CHAPTER 4

RESULTS AND DISCUSSION

4.1 Plant Screening

From the previous report showed that *Sansevieria trifasciata* hort. ex Prain cv. Hahnii Green was the most efficient plant in uptaking toluene in contaminated air (Sriprapat et al., 2014) and in our laboratory found that *Sansevieria kirkii* var. pulchra Coppertone was the best plant in removing trimethylamine in contaminated air. In addition, *Z. zamiifolia* was also reported to be the best plant in uptaking xylene from contaminated air (Sriprapat et al., 2013). Therefore, *S. trifasciata*, *S. kirkii*, and *Z. zamiifolia* were used to study the efficiency of 5 ppm ethylbenzene removal. The result showed that *Z. zamiifolia* had the highest potential in uptaking ethylbenzene from contaminated air. Ethylbenzene removal by *Z. zamiifolia* was about 64% which higher than *S. trifasciata* (48%) and *S. kirkii* (11%) within 24 hours (Figure 4.1A). Comparison of the efficiency of ethylbenzene uptake in nmole/m^2 of leaf area showed that *Z. zamiifolia* could take up ethylbenzene 13.39 µmole/m^2 while *S. trifasciata* and *S. kirkii* could take up about 3.83 and 2.23 µmole/m^2, respectively within 24 hours (Figure 4.1B). The efficiency of each plant in the uptake of ethylbenzene depends on the efficiency of stomata and wax types of leaves (Treesubsuntorn et al., 2012). This result confirmed that *Z. zamiifolia* is an appropriate plant for treatment of ethylbenzene contaminated in air. Therefore, *Z. zamiifolia* was then selected for further study.

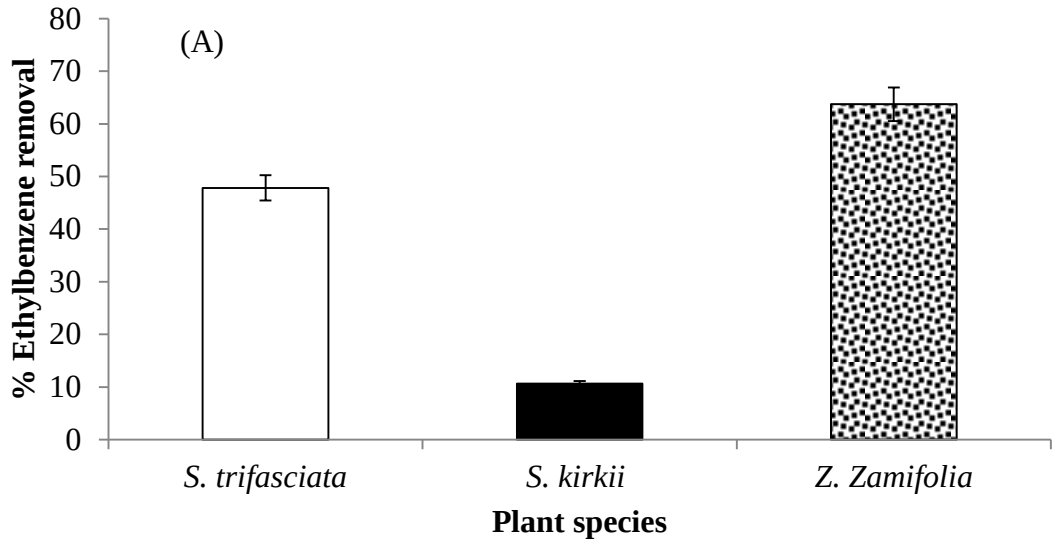


Figure 4.1A Comparison of ethylbenzene removal (%) by various plants within 24 hours (initial ethylbenzene 5 ppm)

