CHAPTER V

DISCUSSION

Diuron has toxic to physically (eye, skin and red blood cell) and genetically (developing embryo and bone marrow cell) in human and animal although, diuron is contaminated in drinking water supplied and environmental fate. The diuron is degraded to 3,4-dichloroaniline moreover, it has a slowly hydrolyzed in natural environment. It is necessary to study a remediation technique to reduce diuron. In this study, three biological treatments including natural attenuation, biostimulation and bioaugmentation, were carried out with two types of soil spiked with diuron.

Bioremediation: Condition A

The comparison of three biological treatments including natural attenuation, biostimulation and bioaugmentation in type-I soil (loam soil) supplemented with 20 ppm diuron was examined. Natural attenuation is the process that diuron was reduced due to naturally-occurring processes by indigenous microorganisms in soil. Biostimulation is the treatment which 20.6, 103 and 206 ppm Triton X-100 (1, 5 and 10 CMC) or 100 ppm *n*-butanol was added. Bioaugmentation was carried out in which approximately diuron-degrading bacteria, *Enterobacter* strain P2 and *Burkholderia capacia* strain P3, were provided at 10^8 CFU/g soil. Abiotic control was also carried out. In this study, natural attenuation treatment demonstrated a high ability to degrade diuron with maximal activity by 17% and the degradation percentage was higher than that in biostimulation and bioaugmentation. Bioaugmentation, with the inoculation of the

Enterobacter strain P2 and *Burkholderia capacia* strain P3 was not more effective in enhancing biodegradation of 20-ppm diuron within 8 weeks due to the limitation degradation ability of the inoculum in soil. This ability may be suppressed by indigenous microorganisms in the non-sterile soil (Yu *et al.*, 2005). Biostimulation has long been used as a strategy to enhance the biodegradation rate. In present study, however, the degradation in biostimulation was also not more effective in enhancing biodegradation when three concentration of Triton X-100 (20.6, 103 and 206 ppm) were added compare to natural attenuation. Similarly, the surfactants including Triton X-100 had a negative effect on atrazine biodegradation (Mata-Sandoval *et al.*, 2001). Furthermore, the negative effects of biostimulation and bioaugmentation on biodegradation had been reported by previous researcher (Braddock *et al.*, 1997). They found that the greatest stimulation of microbial activity in fuel oil contaminated aquifer was at low nutrient addition levels, not at high nutrients addition levels. Otherwise, the supplementation with 100-ppm diuron showed no change in degradation percentage within 8 weeks among three types of bioremediation. The higher concentration of diuron may affect to ability of microbial activity in soil. This activity was determined by power-time curves that were recorded for increasing amounts of diuron, varying from zero to 333.33 μg/g soil. An increase in the amount of diuron in soil caused a decrease of the original thermal effect, to reach a null value above 333.33 μg/g of herbicide (Prado and Airoldi, 2001). The populations of diuron-degrading bacteria determined by plate count technique with the supplementation of 20-ppm of diuron showed that bacterial growth was maintained during 6 weeks among three types of bioremediation, suggesting that microorganisms were able to thrive in the medium. The supplementations with 100-ppm diuron, however, showed that the

populations were reduced at 8 weeks, suggesting that bacteria begun to die. Diuron is able to disrupt the complex ecological community of soil microorganisms and also inhibits microbial activity in soil, even at concentration as low as several parts per million, causing conditions averse to restoring soil fertility (Prado and Airoldi, 2001).

In addition, the comparison of three biological treatments including natural attenuation, biostimulation and bioaugmentation in Type-II soil (silty clay soil) supplemented with 20 ppm and 100ppm diuron were examined. At the end of the 4-weeks experiment, the amounts of diuron were still remained similarity to that of the control in silty clay soil. Theses results may demonstrate that diuron was tightly absorbed on to the silty clay soil. Besides, soil properties and the indigenous soil microbial population affect the degree of biodegradation (Bento *et al.*, 2005). The population density of diuron-degrading bacteria determined by plate count technique with the supplementation of 20-ppm and 100-ppm of diuron in silty clay soil were further examined to observe if a possible relationship existed to microbial activity. The results also showed maintenance of bacterial growth during 4 weeks among three types of bioremediation in both conditions.

Bioremediation: Condition B

The comparison of three biological treatments including natural attenuation, biostimulation and bioaugmentation in loam soil supplemented with 20 ppm diuron was examined. Natural attenuation is the process that diuron was reduced due to naturally-occurring processes by indigenous microorganisms in soil. Biostimulation is the treatment which 1,000 ppm NH_4Cl was added. Bioaugmentation was carried out in which

approximately bacteria consortium A1 were provided at 10^8 CFU/g soil. Abiotic control was also carried out. In this study, natural attenuation treatment and bioaugmentation demonstrated a high ability to degrade diuron with maximal activity by 31% and the degradation percentage was higher than that in biostimulation which showed degradation activity with 26%. Stimulation treatment supplemented by 1,000 ppm NH_4Cl with C:N ratio of 11:1 was less effective biodegradation in loam soil. On the contrary to previous report that the nutrient supplementation at lower nitrogen concentration high C:N ratio was more effective in enhancing biodegradation than that at high N for microbial growth (Atagana *et al.*, 2003). The lower biodegradation may be caused by some abiotic stress (pH, soil, characteristic and environment) and biotic stress (predators and parasites) in soil (Alexander, 1994). The changes in absolute nutrient supply levels, at constant supply ratios, will alter total hydrocarbon degrader biomass, resulting in altered rates of hydrocarbon biodegradation was reported by Smith *et al.* (1998). However, 1,000 ppm NH_4Cl which was added for biostimulation showed the enhancing in dehydrogenase activity about 4-fold.

Additionally, the supplementation with 100-ppm diuron showed changes in percentage of biodegradation among three types of bioremediation including natural attenuation, bioaugmentation and biostimulation. The bioaugmentation with inoculation of bacterial consortium A1 demonstrated a high ability to degrade diuron with maximal activity by 22% and the degradation percentage was higher than that in biostimulation and natural attenuation which showed degradation activity with 18% and 14% respectively. Similar situation was reported for diesel oil-degrading bacteria that were highly able to degrade hydrocarbon in Long Beach and Hong Kong soils (Bento *et al.*,

2005). The populations of diuron-degrading bacteria determined by plate count technique with the supplementation of 20-ppm of diuron showed that bacterial growth slightly increased after the first 5 days because they adapted themselves to the new environment with had some abiotic stress and biotic stress Alexander, 1994), the supplementations with 100-ppm diuron, however, the populations were reduced after the first 5 days.

The comparison of three biological treatments including natural attenuation, biostimulation and bioaugmentation in silty clay soil supplemented with 20 ppm and 100 ppm diuron was further examined. At the end of the 15 days experiment, the levels of diuron were not altered compared to that of the control in silty clay soil. It may possibly demonstrate that diuron was tightly absorbed on to the silty clay soil affected to biodegradation of bacteria. However, the low levels of biodegradation occurred in the control. The populations of diuron-degrading bacteria determined by plate count technique with the supplementation of 20-ppm and 100-ppm of diuron in silty clay soil were further examined. The supplementations with 100-ppm diuron, however, the populations were reduced after 10 days. It might be possible that they could not adapt themselves to the new environment with had some abiotic stress and biotic stress. Moreover, diuron may able to inhibits microbial activity and effect to community of soil microorganisms (Prado and Airoldi, 2001).