CHAPTER 1

INTRODUCTION

1.1 Rational/Problem Statement

Ethylbenzene (C_8H_{10}) is a volatile organic compound (VOCs) in the 'BTEX' (benzene, toluene, ethylbenzene, xylene) substance group (Kaoru et al., 2004.), is produced by alkylating benzene (C_6H_6) with ethylene (C_2H_4) in an acid-catalyzed chemical reaction (Leigh et al., 2007). It is classified as a monocyclic aromatic hydrocarbon containing only carbon and hydrogen atoms (Boonsaner et al., 2011). Ethylbenzene can be detected in air, water and soil. It is naturally found in coal tar, petroleum, paints, inks, insecticides, carpet glues and tobacco products, but it can be also found in the environment from human activities. People can be exposed to the pollutant through breathing and from drinking water. Gaseous ethylbenzene contamination is much higher in urban area, compared to rural area as it was measured five times higher in Bangkok. Concentration of ethylbenzene at a gasoline station on Sukhumvit Road in Bangkok was 6.25± 4.29 ppb (Tunsaringkarn et al., 2012).

According to Henderson et al. (2007), this compound does not seem to induce genotoxic effects on organisms. However, many tests are controversial in this regard (Leigh et al., 2007). The effect of ethylbenzene on human health depends on the level of ethylbenzene contamination is present, how people are exposed to it and the length of exposure (ATSDR 1991). Effects of gasoline on workers were headache, fatigue, throat irritation, nose irritation, nausea and dizziness (Tunsaringkarn et al., 2012). Damage to kidney and cancer were the effects of long-term exposure to ethylbenzene contamination (ATSDR, 2010). Ethylbenzene is commonly found in the air because it is a constituent of automobile exhaust.

Many technologies have been developed to absorb BTEX. However, each technique has its own disadvantages. Air sparing and incineration have high operation costs (Boonsaner et al., 2011) while the biological processes known as bioremediation are regarded as a promising and clean technology, particularly because of their simplicity, low cost and efficacy when compared to other alternatives (Alexander M. 1994). Recently, phytoremediation was purposed as a possible technique to treat the air pollutants by various plant parts (Yadav, et al., 2005). *Zamioculcas zamiifolia* has the highest potential to reduce the concentration of BTEX from contaminated indoor air. Ethylbenzene uptakes per unit area of *Z. zamiifolia* leaf were about 0.92±0.02 mmol m^{-2} at 72 h of exposure. Plants could grow in ethylbenzene concentration, and some of them produced new roots. 75 % of ethylbenzene was removed by stomata, while 25% of it was removed by nonstomata or cuticles (Sriprapat W. and Thiravetyan P., 2013).

However, there are epiphytic bacteria which can promote plant growth and both suppress and stimulate the colonization and infection of tissues by plant pathogens (Irum et al.,2010). Therefore, microorganisms on leaf and soil may be helping degrade

BTEX for metabolisms in plant growth because many reports confirms that some microorganisms in soil such as *Rhodococcus rhodochrous* are able to degrade BTEX under aerobic and anaerobic conditions to carbon source (Corseuil et al., 1998; Deeb and Alvarez-Cohen, 1999; Langenhoffet al., 1996; Schreiber and Bahr, 2002).

In 2007, report study by Anil et al. showed that the pure strain *Bacillus sphaericus* which was isolated from sugar cane bagasse, had higher potential for degrading the mixture of BTEX. *Pseudomonas sp.* NCIB 9816-4 was reported to have efficiency to degrade ethylbenzene. This degradation is initiated by a dioxygenation of the aromatic ring, leading to acetophenone by 1, 2-dioxygenase that is intermediary of energy productize for plant activity (Lee & Gibson, 1996)**.** Thus, some epiphytic bacteria and soil bacteria may have high efficiency to degrade ethylbenzene.

1.2 Literature Review

1.2.1 Treatment of Ethyl Benzene Study

Ethylbenzene can be degraded by photooxidation to 1-phenylethanol and acetophenone but this chemical is no easy to be degraded by photo and have toxic on human. Many clean up techniques have been developed for absorbing BTEX. However, each technique has its own disadvantage. Air sparing and incineration have high operation costs (Boonsaner et al., 2011) while the biological processes known as bioremediation are regarded as a promising and clean technology, particularly because of their simplicity, low cost and efficacy when compared to other alternatives (Alexander M. 1994).

Phytoremediation consists of mitigating pollutant concentrations in contaminated soils, water and air with plants. It able to contain, degrade, or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants from the media that contain them. Phytoremediation have more mechanisms to treatment polluted such as phytoextraction that is plant uptake and concentration of substances from the environment into the plant biomass. Recently, phytoremediation was initialed as a possible technique to treat the air pollutants by various plant parts (Yadav et al., 2005).