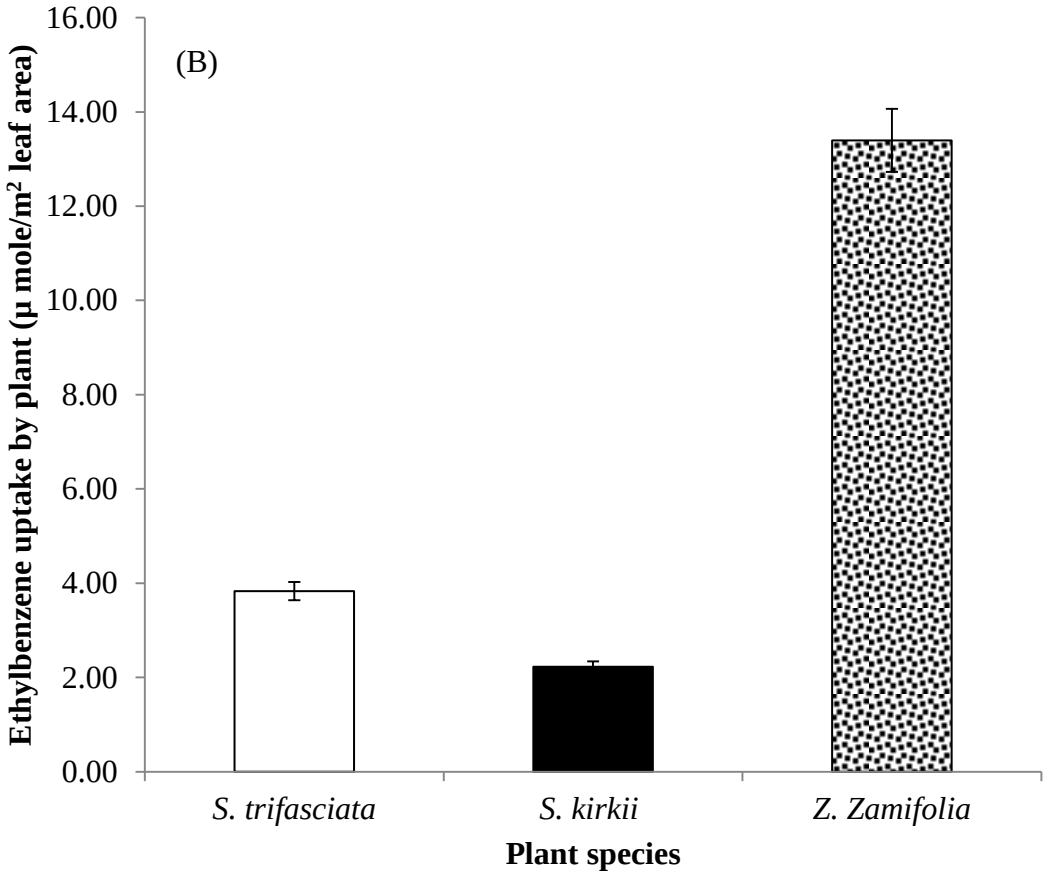


Figure 4.1B Comparison of ethylbenzene uptake by various plants within 24 hours (initial ethylbenzene 5 ppm)

4.2 Ethylbenzene Uptake by Each Part of *Z. zamiifolia*

Z. zamiifolia is an appropriate plant for treatment of ethylbenzene. Within 12 hours, *Z. zamiifolia* leaf and stem could remove ethylbenzene accounted for 26% and 16%, respectively. Within 24 hours, leaf and stem remove ethylbenzene increasing to 45% and 20%, respectively (Figure 4.2). Therefore, ethylbenzene could be taking up via plant leaf either by stomata and cuticles and also absorbed by stem of *Z. zamiifolia*.

From other research explained that stomata and cuticles' wax of plants are important pathways for volatile organic compound uptake (Keymeulen et al. 1993; Kvesitadze et al. 2009; Treesubsuntorn and Thiravetyan 2012). Also plant fiber have performance to remove other chemical compound such as research of Erna et al., 2014 indicated that leaves, stems and flowers of *Stevia rebaudiana* plant which were dried to remove the moisture content, can accumulate heavy metal. Coir pith that is plant fiber is effective adsorbent for the removal of a number of inorganic anions, heavy metals, organics and dyes from water (Namasivayam and Sangeetha, 2006).

