CHAPTER 1

INTRODUCTION

1.1 Rationale/Problem statement

Benzene, which is highly toxic to humans with carcinogenic effects, contaminates many locations around the world as both indoor and outdoor air pollution. Many researchers report that this compound can induce many diseases and symptoms such as allergies, asthma, eye irritation, nervous-system disorders, and also many type of cancer (Wolverton., 1996; Schnatter *et al*., 2005; Sciencelab, 2001). Following extensive research, the IARC (International Agency for Research on Cancer) classified benzene in IA group, which is the group of high potential human carcinogen. The compound is used as an additive or solvent in several industries (Atkinson, 2007). Both benzene emission from industries and leakage from consumer products have been observed and efforts have been made to control the pollution. Guidelines have been outlined by many organization and cities around the world, but higher benzene concentration than standard guideline continue to be reported (Pollution Control Department, 2007). Development of pollution clean-up technologies should be studied and applied in the real world. Nowadays, several technologies had been investigated such as absorption, catalytic combustion and photocatalytic oxidation; however, these technologies have limitations and disadvantages. For example, benzene absorption by activated carbon, which has been widely applied, has high costs associated with the generation process and secondary waste disposal (Aloko and Adebayo, 2007; Kermani, *et al*, 2006). In addition, catalytic combustion and photocatalytic oxidation are known as highly efficient in benzene removal, but these technologies require high cost and specialist maintenance so, these technologies cannot be applied to clean-up indoor benzene air pollution. The use of plants to remove pollutants from soil, water or air, referred to as phytoremediation, might be suitable technology for indoor benzene treatment because rapid benzene removal by some species of plants has already been reported (Wolverton, *et al.*, 1989; Orwell, *et al*., 2004; Liu, *et al*., 2007). Benzene uptake and *in planta* transformation has been investigated. The results suggest that *Acer campestre* and *Viris vinifera* take up gaseous benzene though stomata and the cuticle of the leave surfaces, and completely degrade benzene to carbon dioxide, using benzene metabolites as a carbon source (Ugrekhelidze, *et al*., 1997; Kvesitadze, *et al*., 2009). In experiments under dark conditions, *Spathyphyllum var Petite* could still grow and take up benzene, confirming benzene uptake through cuticle wax, as stomata are closed under dark conditions (Orwell, et al., 2004). Benzene accumulation through plant leaf cuticles has also found in other studies (Gorna-Binkul, *et al*., 1996; U. S. Environment Agency, 2009; Slaski, *et al*., 2000; Tsiros, *et al*., 1999; Collins, *et al*., 2000; Poborski, 1988; Riederer, 1990; Kylin, *et al*., 1994). There is great interest in sustainable and eco-friendly technology. In this present study, removal of benzene by *Draceana sanderiana*, which removes benzene with the highest efficiency, has been investigated. The sustainability of the technology in both light and dark conditions has been confirmed. The applications of both plant leaves and beads packed into a biofilter system have been compared. Twenty two plant leaves materials were screened for benzene adsorption. Hexanes desorption and FT-IR surface functional group analysis was used to estimate adsorption mechanism. The relationship between quantity and composition of wax

and benzene adsorption has been analysed. Towards industrial application, a biofilter was constructed, consisting of benzene absorbent plant leaves grinding material immobilized on glass beads, and a *Pseudomonas putida* bacterium, which has been previously described as efficient in benzene removal (Reardon, *et al*., 2000; Rahul, *et al*., 2013). In biofilter, loading rate and suitable nutrient were evaluated.

