CHAPTER I

INTRODUCTION

1.1 Statement of problem

Environmental pollution can harm the flora and fauna of habitats, resulting in the uptake and accumulation of toxic chemicals in food chain. It is causing serious health problems and/or genetic defects in humans. Although substantial progress has been made in reducing chronic industrial pollution over recent years, major accidents still occur. A considerable number of known polluted sites exist and new ones are continually being discovered. Some of these contaminated sites could become sources of contamination of drinking-water supplies, and thereby constitute a substantial health hazard for current and future generations (Timmis and Pieper, 1999).

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is a substituted phenylurea herbicide used widely for weed control on non-crop areas such as roads, railways and parks and selectively on crops such as citrus, cotton, pineapple and sugar-cane (Cullington and Walker, 1999; Tixier *et al.*, 2002; Widehem *et al.*, 2002). Diuron is very persistent at soil surface partly due to its low solubility (Widehem *et al.*, 2002). The dispersion of diuron in agriculture leads to pollution in soil and aquatic environment by soil leaching (Sumpono *et al.*, 2003; Giacomazzi and Cochet, 2004).

Diuron is harmful to human and animals. The exposure to diuron can cause eye irritation, skin irritation and formation of methemogobin (methemogobin is an abnormal form of hemoglobin). Diuron has caused genetic damage in developing embryos and in bone marrow cells in mice. It also decreased the production of substances necessary for normal immune system function, and caused reduced birth weights when laboratory animals were exposed during pregnancy (Cox, 2003).

Besides, the U.S. Environmental Protection Agency (USEPA) classifies diuron as a "known/likely" carcinogen because it has caused bladder cancer, kidney cancer and breast cancer in studies with laboratory animals (Cox, 2003; Giacomazzi and Cochet, 2004). Due to its toxicity and persistence in the environment, diuron has been classified as one of the priority pollutants. Therefore, it is necessary to clean up contaminated diuron in the environment by appropriate methods.

The degradation can involve abiotic and biotic treatment. Abiotic treatments have been used for diuron removal. However, these methods are known to be expensive than biotic treatments. Biotic treatment is a major treatment in the complete mineralization of aromatic compounds to harmless inorganic products (Alexander, 1994).

Bioremediation is believed to be one of the useful treatments to clean up diuron-contaminated in the environment. Bioremediation is advantageous over other techniques in that it is a natural and safe process. Moreover, the operating cost is comparatively less expensive (Alexander, 1994; Evans and Furlong, 2003). Bioremediation accelerates the natural occurring biodegradation under optimized conditions such as oxygen, temperature, pH, nutrients, moisture and process-related operating conditions such as homogeneity (Hupe *et al.*, 2001).

Three main types of bioremediation today are natural attenuation, biostimulation and bioaugmentation. Natural attenuation utilizes intrinsic degradation capability of the indigenous microorganisms to degrade contaminates and is a natural degradation process (Kaplan and Kitts, 2004). This strategy is advantageous as it avoids damaging the ecologically sensitive habitats. However, intrinsic bioremediation often takes a long time to complete because of population size of the indigenous degrading microorganisms is low.

Biostimulation is a treatment supplying additional nutrients or substrates into the soil to stimulate degradation of native microorganisms (Seklemova and Pavlova, 2001). This treatment requires site adjustments to provide the indigenous microorganisms with a desirable environment in which they can effectively degrade contaminants and improve natural biodegradation rate (Olaniran *et al.*, 2006).

Sometimes, the indigenous microorganisms do not have ability to degrade the toxic compound or the indigenous microorganisms are present in low number or absent. Therefore, it is necessary to add exogenous microorganisms or specialized microorganisms as either a pure culture or a mixed culture for the treatment. This process is called bioaugmentation (Richard and Vogel, 1999).

The present study compared three types of bioremediation techniques to remediate diuron contaminated soil. The efficiency of diuron bioremediation treatments was evaluated by comparing the amounts of diuron after natural attenuation, biostimulation and bioaugmentation. Moreover, the number of diuron degrading bacteria, the total microbial activity and microbial community during bioremediation were monitored.

1.2 Objectives

The main objective of this study was to evaluate the efficiency of three bioremediation treatments including natural attenuation, biostimulation and bioaugmentation for clean up 2 types of diuron contaminated soil.

1.3 Hypothesis

1.3.1 Bioaugmentation should high efficiency treatment to degrade diuron-contaminated in soil more than biostimulation and natural attenuation.

1.3.2 Properties of soil influenced the efficiency of bioremediation treatment used for clean up diuron.

1.3.3 Biostimulation using biostimulating agents could improve biodegradation of diuron in soil.

1.4 Scope of study

1.4.1 The study evaluated the efficiency of three bioremediation techniques.

a. Comparison of three biological treatments; natural attenuation, biostimulation and bioaugmentation.

- Natural attenuation: diuron degradation was occurred by natural processes.
- Biostimulation: diuron degradation was occurred by indigenous bacteria after induction by *n*-butanol, Triton X-100 and NH_4Cl.
- Bioaugmentation: diuron degradation was occurred by the inoculated pure bacterial culture or bacterial consortium.

b. Comparison of two types of soil samples.

Two types of soil samples different in physical and chemical properties were used in the experiment. The samples were collected from agricultural areas in Nakornayok and Nakornratchasrima provinces.

1.4.2 The study of bacterial populations to support the result of diuron degradation.

a. Determination of the number of diuron degrading bacteria during biological treatments.

b. Determination of the total microbial activity by dehydrogenase activity assay.

c. Analysis of the change of microbial communities using Single-Strand Conformation Polymorphism (SSCP) of 16sDNA.