Thereby, due to leaf containing stomata, cuticles' wax and fiber while surface of stem is quite smooth that might be providing small pore for gas exchange. Thus, leaf could remove ethylbenzene higher than stem. The comparison of ethylbenzene removal efficiency between three plants species was showed that within 24 hours, *Z. zamiifolia* leaf could remove ethylbenzene about 13.39 µmole/m^2 while *S. trifasciata* and *S. kirkii* could take up about 3.83 and 2.23 µmole/m^2, respectively (Figure 4.1). Therefore, the ethylbenzene removal efficiency of *Z. zamiifolia* has highest efficiency when compared with *S. trifasciata* and *S. kirkii*.

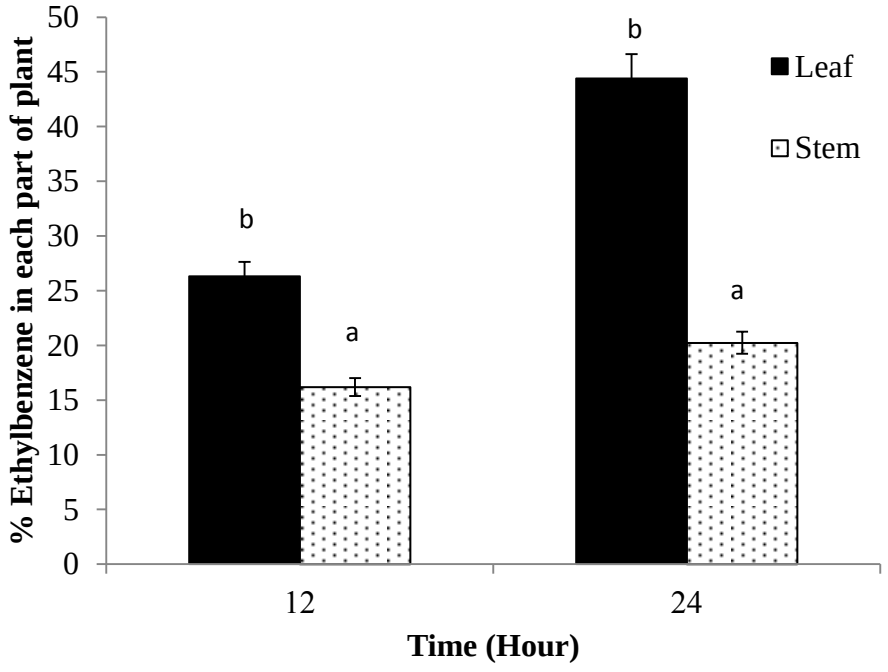


Figure 4.2 Ethylbenzene absorption in leaf, stem

4.3 Ethylbenzene Degradation by *Z. zamiifolia*

Data of HPLC analysis found that ethylbenzene, 1-phenylethanol, and acetophenone presented in some parts of *Z. zamiifolia* which was fumed 300 ppm ethylbenzene and not found in control. Ethylbenzene was found in leaf of day 1 and 5 about 22.44 and 15.78 nmoles/g fresh weights, respectively, and found in stem and root including tuber of day 1, 4 and 5 about 14, 10.50 and 12.16 nmoles/g fresh weight of stem and 11, 3.67 and 4.82 nmoles/g fresh weight of root including tuber, respectively (Figure 4.3a). Moreover, 0.44 nmoles of 1-phenylethanol /g fresh weight of stem was found in day 4 (Figure 4.3b). Acetophenone was found in all plant parts at day 1, 4 and 5 of experiments (Figure 4.3c). Similarly, the result from GC-MS analysis was also found ethylbenzene in leaf of day1 and 5 (Table A9 and A11).