1.2 Literature review

1.2.1 Benzene uptake and transformation in plant

Several experiments propose that plants could take up and completely degrade benzene. Particular experiments of interest are presented in this paragraph. The first uptake and transformation study was carried out in 1996. Ugrekhelidze, *et al*. (1997) studied 1-6^{14}C benzene removal over 8 h by 3 species of plants. In this experiment, benzene uptake through stomata and cuticle were compared by the use of this radioactive technique. The results showed higher benzene uptake by abaxial side (stomata side) than adaxial side (cuticle side) for every plant species, so in 2009, Kvesitadze, *et al*. (2009) concluded that most benzene is generally taken up at the site of stomata. Because of the physical and chemical properties of benzene, the compound could passively and rapidly diffuse into the cell. Distribution of benzene in 4 species of plants has been also studied by a radioactive method, and in every species that the highest percentage of radioactive benzene metabolites was in the cytosol. Chloroplasts also showed high percentage of radioactivity. Because benzene is an organic molecule, *in planta* benzene degradation could possibly yield organic and amino acids. Radioactive techniques together with HPLC have been used to analyse 1-6^{14}C benzene metabolites such as organic acid and amino acid in spinach leaves exposure to 1-6^{14}C benzene for 72 h. The result found that 84% were found as organic acid, and 16% were only amino acid. Of these organic acid analyses, 37% and 24% were muconic acid and fumalic acid, respectively. In amino acid analysis, tyrosine was found as the main benzene metabolite at approximately 34%, and 26% of phenylalanine was found as second amino acid. For study of enzymatic transformation of benzene, 8-Oxyquinoline (a heavy metal enzyme inhibitor) and sodium diethyl-dithiocarbamate (a copper-containing enzyme inhibitor) have been used (Sato, 1966). The intensity of radioactive signal from non-volatile benzene metabolites in spinach chloroplasts, following 3 h of benzene exposure in the presence of the inhibitors, was studied. The presence of 8-oxyquinoline and sodium diethyl-dithiocarbamate inhibited benzene transformation both under light and dark conditions, but a little transformation was reduced by α,α′-dipyridyl, an iron enzyme inhibitor. This experiment concluded that the benzene transformation enzyme contain copper as main component on the enzyme. Phenoloxidases and ascorbate oxidase, copper-containing enzyme in chloroplasts, have been considered as potential benzene-transforming enzymes. However, spinach chloroplasts possess less active ascorbate oxidase than O-diphenoloxidase (Sechneska, *et al*., 1968), so phenoloxidases could be more important enzymes than ascorbate oxidase in benzene transformation. In addition, an interesting result is that NADH and NADPH, common general electron donors in living cells, could enhance benzene degradation (Ugrekhelidze, *et al*, 1996). P450 mono- and di- oxygenases have also been of interest. In 2009, benzene degradation by P450 mono- or di- oxygenase was proposed, and NADH and NADPH are electron donors to P450 mono- and di- oxygenases. This enzyme adds oxygen to hydrophobic molecules, so it is possible that NADH and NADPH can activate CYP P450 to transform benzene and its metabolism. Kvesitadze, *et al*

(2009) reports the mechanism of organic compound catabolism in plant. This review article presents that benzene is degraded by two families of plant enzymes, the phenoloxidases and P450 monooxygenases. P450 monooxygenase can generate a hydroxyl group on the benzene molecule. In this pathway NADH and NADPH provide $2e^-$ for methemoglobin **reductase** (cytochrome b_5 reductase) activation, which cytochrome b_5 reductase allows $2e^-$ to activate P450 monooxygenase. Benzene is changed to phenol, and a hydroxyl group is added to the phenol by P450 monooxygenases again, which is then converted to catechol. Not only P450 monooxygenase but also phenoloxidases could generate catechol. Reactive oxygen species (ROS) are generated by the reaction of o-diphenoloxidase and o-diphenol, and such active oxygen species could directly combine with benzene or phenol to generate catechol. Catechol could then be converted to o-quinone. Muconic acid, which is a common molecule in many organisms, is produced from o-quinone ring cleavage.

1.2.2 Phytoremediation of benzene

Although phytoremediation is not novel topic, and there are many studies that investigate on this method, to clean up air pollution by plant, only a few studies had been done. The first study in 1989 by NASA scientist, 10 species of plant such as *Gerbera jamesonii, Chrysanthemum morifolium, Hedera helix, Sansevieria laurentii, Dracaena deremensis warneckei, Spathiphyllum, Aglaonema* "Silver Queen", *Dracaena marginata, Chamaedorea seifrizii,* and *Dracaena deremensis* have been proposed that they can uptake rapidly benzene at initial benzene concentration of 20 ppm (Wolverton, *et al*., 1989). This experiment, they screen 10 high benzene removal plant species from 50 species. *Gerbera jamesonii* and *Chrysanthemum morifolium* had been reported as high benzene removal plant. These two species can uptake around 23.5 µg cm^{-2} and 18.2 µg cm^{-2} of benzene, respectively. In 2004, Orwell, *et al*. (2004) studied 7 plants for benzene removal such as *Dracaena deremensis, Epipremnum aureum, Dracaena marginata, Schefflera* "Amate", *Spathiphyllum* "Petite", *Spathiphyllum* "Sensation", *Howea forsteriana* at initial concentration of 25-50 ppm. *Dracaena marginata* can uptake benzene 23.23 µg cm^{-2} that is the most efficiency plant in this experiment. However the similar species was found that can remove only 4 µg cm^{-2} in Wolverton study. *Dracaena deremensis* in Wolverton study can remove 1.7 µg cm^{-2}, but 13 µg cm^{-2} benzene removal was found in Ralph study. The results from these 2 studies suggested that there are many affecting factor on benzene phytoremediation efficiency which is not only plant species. In 2007, low concentration of benzene had been investigated. Liu, *et al.* (2007) screened 72 species for benzene removal by a dynamic system. Benzene was injected though a 75.36 L glass chamber, and initial benzene was 150 ppb. In the same year, Tarran, *et al.* (2007) showed that in 2 plant species such as *Zamioculcas* sp. and *Aglaonema* sp., they request time to adapt themselves under benzene stress condition, so these plants can uptake slowly benzene when exposure exceeds first time. This tendency suggests that plant required the time for its adaptation. Wood, *et al.* (2001) had studied on the benzene removal efficiency of *Spathyphyllum var Petite* plants when they were exposure with 25-30 ppm of benzene under light and dark conditions. Under light conditions, the tendency of benzene removal efficiency was similar to Tarran's study. However, slower benzene removal tendency had been found under dark conditions. This experiment had reported firstly about effect of light on benzene uptake by plants.

