e., kaffir lime peel to replace toluene for inducing TCE degradation.

The ultimate goal of this study was therefore to efficiently remove TCE from soil by using bioaugmentation and biostimulation techniques. The ability of mixed cultures obtained from acclimatized activated sludge to degrade TCE in soil in comparison to pure culture was investigated. The potential use of immobilized mixed culture obtained from acclimatized activated sludge compared with pure culture was also studied. Microorganisms were induced and stimulated to degrade TCE by supplementing with kaffir lime peel and cassava pulp, respectively. Results from this study would provide necessary information for the possible *in situ* bioremediation of TCE in contaminated sites.

1.2 Objectives

1.2.1 Ultimate goal

To efficiently remove TCE from soil by bioaugmentation and biostimulation techniques.

1.2.2 Sub objectives

1.2.2.1 To examine the ability of mixed culture obtained from acclimatized activated sludge to degrade TCE in soil in comparison to pure culture.

1.2.2.2 To search for an effective natural support materials for immobilizing the inocula.

1.2.2.3 To examine the ability of immobilized cell in comparison to free cell on TCE degradation in soil.

1.2.2.4 To examine the effect of different concentrations of kaffir lime peel as potential primary substrate on TCE biodegradation in soil.

1.2.2.5 To examine the effect of different C:N ratios amended with cassava pulp as organic amendment to biostimulate TCE biodegradation in soil.

1.3 Hypotheses

1.3.1 Microbial consortium in acclimatized activated sludge can degrade TCE in soil.

1.3.2 Corncob and coir have porosity that are able to absorb microbial cells.

1.3.3 Immobilization of cells can enhance survival of microorganisms with the remaining of TCE biodegradation capability.

1.3.4 Kaffir lime peel can be used as potential primary substrate on TCE biodegradation in soil.

1.3.5 Cassava pulp is able to stimulate TCE biodegradation in soil.

1.4 The scope of the study

This study investigated the abilities of mixed culture obtained from acclimatized activated sludge to degrade TCE in soil in comparison to pure culture in both forms of immobilized cell and free cell. Corncob and coir were used as support materials. Survival of microorganisms and cell leakage from the support materials were examined in order to evaluate the effectiveness of the support materials. Cassava pulp at different C:N ratios were used to stimulate microorganisms to remove TCE from soil. Kaffir lime peel and its optimal concentration were investigated if they could be used as primary substrate for TCE degradation.