This result confirmed that ethylbenzene is taken up by *Z. zamiifolia* through plant leaf and stem and when was translocated to root including tuber. Ethylbenzene might be degraded by plant enzyme or other mechanisms in plant to 1-phenylethanol and acetophenone (Figure 4.4). As previously published work reported that bacteria such as *Pseudomonas sp.* NCIB 9816-4, *P.putida* 39/D, and *Pseudomonas* sp. NCIB 10643 has oxygenase enzyme that could be used to degrade ethylbenzene (Lee & Gibson 1996). Monooxygenase and dioxygenase enzymes are important enzymes in plant. They are generally in the family of heme proteins collectively called cytochromes P450 ((Mary A. Schuler, 1996). Plant cytochromes P450 are involved in a wide range of biosynthetic reactions, leading to various fatty acid conjugates, plant hormones, defensive compounds, or medically important drugs (Mary A. Schuler, 1996). Therefore, *Z. zamiifolia* might be used oxygenases to degrade ethylbenzene as a carbon source for its growth.

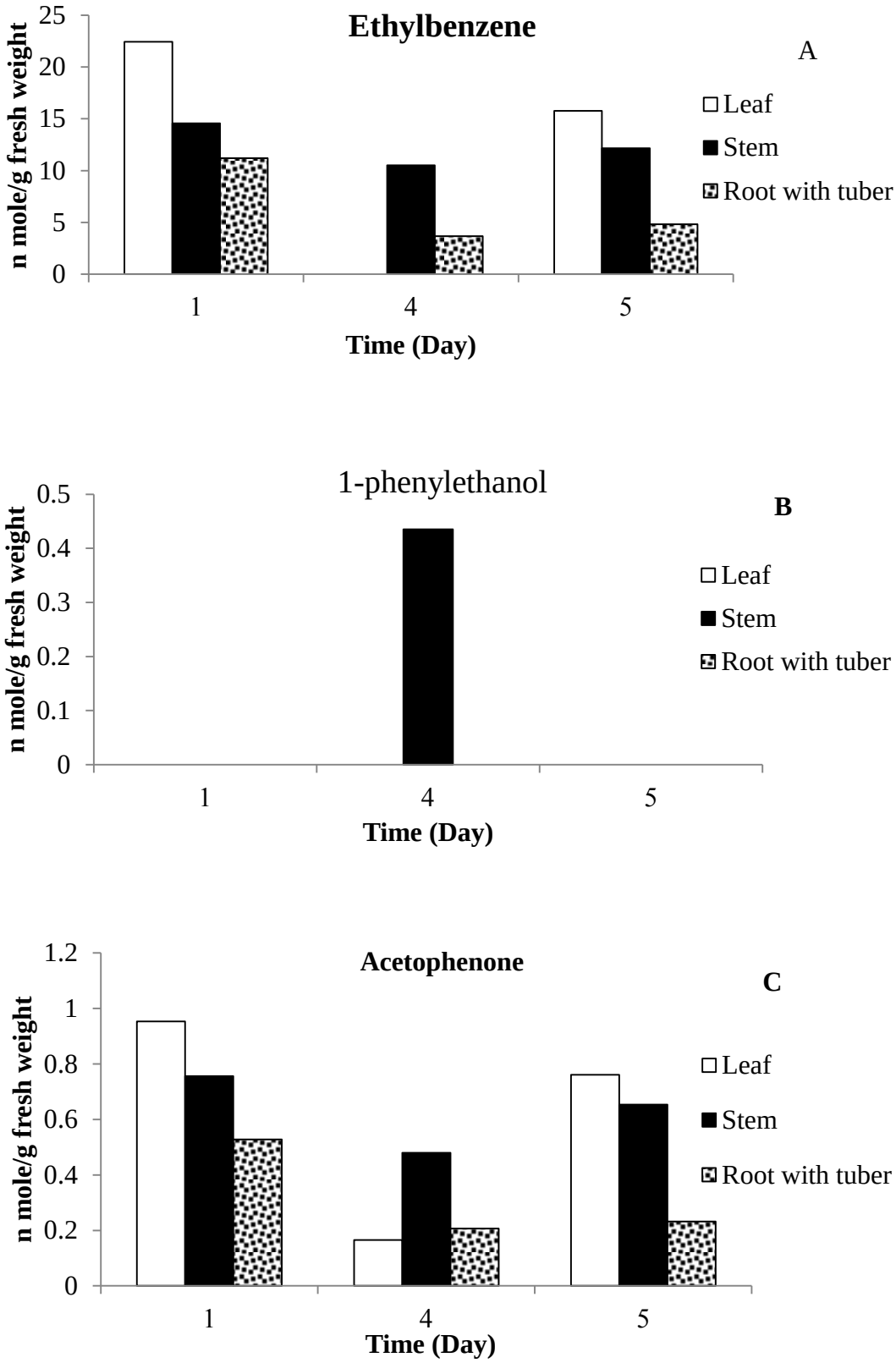
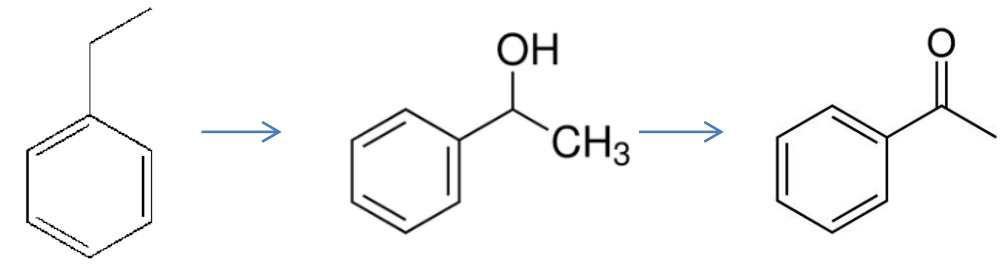