In 2013, *Z. zamiifolia* has the highest potential to reduce the concentration of BTEX from contaminated indoor air (Sriprapat W. and Thiravetyan P., 2013). According Sriprapat et al. in 2014, the highest toluene removal was found in *Sansevieria trifasciata*, while the highest ethylbenzene removal from air was *Chlorophytum comosum*. Toluene and ethylbenzene can penetrate the plant's cuticle (Sriprapat W. and Thiravetyan P., 2013). However, the removal rates do not appear to be correlated with numbers of stomata per plant.

1.2.2 Epiphytic Bacteria Study

The aerial parts of plants including leaf, stems, buds, flowers and fruits provide a habitat for microorganisms termed the phyllosphere (Duetz et al., 1994, Leigh et al., 2007). Some epiphytic bacteria species are present in soil. Members of all plant phyla are colonized by microbial epiphytes, with more than 85 different species of microorganisms in 37 genera recovered from the phyllosphere of rye, olive, sugar beet, and wheat despite the hostile environment of the leaf surface (Morris et al., 1998; Yang et al., 2001). The most common bacterial such as *Pseudomonas fluorescens, P. corrugata, P. tolaasii, P. paucimobilis, X. campestris,* and *Enterobacter cloacae*, species also found on plant surfaces (Tanprasert and Reed, 1998). Recently, hydrocabon-degrading bacteria (up to 9×10^7 cells/g) were isolated from the surfaces of leaf of two legumes, peas and beans (Ali et al., 2012).

These microorganisms are able to break down compounds to be used as energy source, thereby eliminating them from contaminated environments. Some epiphytes bacteria from poplar trees exhibited the ability to degrade benzene, toluene, ethylbenzene and xylene (BTEX) compounds at one contaminated site (Lindow, S.E. and M.T. Brandl., 2003). However, the ability of BTEX degradation of certain bacteria is already known such as bacteria of the genus *Pseudomonas* are often cited, such as *P. putida*, *P. fluorescens*, *P. oleovorans*, *P. aureofaciens*, and *P. aeruginosa*, including *Microbacterium lactuim*, *Bacillus cereus*, *Stenotrophomonas maltophilia*, *Rhodococcus rhodochrous* (Dânia et al., 2010).

1.2.3 Soil Bacteria Study

Bacteria are most abundant microbes in the soil. In a single gram of soil, there can be billions of bacteria or estimated 60,000 different bacteria species, most of which have yet to be even named (Greg and Percy, 2005). The potential of soil bacteria for use in biofilter for the remediation of BTEX contaminated environments was studied by Anil et al. (2007) who found that *Bacillus sphaericus* had a high BTEX-degrading activity. Other bacteria were isolated from soil contaminated such as *Pseudomonas* strains were able to degrade BTEX from polluted soils (Duetz et al., 1994). *Pseudomonas* strains such as *P.putida* OlG3 from soil found to be able to metabolize different substrates. Bacteria from polluted soil are good bacteria for treating environment such as bacteria from petroleum refinery soil. In 1990, Gibson et al. report that *P. putida* from effluents of petroleum refinery is known as an aromatic hydrocarbon degrader, capable of using benzene, toluene, ethylbenzene, phenol and other aromatics as the only carbon and energy sources. However, they conclude that the bacteria *P. putida* is an efficient microorganism for BTEX biodegradation and suitable for remediation of environments contaminated with this compound (Gibson et al., 1990). According to Corseuil et al. (1998), aerobic bioremediation of benzene generally exhibits faster degradation rate than anaerobic systems (Corseuil H et al., 1998). In 2010, a pure culture using benzene as sole carbon and energy sources was isolated by screening procedure from gasoline contaminated soil. The isolated strain was a member of genus *Bacillus cereus* which is effective in the degradation of benzene (Junfeng et al., 2010).

1.3 Research Objectives

1.3.1 To screen the suitable plant with high efficiency for ethylbenzene removal.

1.3.2 To study the degradation of ethylbenzene in *Zamifolia zamiffolia*.

1.3.3 To screen bacteria from *Zamifolia zamiffolia* leaf and soil contaminated pesticides and their efficiency to remove ethylbenzene in contaminate air

1.4 Scope of This Study

1.4.1 The efficiency of plants to remove 5 ppm of ethylbenzene was studied by using close chamber in laboratory room and comparing efficiency by percentage ethylbenzene removal per leaf area.

1.4.2 Microorganisms were isolated from *Z. zamiffolia* leaf and pesticides contaminated soil in Bangkok.

1.4.3 The efficiency of microorganisms-associated leaf was compared with natural plant and steriled plant.

1.4.4 Data were analyzed by one way analysis of variance (ANOVA) using Statistical Program for Social Sciences (SPSS).