1.2.3 Benzene uptake by wax

Benzene shows as a hydrophobic compound. Accumulation of benzene on cuticle of plant that have strong hydrophobic property had been interested to study. Interesting investigation had been reported by many researchers. This topic had been started in 1996. Ugrekhelidze, *et al.* (1997) studied on the 1-6^{14}C benzene uptake pathways in 8 h exposure in 3 species of plants by the use of radioactive technique. The results suggested that plant uptake gaseous benzene though stomata and cuticle on the surface of leaves. In addition, although plant had been grown under dark condition, plant could still grow and uptake benzene that this event should be the benzene uptake by only cuticle because of the close of stomata under dark condition (Orwell, et al., 2004; Wood, et al., 2001). The benzene accumulation in cuticle of plant leaves had been found also in many researches (Gorna-Binkul, *et al*., 1996; U. S. Environment Agency, 2009; Slaski, et al., 2000; Tsiros, *et al*., 1999; Collins, *et al*., 2000; Poborski, 1988; Reiderer, 1990; Kylin, *et al*., 1994). For example, in 1996, Gorna-Binkul A., *et al*. (1996) found the benzene and it derivatives contamination on orange shell, and parsley. The part of black berry, apple, and cucumber had been observed benzene accumulation, and the result found that high benzene accumulation occurs in black berry and apple (Collins, *et al*., 2000). Not only benzene but also poly-aromatic-hydrocarbons (PAHs) were also found the contamination in many part of plant that grown in contaminated land (Slaski, *et al*., 2000). Possible reason of benzene accumulation in plant cuticle had been reported in 2004. Benzene that contains 2.13 of log K_{ow} can transport easily in to the plant (Kamath, *et al*., 2004). Moreover, Ugrekhelidze, *et al*. (1997) observed the ^{14}C-benzene and non-volatile benzene metabolites in old and young plant leaves. The result suggested that although high quantity of cuticle was found in old leaf, young leaf have more benzene and it metabolites accumulation intensity than old leaf. This result, the composition of wax might be affecting factor for benzene accumulation.

1.2.4 Benzene removal by *Pseudomonas putida.*

P. putida was classified in Genus: *Pseudomonas* and Species: *Pseudomonas putida. Pseudomonas putida* is a rod-shaped, nonsporeforming, gram-negative bacteria that utilizes aerobic metabolism. This bacterium also has multiple polar flagella for motility. The flagella have a waveform that is usually 2 to 3 wavelengths long. *Pseudomonas putida* is sensitive to the environment and suppresses the changes in the direction of flagella rotation upon sensing chemoattractants. This is very helpful in guiding the *Pseudomonas putida* to propel towards the seeds of the plants, which provides nutrients to the bacterial cells (Harwood, 1989). It grows optimally at 25-30 ^{o}C and can be easily isolated. Certain strains of *Pseudomonas putida* are not pathogenic due to lack of certain genes including those for enzymes that digest cell membranes and walls of humans and plants. *Pseudomonas putida* has a very diverse aerobic metabolism that is able to degrade organic solvents such as benzene, toluene, etc. (Reardon *et al*., 2000; Rahul *et al*., 2013; Heald and Jenkins, 1996) and also to convert styrene oil to biodegradable plastic Poly-hydroxy-alkanoates (PHA) (Ribera, 2001).

1.3 Research objectives

1.3.1 To screen ornamental plants for benzene removal and study benzene uptake pathway on plant

1.3.2 To screen plant leaf materials for benzene adsorption and analyse the relation of cuticle quantity and composition on benzene adsorption.

1.3.3 To apply leaf material as a packing bead in biofilter with *P. putida* and investigate the suitable flow rate and nutrient

1.4 Scope of experiment

1.4.1 Eight ornamental plants were screened to remove gaseous benzene in close system.

1.4.2 Benzene removal under light and dark conditions by *Dracaena sanderiana*, which is the highest benzene removal plant, was studied.

1.4.3 Sustainability of benzene removal by *Dracaena sanderiana* was investigated.

1.4.4 Wax quantity, stomata pattern, and PhotosystemII activity were analysed to study factor affecting on benzene removal efficiency.

1.4.5 Leaf materials from 21 species of plants were prepared and screened to adsorb benzene.

1.4.6 Wax quantity and composition were analysed and used to calculate the relationship between benzene removal efficiency and wax quantity and wax composition.

1.4.7 Effective 6 plant leaf materials were applied to use in continuous system.

1.4.8 Benzene adsorption mechanism of plant leaf materials was studied.

1.4.9 Two plant leaf materials were used to be packing bead in biofilter system that was inoculated with *P. putida.*