Figure 4.3 Plant metabolites obtained from ethylbenzene degradation by each part of *Z. zamiifolia*. (A) Ethylbenzene, (B) 1-phenolethanol, and (C) acetophenone



Ethylbenzene 1-phenylethanol Acetophenone

Figure 4.4 Degradation of ethylbenzene in leaves, stems, and roots of *Z. zamiifolia*

4.4 Microbial Screening

Six bacteria strains including, *Bacillus amyloliquefaciens* subsp. plantarumNAU-B3, *Bacillus cereus* E33L, *Bacillus* sp. N6, *Exiguobacterium* sp. MH3, *Acinetobacter* sp. 10095, and *Acinetobacter calcoaceticus* HPC253 were isolated from *Z. zamiifolia* leaf under natural condition (Table 4.1).

In addition, six bacteria isolated from pesticide-contaminated soil were identified as follows: *Bacillus sp.* N6, *Serratiamar cescens* DHU-35, *Acinetobacter calcoaceticus* HPC253, *Bacillus cereus* ZQN5, *Bacillus sp.* 6B254-3L, and *Bacillus megateriumstrain* X14 (Table 4.1). In addition, *P. putida* TISTR1522, purchased from MIRCEN, Thailand and *P. aeroginosa* isolated from pesticide-contaminated soil were used in this study (Table 4.1). The types of epiphytes and soil bacteria is dependent on atmosphere, a variation in climatic conditions including moisture, humidity, temperature, wind speed, radiation and rainfall may influence epiphyte diversity (Hirano SS and Upper, 1983).

Table 4.1 Microorganism screening from leaf of *Z. zamiifolia* and contaminated soils

Bacteria species	**Gram**
Epiphytic bacteria	
Bacillus amyloliquefaciens subsp. plantarum NAU-B3	Gram positive (+)
Bacillus cereus E33L	Gram positive (+)
Bacillus sp. N6	Gram positive (+)
Exiguobacterium sp. MH3	Gram positive (+)
Acinetobacter sp. 10095	Gram negative (-)
Acinetobacter calcoaceticus strain HPC253	Gram negative (-)
Soil bacteria	
Bacillus sp. *N6*	Gram positive (+)
Serratia marcescens strain DHU-35	Gram negative (-)
Acinetobacter calcoaceticus strain HPC253	Gram negative (-)
Bacillus cereus strain ZQN5	Gram positive (+)
Bacillus sp. 6B254-3L	Gram positive (+)
Bacillus megaterium strain X14	Gram positive (+)
Other bacteria	
Pseudomonas putida TISTR1522	Gram negative (-)
Pseudomonas auroginosa	Gram negative (-)

4.5 The Population of Bacteria on *Z. zamiffolia* Leaf

Microorganisms was sprayed on *Z. zamiifolia* leaf about 10^2-10^3 colony forming unit (CFU) /ml, after three cycle experiment they increased to 10^5-10^8 CFU/ml (Table 4.2). It was found that *P. aeroginosa* grew more slowly than other strains but had the highest efficiency in enhancing ethylbenzene removal. These results implied that number of microorganisms was not the major factor in ethylbenzene removal. The efficiency of ethylbenzene removal in each bacterium might depend on specific mechanisms of each bacterium such as efficiency of oxygenase enzymes.

Table 4.2 Number of microroganisms on the leaf of *Z. zamiifolia* before and after treatment of ethylbenzene after 3 cycles

Microorganisms	**Treatment**	
	Before Treatment CFU/ml	**After 3 cycle treatment CFU/ml**
B. cereus E33L	2×10^2	1.3×10^8
Bacillus sp. N6	2.3×10^3	1.4×10^7
B. cereus strain ZQN5	7×10^2	4×10^7
Bacillus sp. 6B254-3L	1.1×10^3	1×10^7
P. aeroginosa	1×10^2	4×10^5
*P. putida*TISTR1522	3.1×10^3	1.1×10^7

4.6 Removal of Efficiency by *Z. zamiffolia* and Associated Microorganism on The Leaf

The enhancing ethylbenzene removal by microorganisms isolated from *Z. zamiifolia* leaf and soil was studied. The result showed that 5% of $NaClO_2$ solution for *Z. zamiifolia* sterilization was not affected on ethylbenzene removal. Steriled *Z. zamiifolia* has the potential to remove 5 ppm of ethylbenzene within 60 hours, which no significantly difference compared with natural *Z. zamiifolia*. (Figure 4.5). This might due to natural microorganisms on natural leaf of *Z. zamiffolia* was not high enough and some strains also could not degrade ethylbenzene or produce some chemical to inhibit the degradation of other microorganisms. Result was also found that epiphytic bacteria such as *B. amyloliquefaciens* subsp. plantarum NAU-B3, *Exiguobacterium* sp. MH3, *Acinetobacter* sp. 10095, and *A. calcoaceticus* strain HPC253 had no effect on ethylbenzene removal (Table 4.3). As a result, the efficiency of natural and sterile plant to remove ethylbenzene was not significantly different.

In addition, sterile plant was able to take up only 46% of ethylbenzene, while sterile plant inoculated with *P. aeroginosa*, *P. putida* TISTR1522, *B. cereus* E33L, *Bacillus* sp. N6, *B. cereus* strain ZQN5, and *Bacillus* sp. 6B254-3L were able to take up higher ethylbenzene (65%, 53%, 66%, 65%, 51% and 63%, respectively) within 12 hours (Table 4.3). Sterile plant was able to take up ethylbenzene completely within 60 hours, while sterile plant with inoculated *P. aeroginosa* and *B. cereus* ZQN5 were able to remova ethylbenzene completely in a shorter time (36 hours). In addition, plants inoculated with *P. putida* TISTR1522 , *B. cereus* E33L, *Bacillus* sp. N6, and *Bacillus* sp. 6B254-3L were able to remove ethylbenzene within 48 hours, but other isolated microorganisms were not able to enhance the plants to remove ethylbenzene (Table 4.4). It was explained that some microorganisms sprayed on the leaf of plants were able to enhance ethylbenzene removal, especially *P. aeroginosa* and *B. cereus* strain ZQN5, which had the highest efficiency in removing ethylbenzene.

For the second cycle, *P. aeroginosa*, *B. cereus* E33L, *Bacillus* sp. N6, and *B. cereus* ZQN5 took up ethylbenzene completely within 36 hours, while *P. putida* TISTR1522 and *Bacillus* sp. 6B254-3L took up ethylbenzene completely within 48 hours (Table 4.5). This result was not different compared with the third cycle (Table 4.6). It was showed that all treatments were efficient in removing ethylbenzene. Steriled plant with *P. aeroginosa* and *B. cereus* ZQN5 had stable efficiency to remove ethylbenzene in the air.

The efficiency of microorganisms without plant was found that in the first cycle, the percentage of ethylbenzene removal by bacteria within 12 hours was contributed from *P. aeroginosa* 68%, *Bacillus* sp. N6 65% , *B. cereus* E33L 64%, and *Bacillus* sp. 6B254-3L 62%, while *P. putida* TISTR1522 and *B. cereus* ZQN5 could remove 53% of ethylbenzene after 24 hours. It was found that *P. aeroginosa* and *B. cereus* ZQN5 could remove ethylbenzene at a very high rate in the first cycle were 77% and 73% of ethylbenzene, respectively. The result showed that microorganisms on leaf at the first cycle of treatment could remove ethylbenzene at a very high rate because this duration is a log phase of microorganisms. After that, at cycle 2 and 3, the efficiency was stable due to reaching a stationary phase of microorganisms (Figure 4.6).

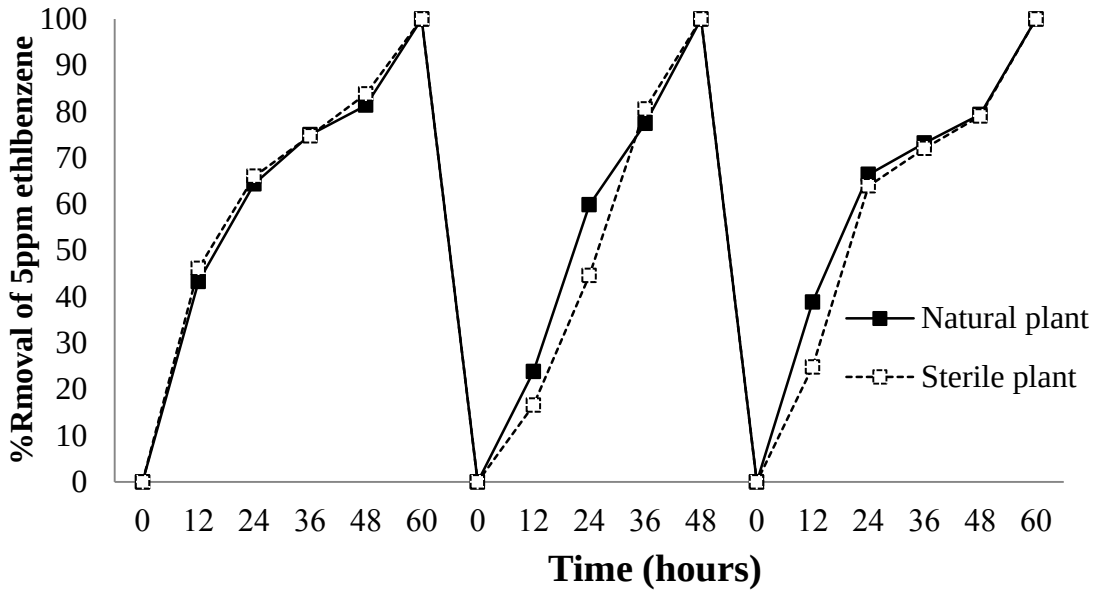


Figure 4.5 Comparison of ethylbenzene removal by natural plant and sterile plant